The genetic basis and effect of the few-branched-1 (*Fbr1*) mutant tassel trait on grain yield and seed production dynamics in maize

by

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DECLARATION

I declare that the thesis hereby submitted by me for the degree of Philosophiae Doctor in Agriculture at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I further cede copyright of the thesis in favour of the University of the Free State.

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Shorai Dari Date

DEDICATION

This piece of work is dedicated to my father Stanford Takadurai Munjoma, and my mother Sophie Munjoma who have taught me the value of hard work. Their mentorship has made me whom I am today.

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ABBREVIATIONS AND SYMBOLS

AEA	Average environmental axis
AEC	Average environmental coordinate
AFLP	Amplified fragment length polymorphism
AMMI	Additive main effect and multiplicative interaction
ANOVA	Analysis of variance
ASI	Anthesis silking interval
ASO	Allelic specific oligonucleotides
ASV	AMMI stability value
BeCA	Bioscience for Eastern and Central Africa
bi	Regression coefficient
bp	Base pair
CD	Cob diameter
CIMMYT	International Maize and Wheat Improvement Centre
cm	Centimetres
cM	Centimorgan
CML	CIMMYT maize line
CTAB	Cetyl Trimethyl Ammonium Bromide
CV	Coefficient of variation
CW	Cob weight
Df	Degrees of freedom
DNA	Deoxyribonucleic acid
DTA	Days to anthesis
DTMA	Drought tolerant maize for Africa
DTS	Days to silking
EDTA	Ethylenediaminetetraacetic acid
EL	Ear length
EMS	Ethyl methanesulfonate
ERN	Ear row number
EW	Ear weight
F	Inbreeding coefficient
F_1	First filial generation
FAM	5-Carboxyfluorescine
FAOSTAT	FAO statistical database
Fbr1	Few-branched-1 tassel mutation
FM	Floral meristem
FRET	Florescence resonance energy transfer
g	Gram
GCA	General combining ability
GD	Genetic distance

GGE	Genotype and genotype x environment interaction
GLM	General linear model
GSI	Genotype selection index
GxE	Genotype by environment interaction
GYC	Grain yield components
GYLD	Grain yield
H^2	Broad sense heritability
h^2	Narrow sense heritability
ha	Hectare
HP	High parent
HPH	High parent heterosis
IAA	Indol-acetic-acid
IITA	International Institute for Tropical Agriculture
InDels	Insertions and deletions
IPCA	Interaction principal component
Kg	Kilogram
KPE	Kernels per ear
KRN	Kernel row number
KW	Kernel weight
l	Litre
LSD	Least significant difference
m	Metre
MALDI-TOF	Matrix-assisted laser desorption/ ionisation time-of-flight
MAS	Marker assisted selection
masl	metres above sea level
MET	Multi-environmental trials
MP	Mid parent
MPH	Mid parent heterosis
MRD	Modified Roger's distance
N	Nitrogen
^oc	Degrees Celsius
OLA	Oligonucleotide ligation assay
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PIC	Polymorphic information content
PYC	Pollen yield components
PYLD	Pollen yield
QTL	Quantitative trait loci
r^2	Coefficient of determination

RAPD	Random amplified polymorphic DNA
$RASV_i$	Rank of AMMI stability value
REML	Restricted Maximum Likelihood
RFLP	Restriction fragment length polymorphism
ROX	Rhodamine X dye
S_1	Inbred lines from first selection cycle
SAM	Spikelet apical meristem
SCA	Specific combining ability
SHMM	Shifted multiplicative model
SM	Spikelet meristem
SNP	Single nucleotide polymorphism
SPM	Spikelet pair meristem
SSR	Simple sequence repeat
TBN	Tassel branch number
TE	Tris EDTA
TTL	Total tassel length
TWL	Tassel weight loss
UPGMA	Unweighted pair group method with arithmetic averages
US	United States
VIC	2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein
Y_i	Mean grain yield
YS_i	Yield stability statistic
σ^2_A	Additive genetic variance
σ^2_D	Dominance genetic variance
σ^2_e	Environmental or error variance
σ^2_g	Genetic variance
σ^2_p	Phenotypic variance
σ^2_{sca}	SCA variance
σ_p	Phenotypic standard deviation
%	Percent
µl	Micro litre

Chapter 1

General introduction

Maize (*Zea mays* L) was introduced into Africa by the Portuguese at the beginning of the 16th century (Reader, 1997), and it has since become Africa's second most important food crop, after cassava. The popularity of maize among African farmers grew slowly until the early part of the 20th century. Maize cultivation in southern Africa was initially linked to the spread of commercial mining, as maize required less labour to grow and process than the traditional grain crops, millet and sorghum (Byerlee and Heisey, 1997). Per capita consumption of maize in Africa is highest in eastern and southern Africa (De Vries and Toenniessen, 2001). It is grown in major agro-ecological zones in southern Africa covering millions of hectares (ha) and is the staple food for more than 200 million inhabitants in the region (FAOSTAT, 2003). In sub-Saharan Africa alone, the demand for maize is projected to increase from the 1995 level to 93% by 2020 (Pingali and Pandey, 2001).

Small- and medium-scale farmers produce up to 95% of maize in Africa. The farms are usually 10 ha or less and yields on these farms are usually low, averaging 1.2 t ha^{-1} (Byerlee and Heisey, 1997). Compared with traditional crops, maize is relatively susceptible to moisture and nutrient stress. In tropical sub-Saharan Africa, small-scale farmers dominate production of maize under stressful conditions of disease, low soil fertility and drought, and with limited access to the essential inputs (Bänziger and de Meyer, 2002). In most cases, these farmers have either little or no access to improved technologies. Drought and low soil fertility are the biggest production constraints on small-scale farmers' fields in Africa, and they are ever present (Edmeades et al., 1994). Frequent droughts that reduce maize production, are common in southern Africa. The weather patterns are variable, such that highly favourable seasons are often followed by unfavourable drought years. The average annual loss of maize production due to moisture stress in eastern and southern Africa is 13% of total production, which translates to 1.8 million tons per year (Waddington et al., 1994). Drought affects 36% of estimated area in lowland tropics, 21% of area in the sub-tropical mid-altitude mega-environment and 0% in the highland mega-environment (CIMMYT, 1988). In Malawi, Zambia and Zimbabwe, there have been fluctuations

in grain production, which was attributable to rainfall variation, among other factors, from 1961 to 2003. Thus severe droughts have periodically reduced grain production since more than 93% of the crops are not irrigated (Bänziger and Diallo, 2002; Pingali and Pandey, 2001). Therefore, Campos et al. (2004) suggested that appropriate cultivars for release should carry base-line drought tolerance, regardless of the area of their deployment.

Maize is produced in three mega-environments i.e. highland, mid-altitude and tropical lowland. Low and declining soil fertility is the biggest production constraint across all these three environments. Especially low nitrogen (N), remains one of the biggest constraints as farmers usually do not have access to fertilizer in developing countries. Although low soil fertility is a serious threat to regional food security, it is a factor that farmers are aware of on their own farms, and which they can take into account when they plant. But on the other hand, tolerance to low soil N has been observed to be associated with drought tolerance in maize. Drought stress has been exacerbated in recent decades of declining soil fertility, which is often associated with reduced soil water-holding capacity (Derera, 2005).

Maize is one of the most important cereals in the world, and it is also one of the crops which has been most frequently improved in breeding programmes. Up to the early 1900s breeding was limited to recurrent selection methods (De Vries and Toenniessen, 2001). Population improvement is done through a series of recurrent selection procedures. The aim of this is to combine as many as possible favourable alleles for superior crop performance at each locus to maximise yield in a given environment. Hybrid varieties are still not in use in many maize producing African countries. It is estimated that 63% of maize grown in Africa is of unimproved, or landrace, varieties. The lack of hybrid varieties is largely due to poorly developed seed industries. This is often linked to poorly developed economies in these countries. Investments in breeding and in the seed industry in Kenya and Zimbabwe lead to early adoption of hybrid maize varieties by farmers in these countries (Gerhart, 1975; Rattray, 1969). The first commercial use of a single-cross hybrid in the world was achieved in 1960 in Zimbabwe when breeders released the single-cross hybrid ‘SR52’ (De Vries and Toenniessen, 2001). This is one of the indications of the role that conventional plant breeding programmes in Africa can play in food security.

The breeding efforts at the International Maize and Wheat Improvement Center (CIMMYT) have focused on incorporating drought tolerance into elite germplasm (Monneveux et al., 2006). The improvement of drought-tolerance relies on manipulation of adaptive traits, that limit yield under the target stress. Under drought conditions, as resources become limited, a hypothesis has been put forward that tassel size influences the development of the ear and silk (Ribaut et al., 2004). The tassel can dominate the ears and thus limit grain yield by three different mechanisms: (1) shading of the upper leaves (Duncan et al., 1967; Hunter et al., 1969); (2) acting as a competitive sink (Anderson, 1972) and (3) modifying the supply of growth regulators (especially auxins) and CO_2 acceptors (Seyedin et al., 1980). The degree of competition between tassel and ear development is highly related to the plant's environment (Sangoi and Salvador, 1996).

Under favourable conditions (water, light and nutrients) there is less competition between male and female inflorescences, but under stress conditions (high populations and drought stress), apical dominance is increased and ear development decreases resulting in barrenness and decreased grain yield (Sangoi and Salvador, 1996).

Plant morphology and yield components that develop during growth play a significant role in determining yield (Ledent, 1984). In maize, tassel morphology has an effect on grain yield as it intercepts radiation to the canopy (leaves) and diverts available photosynthates away from the developing grain (Ribaut et al., 2004). The negative effect of the tassels on yield was demonstrated when de-tasselled plants yielded 19% more grain than plants that had not been de-tasselled or had tassels removed and then re-joined (Hunter et al., 1969). This yield increase was attributed to interception of radiation by the tassels. Other studies have shown a correlation between detasselling and reducing the number of tassel branches with a positive effect on yield (Lambert and Johnson, 1977; Geraldi et al., 1985). In tropical maize, unlike in temperate maize, the indirect pressure of selection for reduced tassel size by selecting for increased grain production has had relatively modest effects on tassel size. Most tropical inbreds still possess a relatively large tassel (12 to 20 branches), except for highland germplasm (1 to 10 tassel branches). Tassel morphology also has an effect on maize intercrops as it determines the aggregate amount of photosynthetically active radiation reaching the crop under the cereal foliage. In Zimbabwe, smallholder farmers routinely intercrop maize with cucurbits, cowpeas,

beans and groundnuts, thus breeding for small tassel morphology may increase the yield of these intercrops.

Single characters often relate strongly to yield and their selection may improve yield, but long-term yield improvement probably results from coordinated improvement in all yield components (McNeal et al., 1978; Vidal-Martínez et al., 2001). Numerous studies have been done on yield components in maize but very little research has been done on pollen. Individual tassel traits have been regularly related to grain yield but not to pollen yield components. Sharma and Dhawan (1968) pointed out the importance of considering certain tassel and ear characters simultaneously when creating new inbred lines.

Based on theory rather than experimental evidence, breeders have not taken pollen production into account and have not considered it as a limitation to kernel set. Selection has therefore been more in the direction of plants with small tassels (Fischer et al., 1987) to reduce their dominance over the ear. Tassel size, tassel weight, and tassel branch number have been found to be negatively associated with grain yield, therefore breeders have indirectly selected smaller tassels (Lambert and Johnson, 1977; Geraldi et al., 1985; Fischer et al., 1987). As such, tassel weight of Pioneer hybrids decreased by 36% from 1967 to 1991 (Duvick and Cassman, 1999), while yielding ability has also increased (Kisselbach, 1999). Pollen production has been found not to limit kernel set (Bassetti and Westgate, 1994; Otegui et al., 1995). Yet, if the tassel size is reduced very much, there may not be enough pollen produced per plant to produce an adequate kernel number. Pollen production could be particularly important in certain specific production systems, like the seed industry and high-oil maize, where only a small proportion of plants (usually less than 20%) are used as pollinators (Uribelarrea et al., 2002). Limited information is available, however, on pollen production of modern hybrids and the effect of breeding for reduced tassel size on seed production.

Working with the hypothesis of tassel size effect on yield under stress, CIMMYT breeders have successfully introduced an ethyl-methanesulfonate (EMS) induced, few-branched-1: designated as *Fbr1* by Neuffer (1989), tassel mutation from a Mexican donor line of tropical adaptation into elite CIMMYT maize lines by backcrossing. The *Fbr1* mutation in maize is seen as a reduced

number of tassel branches, usually less than three. Plants are usually quite normal, although the second tassel branch from the base is often replaced by a small leaf bract. In some plants irregularly formed awns appear on the tips of the glumes. Neuffer (1989) found the homozygotes to have slightly more extreme tassel characteristics than the heterozygotes. Dr. John MacRobert (Personal communication, 2009) also observed that it is consistently a dominant mutation, which has demonstrated additive effects in certain genotypes. This *Fbr1* tassel mutation seems to be a potentially useful morphological trait under stress environments as the improvement of stress-tolerance relies on manipulation of adaptive traits that limit yield. Evaluation of *Fbr1* populations under drought and low N stress conditions allows the determination of effects of the tassel mutation under these conditions. These particular populations can be of interest if yield advantages over the normal tasselled types under stress outweigh presumed pollen reductions due to reduced tassel size.

SNP markers have become an ideal marker system for genetic research in many crops. SNPs are abundant and evenly distributed throughout the genomes of most plant species (Yan et al., 2009). Several high throughput platforms have been developed. These allow rapid and simultaneous genotyping of up to a million SNP markers (Yan et al., 2010). SNPs can be used in the same manner as other genetic markers for a variety of functions in crop improvement, including linkage map construction, genetic diversity analysis, marker-trait association and marker-assisted selection (MAS). More than 30 different SNP detection methods have been developed and applied in different species (Gupta et al., 2008). SNP markers were used in this study to characterize the backcross-converted *Fbr1* CIMMYT maize lines (CMLs) to assess the level of inbreeding and relatedness of these lines to the recurrent normal-tasselled CMLs.

The main objective of this research was to genetically characterise the *Fbr1* maize lines, do a genetic analysis on yield performance, and study the effects of the *Fbr1* dominant mutant tassel trait on maize yield (under stress and optimal conditions). This will help in developing recommendations on breeding for the *Fbr1* trait in maize improvement programmes.

Specific objectives of this study were:

(i) To assess relatedness and level of homozygosity of *Fbr1* and non-*Fbr1* CIMMYT maize lines by genetic fingerprinting using SNP markers .

(ii) To do a genetic analysis and yield evaluation of CIMMYT *Fbr1* maize inbred lines and hybrids under stress and non-stress environments.

(iii)To evaluate phenotypic relationships between grain yield and tassel size in *Fbr1* maize genotypes under abiotic stress and optimal conditions.

(iv) To determine yield stability of *Fbr1* maize lines and hybrids across optimal and stress environments using AMMI and GGE models.

(v) To investigate SNP- based genetic diversity among *Fbr1* maize lines and its relationship with heterosis, combining ability and grain yield of *Fbr1* testcross hybrids.

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Chapter 2

Literature review

2.1 The development of the maize inflorescences

Two types of inflorescences develop on monoecious maize plants – the tassel bearing male flowers, and the ear bearing female flowers. The tassel arises directly from the spikelet apical meristem (SAM) after it has ceased producing leaves, whereas the ear develops from the tip of an auxillary branch. Both of these distinct florescences develop in the same manner after each meristem undergoes a series of branching, and transitions of their identity (Irish, 1997).

The first event is in the change in the identity of the meristem to an inflorescence meristem, and this occurs after the plant develops from the vegetative to the reproductive phase in response to intrinsic and extrinsic factors. Once an inflorescence meristem is initiated, it produces a second type of meristem – the spikelet pair meristem (SPM); these arise in multiple rows (polystichous) of SPM and in an acropetal manner, that is, the meristems are initiated from the base towards the tip. In tassels, the SPMs that arise first give rise to branch meristems that initiate tassel branches bearing more SPMs. Each of the remaining SPMs produces a third type of meristem – the spikelet meristem (SM). Each SPM produces one SM before it too is transformed to an SM. In the tassel, each SM produces a pair of bract like organs, the glumes, and initiates the lower floret meristems (FMs) before becoming the upper floret meristem. Each FM then gives rise to the terminal floral organs; in tassels, the pistils abort, while in ears, the lower pistil and the anthers abort (Turnbull, 2005).

The maize tassel and ear are organs that come out as separate inflorescences that carry male and female flowers respectively. They are formed from a developmental system that involves a number of meristem identities. Phenotypic and genetic studies of mutants that affect meristem initiation, size, determinacy and identity have been done. This information generated insights into genes and gene interactions affecting these traits. There is a whole collection of mutants. They are included in the databases of ethyl methanesulfonate (EMS) and transposon-based

screens. This mutant collection will in future be used to provide information for geneticists and developmental biologist (Bennetzen and Hake, 2009).

There is a large amount of variation in the tassels and ears of various inbred genotypes. This also reflects the large amounts of allelic diversity found among these inbreds (Liu et al., 2003). The number of tassel branches varies from three to 20, while tassel length, angle of tassel branches and the size and number of ears per plant varies greatly. Natural variation in maize is used as the basis to find quantitative trait loci (QTL) (Upadyayula et al., 2006; Zhao et al., 2006) and this variation makes association mapping possible. Association mapping identifies statistical associations between traits and genetic markers.

Maize inflorescence development is influenced by a number of mutations. Many of these classical mutants have been described (Coe et al., 1988). Some of these mutations have influenced sex determination, while others affect inflorescence morphology. Some mutations influence specific combinations of features. Mutation effects are a result of changes in meristem functions during the development of the inflorescence, or changes in the differentiation of organs produced by meristems, or both. These changes affect meristem initiation, size and maintenance, meristem identity or determinacy or features of sex determination and floral organ specification (Table 2.1) (Bennetzen and Hake, 2009). Additional functions of these genes are revealed when mutants are introgressed into different genetic backgrounds.

2.2 Flowering and determinacy in maize

Plants produce new organs and structures throughout their growing and production cycle. This is done through the action of meristems. Meristems are concentrations of self-regenerating stem cells found at the apex of shoots and roots (Steeves and Sussex, 1989). Divisions in the meristem result in cells with different functions. The central zone consists of cells in the centre of the meristem. These cells refill the meristem, so that it maintains a distinct size. The morphogenetic zone contains the cells in the periphery of the meristem. These cells lead to the development of different organs (Bortiri and Hake, 2007).

Table 2.1 Selected mutants in maize that affect the inflorescence development in maize

Mutant[†] symbol	Map location	Meristem function	Meristem identity	Organ dev	Sex determ	Gene product
an1	1.08	-	-	√	√	Ent-kaurene synthase
Bif1	8.02	√	-	-	-	-
bif2	1.05	√	-	-	-	S-T kinase
fea3	3.04	√	-	√	-	-
Fas1	9.05	√	-	√	-	-
Fbr1	Unplaced	√	-	-	-	-
ra2	3.04	-	√	-	-	LOB domain (TF)
ra3	7.04	-	√	√	-	Trehalose phosphatase
tsh2	Unplaced	√	-	√	-	-
te1	3.05	-	√	√	√	RNA binding

[†]Mutant symbol, is the shortened symbol for the most common mutant alleles (dominant alleles start with uppercase and recessive alleles start with lowercase); map location is chromosome and bin in which the gene has been cloned. Gene names for each mutant symbol: *an1=anther ear1*, *Bif1=barren inflorescence1*, *fea3=fasciated ear3*, *Fas1=fascicled ear1*, *Fbr1=few-branched1*, *ra2=ramosa2*, *ra3=ramosa3*, *tsh2=tassel sheath2* and *te1=terminal ear1* (Bennetzen and Hake, 2009).

Organogenesis and self-perpetuation are balanced processes and this balance leads to prolonged activity resulting in an indeterminate meristem. The alternative to indeterminate meristems is determinate meristems. One example is flower production, where the process ends after a certain number of organs have been made.

Both indeterminate and determinate meristems influence the formation of maize inflorescence. A number of mutations affect various stages of inflorescence development (Neuffer et al., 1997) resulting in mutants with abnormal meristem size or miss-specification of organ identity, or both. The genetics of inflorescence and flower development in maize and other grasses has been extensively studied (McSteenet al., 2000; Bommert et al., 2005).

The spikelet is a compact axillary branch with two bracts, each subtending to several reduced flowers and is the basic unit of grass inflorescence architecture (Clifford, 1987). Maize is a monoecious plant that produces male flowers on a terminal tassel, and female flowers on lateral ears. The ears arise in the axils of vegetative leaves and the tassel have several long, indeterminate branches at the base while the ear is made up of a single spike with no long branches (Bortiri and Hake, 2007). The main spike and branches of the tassel, and the whole ear, produce short branches called spikelet pairs and these bear two spikelets. The branches and spikelet pairs develop in the axils of bracts: the small, undeveloped leaves. In maize, spikelet and spikelet pair meristems are determinate since they produce a defined number of organs (Vollbrecht et al., 2005).

2.3 Genetic regulation of inflorescence architecture

Inflorescence architecture is being studied in several model species for which mutants with defective inflorescences are known. The application of insertion mutagenesis with transposons, or T-DNAs, available for some of the plant models has facilitated the isolation of mutants for known target genes and also the identification of novel genes influencing inflorescence architecture (Turnbull, 2005). A candidate gene approach focusing on key regulators of inflorescence form has been successfully applied to pea (Hofer et al., 1997; Foucher et al., 2003), which has a rich collection of inflorescence architecture mutants.

2.4 Quantitative trait loci for tassel traits in maize

A large amount of the natural variation in inflorescence shape, which can be seen in maize and other grass species is usually a result of a number of genes that have a cumulative effect at several loci. In maize there are four different reproductive meristem types. They are the inflorescence meristem, the spikelet pair meristem, the spikelet meristem, and the floret meristem (Irish, 1997). Tassel branch number and tassel weight are determined by several loci with quantitative effects, and these cause changes in the growth of one or more meristem types. These loci include *ramosa1 (ra1)*, *ramosa2 (ra2)*, *ramosa3 (ra3)*, *barren stalk2 (ba2)*, *Tassel seed6 (Ts6)*, and *branched silkless1 (bd1)* (Coe et al., 1988). The locus *Ts6*, for example, causes extra branches to form in the tassel. In a study by Geraldi et al. (1985) on the inheritance of tassel characters in different maize populations, value (h^2, single plant basis) of 36.1% was found

for tassel weight and 45.8% for branch number averaged over the three populations. There was a high negative correlation (r = -0.65) between branch number and grain yield. In another study, branch number inheritance was determined from two inbred lines differing in branch number. The generation means of their progeny was analysed (Mock and Schuetz, 1974). Heritability on single plant basis was 0.50. Branch number was mainly determined by additive gene effects but there was also some dominance gene effects involved. Fischer et al. (1987) reduced branch number by 7.7% per cycle averaged over three tropical maize populations when they conducted six cycles of selection for reduced branch number. Bolaños et al. (1993) did eight cycles of selection for drought tolerance to determine how this influenced branch number. The selection process decreased branch number by 2.6% per cycle, from 19.1 branches in cycle 0 to 14.8 branches in cycle 8.

The identification of a QTL for a quantitative trait is dependent on sample size (N) from the original population and the heritability of the trait (Beavis et al., 1994). The fraction of the additive genetic variance explained by detected QTL is inversely related to the product h^2N (Melchinger et al., 1998). For a trait with moderate or low h^2, and working with a sample size of N is 100-200, the chances of detecting a QTL in a population are quite low unless if the trait is a major QTL: if it explains a greater fraction of the genetic variation within the population (Berke and Rocheford, 1999).

A study on a population of 200 S_1 lines derived from a single F_1 plant from a cross of Illinois High Oil (IHO) by Illinois Low Oil (Early Maturing) found that the QTL showed both additive and dominant gene effects (Berke and Rocheford, 1999). The measured traits such as branches per tassel, tassel weight, and tassel angle had varied direction in different genomic regions of dominance and type of gene effects of the QTL.

2.5 Morphology of tassel components and their relationship to some quantitative features of maize

In maize breeding, increased attention is being paid to the selection of features that can help reach maximum yield with regulation of energy conversion (Bódi et al., 2008). In addition to plant height, ear height, leaf number and leaf area, tassel characteristics can influence plant

performance and productivity significantly. Morphology of tassel components influencing primarily pollen amount can be significant factors determining the success of seed production and selection. Several researchers studied relations between pollen and tassel components (Vidal-Martínez et al., 2001a; b; 2004; Rácz et al., 2006; Hidvégi et al., 2005, 2006) and found that pollen yield is affected by tassel size. A number of authors examined the inheritance of tassel characteristic. Mock and Schuetz (1974) researched the inheritance of tassel branch number and found that it was quantitatively inherited with a high heritability estimate. Geraldi et al. (1978) found 86.1, 45.8 and 28.8% heritability for tassel weight, tassel branch number and tassel length, respectively. Inheritance of tassel characteristics is not fully clarified according to Berke and Rocheford (1999). Work done by Geraldi et al. (1978; 1985), Vidal-Martínez et al. (2001a), Gyenesné Hegyi et al. (2001) and Hegyi (2003) showed that selection targeted on the decrease of tassel branch number and tassel size may indirectly increase yield. Selection for smaller tassels decreases the energy of the plant consumed for tassel formation and the shading of flag and upper leaves (Lambert and Johnson, 1977). Smaller tassel size in the case of male parental lines, however, can cause problems in F_1 seed production and the maintenance of male lines due to unsatisfactory pollen production and shed (Wych, 1988). Tassel branch number is a determinant of pollen amount (Vidal-Martínez et al., 2001a). In hybrid breeding programmes an ideal male parent should have large tassels that can produce large amounts of pollen. An ideal female should partition more assimilates towards big ears and hence should possess small tassels (Upadyayula et al., 2006). Bódi et al. (2008) compared tassel components and some quantitative features of maize grain yield using Pearson's correlation coefficient. The strength of relations between traits and directions of interactions were determined. They concluded the importance of correlation studies of tassel components as indirect selection criteria in maize breeding and seed production.

2.6 Effect of tassel size on grain yield and genetics of tassel branch number in maize

Increasing solar-energy interception by the maize canopy is one solution to the problem of increasing the efficiency with which maize converts solar energy into grain (Schuetz and Mock, 1978). Most maize genotypes are barren when grown at high plant densities that maximise solar-energy interception; thus barrenness must be overcome to maximise grain yield. Small tassels are associated with density tolerance (decreased barrenness at high densities) in maize. For example,

Buren et al. (1974) found correlations between dry weight of the tassel at pollen shed and grain yield ranging from -0.41 to -0.81 for three sets of maize hybrids grown at a plant density of 98 800 plants ha^{-1}. A correlation of -0.82 between mean tassel branch number of pairs of inbred parents and grain yield of their respective F_1 hybrids was reported by Sharma and Dhawan (1968). Evaluation of correlated responses to recurrent selection for grain yield in three Iowa maize breeding populations showed that six to seven cycles of selection had increased grain yield and decreased both tassel branch number and tassel dry weight significantly (Fakorede and Mock, 1978). Several studies have demonstrated that decreasing tassel size, rather than completely eliminating tassel or pollen production, has a positive effect on yield.

Small-tasselled single-cross hybrids must be produced after small-tasselled inbreds are developed if maize breeders are to significantly increase the density tolerance of the maize crop. Evidence suggests that choice of a line to be used as male or female has little bearing on the tassel size of the hybrid progeny. Schuetz and Mock (1978) found no evidence of reciprocal effect between two crosses involving BSSS-36 and BSSS-78, and mean numbers of tassel branches did not differ significantly for B75 x H19 (7.35±0.18) and H19 x B75 (7.74±0.21). Mock and Schuetz (1974) found no evidence for a reciprocal effect for crosses involving BSSS-11 and BSSS-26.

The nature of gene action involved in inheritance of tassel traits can help breeders to devise better selection strategies, and to seek improvement in these traits in the desired direction (Sofi, 2007). Most of the studies have shown that additive gene action is predominant in the inheritance of tassel and ear traits whereas few studies have come up with evidence for non-additive gene action such as dominance and epistasis (Schuetz and Mock, 1978; Guei and Wasson, 1996; Berke and Rocheford, 1999; Wolf and Hallauer, 1997; Hinze and Lamkey, 2003).

2.7 Pollen production and kernel set in maize

In maize breeding there has been selection toward reduced tassel size. It is generally accepted that maize pollen production does not reduce kernel set, but very little is known about pollen production of modern hybrids and the effect of reduced tassel size on this trait (Uribelarrea et al., 2002). A short anthesis-silking interval (ASI = silking date minus anthesis date) is an important

trait for increasing grain yield in maize (Bolaños and Edmeades, 1993a; b). An increase in ASI from -0.4 to 10 days, caused a decline in yield of 8.7% per day. Increased ASI under water-stressed conditions could reduce kernel number because there is no pollen for late-appearing silks (Hall et al. 1981; 1982). Therefore, a short ASI should contribute to the pollination of a larger number of differentiated florets (Uribelarrea et al., 2002). However, Otegui et al. (1995) found that the addition of fresh pollen in ovaries of late-pollinated silks did not improve kernel set in maize. Thus, under stress conditions, the availability of pollen does not seem to be the cause of reduced kernel number. A short ASI improves synchronous pollination among ovaries within and between ears (Uribelarrea et al., 2002) and this increased grain yield (Sarquis et al., 1998) and kernel number (Carcova et al., 2000) of different maize genotypes planted at different plant densities in different environments. Breeders have in recent years ignored pollen production as a limitation to kernel set (Uribelarrea et al., 2002). Therefore most breeders will select plants with small tassels (Fischer et al., 1987) to reduce their dominance over the ear. Normally, under increased plant density, tassel dominance is enhanced (Edmeades and Daynard, 1979a; Edmeades et al., 2000a; b) and the effects on yield are significant.

Although it is assumed that pollen production does not limit kernel set (Bassetti and Westgate, 1994; Otegui et al., 1995), continued reduction of tassel size could reduce the amount of pollen produced per plant and consequently reduce kernel number. Pollen production is critical in production systems like the seed industry and the high-oil maize, where a small fraction of plants (normally less than 20%) are used as pollinators. In this situation it is important that breeders understand the dynamics of pollen production, so that there is enough pollinators in the population to guarantee maximum kernel set (Uribelarrea et al., 2002).

In maize, the quantification of pollen under field conditions is difficult considering the availability of airborne pollen that could be floating in the field, and only limited data is available on pollen quantification (Uribelarrea et al., 2002). Hall et al. (1982) described pollen production of plants grown in pots under different water treatments. They bagged the tassels to collect pollen and sub-sampled pollen samples to quantify the number of pollen grains. Struik and Makonnen (1992) removed the tassels of plants in the field, and grew them on water in a greenhouse. They also bagged the tassels and collected pollen every second day. They weighed

the amount of pollen but did not count the number of pollen grains per unit area or per plant. In both studies however, tassels were bagged and cut, which could be traumatic, or to artificial environmental conditions, which could have decreased pollen production relative to the natural field conditions. Bassetti and Westgate (1994) alternatively used pollen traps of the kind described by Sadras et al. (1985) for collecting pollen. This method did not affect normal tassel development and also the method provided information on pollen availability per unit land area.

It is very important to make sure that selection for reduced tassel size is not accompanied by a reduction in pollen shedding period of the plants since no pollen would be available for late-appearing silks from the late-silking plants in the population (Uribelarrea et al., 2002). Thus, selection for characteristics associated with tolerance to stress, like increased plant density, reduction in ASI, interplant variability in silking date and ASI of individual plants, should include evaluation of secondary traits like reduction in tassel size and pollen production with no reduction in the pollen shedding duration (Uribelarrea et al., 2002).

2.8 Breeding maize for abiotic stress

2.8.1 Drought and low N tolerance improvement in maize

There is large variability between plants for abiotic stress tolerance, both between species and within populations of a single species (Ribaut et al., 2002). Abiotic stresses are the biggest constraints in crop productivity of almost all crops globally, but the nature of tolerance is not well characterised. Crop productivity can be improved through a better understanding of tolerance mechanisms. Characteristics associated with tolerance to abiotic stresses include morphological and physiological traits such as the morphology and depth of root the system, the architecture of the plant, regulation of the stomata, variation in the thickness of leaf cuticle, osmotic adjustment, antioxidant capacity, regulation of hormonal system, tolerance of the plant to desiccation: membrane and protein stability, maintenance of photosynthesis, and control of reproductive events (Bohnert et al., 1995; Shinozaki and Yamaguchi-Shinozaki, 1996; Bray, 1997; Nguyen et al., 1997; Edmeades et al., 2001). The large number of related characteristics is to be expected as plants under stress conditions have to tolerate differences in soil composition, temperature and water potential during development (Ribaut et al., 2002). Breeding for drought tolerance is a challenge due to its unpredictable nature. It is also a challenge to select the correct

environment for selection for drought tolerance as environments can vary considerably (Ribaut et al., 2002).

Average annual yield losses in maize caused by drought are estimated at 17% in tropical regions (Edmeades et al., 1989; Monneveux et al., 2006; Shirani Rad et al., 2012). In southern Africa for example, loss in individual seasons can reach up to 60% (Rosen and Scott, 1992). Maize in developing countries is mainly produced under low N conditions (McCown et al., 1992; Oikeh and Horst, 2001) because of limited N use and reduced N uptake in drought prone environments. Also the high price of fertilizer, which is not comparative to the low value of the grain harvested, the lack of availability of fertilizer, or lack of credit to farmers (Bänziger and Lafitte,1997) makes N use limited. Thus, for the past several decades, maize breeding programmes at CIMMYT have focused on breeding for drought and low N tolerance (Monneveux et al., 2006).

Maize is very sensitive to water stress a week before to two weeks after flowering (Grant et al., 1989). Drought during this period causes a delay in silk emergence and consequently an increase in the ASI (Edmeades et al., 2000a) and grain aborts (Boyle et al., 1991). Abortion of grain normally occurs during the first 2 to 3 weeks after silking (Westgate and Boyer, 1986; Schussler and Westgate, 1991). If canopy photosynthesis is reduced by any kind of stress, grain abortion increases. Movement of assimilates to the developing ear can also be reduced resulting in the fall of assimilate levels to levels below a threshold required to sustain formation of grain and growth (Edmeades and Daynard, 1979b; Tollenaar et al., 1992). A decrease in photosynthesis could be a result of a decrease in radiation interception, associated with reduced leaf expansion, rolling of the leaves (Bolaños et al., 1993) and foliar senescence (Wolfe et al., 1988). Photosynthesis reduction could also be a result of the reduction in carbon fixation per unit leaf area because of closure of the stomata or a decline in carboxylation capacity (Bruce et al., 2002). Barrenness, ASI, leaf senescence, and leaf rolling are important secondary traits that are useful for improving maize yield under drought environments because of their high heritability and correlation with yield under stress conditions (Bänziger et al., 2000). Under N stress, final grain number is also reduced due to increased kernel abortion (Uhart and Andrade, 1995a). The approximately 85% of the abortion that occurred during the first 20 days after female flowering, reported by Monneveux et al. (2005) was closely related to a lack of post-flowering N uptake by the crop

(Below, 1997). A lack of N causes reduced leaf area index and consequently radiation interception. It also increases rate of senescence in lower leaves (Wolfe et al., 1988; Moll et al., 1994), decreases radiation use efficiency (Uhart and Andrade, 1995b), and increases ASI (Jacobs and Pearson, 1991; Edmeades et al., 2000b). Bänziger and Lafitte (1997) and Bänziger et al. (2000) suggested that ASI and foliar senescence could be useful secondary traits for improving maize for low N tolerance.

The growth and development of maize plants is affected by differences in N supply (McCullough et al., 1994). N supply is very critical during the beginning of grain filling within the maize plant (Christensen et al., 1981). N affects a range of characteristics such as photosynthetic rate, leaf area, size of the sink and thus yield (Dass et al., 1997). When there is a shortage of N, leaves become the main source of mobilized N to the ear (Below, 1997). The reduction in chlorophyll concentration and yellowing of the leaves are good indicators of N remobilization (Dwyer et al., 1995). N deficiency increases the rate of leaf senescence by reducing chlorophyll concentration (Monneveux et al., 2005). Kernel abortion results when N is lacking in a plant (Pearson and Jacob, 1987) causing a reduction in the final grain number (Lemcoff and Loomis, 1986; Uhart and Andrade, 1995a; b; Monneveux et al., 2005) and grain yield (Monneveux et al., 2005). Reduction of grain weight under low N conditions is the result of reduction in grain filling period rather than a reduction in growth rate (Monneveux et al., 2005).

2.8.2 Target secondary traits identification under drought/low N stress conditions

The use of secondary traits in selection has the potential to improve the efficiency of selection under stress conditions, whether it is low N or drought stress. Bänziger and Lafitte (1997) reported that the use of secondary traits: ASI, leaf senescence, ears per plant and leaf chlorophyll concentration, increased selection efficiency for grain yield when broad-sense heritability of grain yield was low under low N. Moll et al. (1987) found that selection for prolificacy under low N improved the identification of superior genotypes. Bänziger and Lafitte (1997) used prolificacy and leaf senescence to discriminate genotypes that were superior under low N stress. A higher number of ears per plant under low N stress indicate the ability of a plant to yield more under low N stress (Wolfe et al., 1988).

Some key secondary traits that control the response of plants under drought or low N stress environments have been identified in different crops (Ribaut et al., 2002). If a trait is associated with grain yield under drought or low N, then it qualifies to be a useful secondary trait for selection under these conditions. A suitable secondary trait must also have a high heritability value, be inexpensive and quick to measure, must be stable and be observed at or before anthesis and must not be associated with yield reduction under optimal conditions (Edmeades et al., 2001). Maize yield is reduced dramatically when drought or N stress occurs just before and during flowering. This is because silking is delayed, resulting in an increase in the length of the ASI (Hall et al., 1982; Westgate and Bassetti, 1990; Bolaños and Edmeades, 1993a). This asynchrony between male and female flowering has been associated with reduction in grain yield under drought (Westgate and Boyer, 1986; Edmeades et al., 2000b). ASI is simple to measure in the field, and is highly heritable under stress environments. The "stay green" characteristic is a tolerance mechanism where stems and upper leaves stay green when water availability is limited at grain filling (Ribaut et al., 2002). As this phenomenon occurs after flowering it is very important, because drought has a very negative influence on yield at this stage. Stay green genotypes maintain more photosynthetically active leaves than genotypes without the trait (Rosenow et al., 1983). This increased photosynthesis period could lead to increased yield in crops where the harvest component consists mainly of carbohydrate (Thomas and Smart, 1993).

A study was done on two drought tolerant CIMMYT maize germplasm populations 'DTP1' and 'DTP2' to evaluate direct and correlated responses to recurrent selection for drought tolerance (Monneveux et al., 2006). The improved tolerance over cycles of selection was a result of increased partitioning of biomass towards the developing ear (Bolaños and Edmeades, 1993b), rather than changes in water status or senescence. The same findings were reported in Argentinean hybrids where increased tolerance was mainly due to increased partitioning of dry matter to the ear. The increase in ear growth was a result of considerable decrease in tassel and stem weight. There was also successful competition between the ear at flowering and other organs for available carbon products (Monneveux et al., 2006) . A decrease in the number of ovules at silking caused a reduction in grain abortion in advanced cycles of selection for drought tolerance, resulting in reduced competition among developing grains: sink reduction. Monneveux et al. (2006) pointed out that further research on drought tolerance in tropical maize

should focus on reducing competition between developing grains and other organs growing at a time that concurs with kernel set.

2.8.3 Genotype by environment (GxE) interaction, combining ability and heterosis under stress and non-stress conditions

2.8.3.1 GxE interaction

GxE refers to differential responses of genotypes or cultivars across a range of environments (Kang, 1998; 2004). GxE interaction complicates the selection of superior genotypes (Magari and Kang, 1993; Ebdon and Gauch, 2002a; b) and reduces correlation between phenotypic and genotypic values, thus hindering selection progress (Comstock and Moll, 1963). It is a problem when breeders ignore GxE interaction especially when it is significant and larger than the genotype main effect, which common in yield trials (Gauch and Zobel, 1996). The existence of GxE interaction justifies the need for additional broad-based testing in different environments and predict the variability expected among farms (Busey, 1983).

GxE interactions are of major consequence to the breeders in the process of evolution of improved varieties. When varieties are grown at several locations to test their performance, their relative rankings usually do not remain the same (Dabholkar, 1999). This causes difficulty in demonstrating significant superiority of any variety. GxE interaction is present whether varieties are pure lines, single-crosses, double-crosses, top-crosses, S_1 lines or any other material with which the breeder is working. Stratification of the environment has been recommended to reduce the GxE interaction, e.g. large and heterogeneous geographical region may be subdivided such that environment within each sub-region is relatively homogeneous. The stratification is usually based on such macro-environmental differences as temperature gradients, rainfall distribution and soil types. However, even with this refinement technique, interaction of genotypes with environment in a given sub-region remains large.

Genotype and environment may exhibit their interaction in several ways (Mather and Jinks, 1982). Environment may cause change in the genetic constitution of a population by pressure of the selection (e.g. differential fertility and/or viability) it exercises on the population, such that in

the long run it may lead to evolutionary changes. In the short term, however, pressure of selection from the environment may alter the genetic constitution of segregating material. Therefore, differential fertility and/or viability of various genotypes may change the genetic constitution of segregating material (Dabholkar, 1999).

Most important traits such as grain yield are quantitatively inherited and therefore show large GxE interactions. It is therefore necessary to evaluate genotypes across multiple environments, which are referred to as multi-environmental trials (MET) in the advanced stages of selection (Annicchiarico, 2002; Kang et al., 2004). In MET, varying genotypic responses to the different environmental conditions, especially when rankings of the genotypes change, prevents the identification of superior, stable hybrids (Epinat-Le Signor et al., 2001). When GxE interaction for a trait is significant, the usefulness of overall genotype means is reduced (Kang, 1998, 2002; Annicchiarico, 2002). When cultivars are grown in different environments, the identification of the highest performing and most stable cultivars is possible (Lu'quez et al., 2002). When breeders are looking for genotypes that show wide adaptation, they should identify those genotypes that do not show any GxE interaction or those that show non-crossover GxE interaction (Matus-Cádiz et al., 2003). As a result, estimating stability of performance becomes important in breeding programmes in order to identify genotypes that are consistent in performance and also that are high-yielding (Kang, 1998).

Under drought stress conditions, GxE interactions are common and they make breeding progress difficult (Bänziger et al., 2004). A number of factors can cause GxE interactions such as variation in the timing and severity of water deficits, genetic variation in flowering time, and nutrient deficiencies (Bänziger and Cooper, 2001; Cooper et al., 1999). High error variances e.g. those induced by variable plant stand or variable soil water holding capacity, are common in field trials grown under drought. This can make selection decisions difficult as these trials are often planted in adverse conditions which differ from conditions experienced at research stations (Bänziger et al., 2004).

Environmental factors which lead to GxE interactions can be classified as predictable and unpredictable (Allard and Bradshaw (1964). The contribution of predictable environmental

variation to GxE interactions can be reduced by assigning specific cultivars to specific environments. Unpredictable environmental variation is more complex and often leads to large genotype x year and genotype x year x location interactions (Allard and Bradshaw, 1964). The level of these interactions can be reduced by selecting stable cultivars that perform consistently across environments.

A basic principle indicated by the GxE interaction is that even if all plants are from the same genotype, they will not necessarily express their genetic potential in the same way when environmental conditions (drought, temperature, disease pressure, stress, etc.) vary. Genotypes are normally tested over a wide range of diverse environments (for example, locations, years, and growing seasons), and agricultural experiments involving GxE interactions may involve a large number of genotypes. Studies have been done to solve the problems caused by GxE interactions (Comstock and Moll, 1963). Stability analysis has been done to determine if cultivars evaluated in MET were stable (Lin et al., 1986; Hühn, 1996; Flores et al., 1998; Hussein et al., 2000; Robert, 2002; Sabaghnia et al., 2006). Usually genotypes that are stable may not be the highest yielding, so methods that integrate yield performance and stability must be used in the selection of superior genotypes (Kang, 1988; Pham and Kang, 1988; Kang and Pham, 1991; Kang, 1993; Kang and Magari, 1996).

Most estimates only provide information on the presence and magnitude of GxE, but give no measurements on the response of the individual genotypes within the environment. They therefore do not give information on stability of individual cultivars. Research has focused on regression analysis, an approach originally proposed by Yates and Cochran (1938) and later modified by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). Regression analysis has been widely used in comparing and measuring genotypic performances of crops like common beans.

Various statistical methods have been proposed to analyse GxE interaction data. These methods include analysis of variance (e.g. Least Squares, Restricted Maximum Likelihood = REML), regression (e.g. joint regression analysis, partial least-squares regression, factorial regression), shifted multiplicative model, variance components, cluster analysis, factor analysis, and additive

main effects and multiplicative interaction effects (AMMI model). To apply the AMMI model, the conventional analysis of variance for the additive main effects is combined with principal component analysis for the multiplicative interaction (non-additive residual) effects to analyse the matrix of two-way means. The AMMI model can effectively be used for stability analysis because it captures a large portion of the GxE sum of squares. Main and interaction effects can be distinguished from this analysis and it provides agronomically meaningful interpretation of the data (Ebdon and Gauch, 2002a; b). The results obtained from AMMI analysis can be used in breeding programmes to identify genotypes with specific adaptation and to select the most favourable environments where genotypes can be grown (Gauch and Zobel, 1997). The results of AMMI analysis are shown in common graphs called biplots. In the biplots, genotype and the environment values and their relationships are shown using the singular vectors technique. The AMMI model has been extensively used in the statistical analysis of multi-environment cultivar trials (Kempton, 1984; Gauch and Zobel, 1989; 1997; Crossa et al., 1990a).

The advantages of the AMMI model or its variants are that they use overall fittings, impose no restrictions on the multiplicative terms and result in least square fit (Freeman, 1990). Within limits, any model may be expected to fit the data from which it was derived. With the AMMI model, predictions for new sites and new years are possible (Gauch, 1988). The principal component analysis of AMMI partitions GxE interactions into several orthogonal axes, the interaction principal component analyses (IPCA). Gauch and Zobel (1996) showed that AMMI1 with IPCA1 and AMMI2 with IPCA1 and IPCA2 are usually selected and the graphical representation of axes, either as IPCA1 or IPCA2 against main effects or IPCA1 against IPCA2 is generally informative. When AMMI3 and higher models are presented in an analysis, the third and higher IPCA axes are dominated by noise and have no predictive value (van Eeuwijk, 1996). Genotypes with PCA1 scores close to zero have general adaptation. A larger genotypic PCA1 score indicates that genotypes are specifically adapted to environments with PCA1 scores with the same sign (Xu, 2010). When varieties are placed close to environments (therefore having similar IPCA1 loadings), it means those are the environments where those varieties yield well. Ideal test environments should exhibit small (absolute) IPCA2 (meaning it is more representative of the overall environment) and large IPCA1 (absolute) scores, in order to have more power to discriminate genotypes in the terms of the genotypic main effect (Gauch and Zobel, 1996).

The shifted multiplicative model (SHMM) proposed by Cornelius et al. (1993) clusters genotypes into groups within which crossover interactions do not exist. Within such groups, the genotypes with the best mean would be the most desirable genotypes having high mean performances and low coefficient of variation (CV) values. The regression methods of analyses involve the regression of genotype means on the environment means and the regression coefficient is used as a measure of the consistency of the genotypic performance over environments (Aremu et al., 2007).

The yield stability statistic (YS_i) (Kang, 1993) and the genotype and genotype x environment interaction (GGE) distance (i.e., the distance from the markers of individual genotypes to the ideal genotype: an ideal genotype has the highest yield and is highly stable) in GGE biplot analysis (Yan, 2001; Yan and Kang, 2003) help in the selection for yield and stability. The GGE biplot analysis is based on singular-value decomposition or principal component analysis (Yan and Kang, 2003). The GGE biplot method was used to evaluate test environments in soybean (Yan and Rajcan, 2002), cotton (Blanche and Myers, 2006), and common bean (Kang et al., 2006), to characterise end-use quality in wheat (Morris et al., 2004); and to breed cultivars with specific adaptation to specific environments in rice (Samonte et al., 2005). Ober et al. (2005) used the GGE biplot analysis to evaluate some physiological traits and found that these could be used as indirect selection criteria for drought tolerance.

2.8.3.2 Significance of GxE interaction and stability

According to Allard and Bradshaw (1964) ‘a variety which can adjust its genotypic or phenotypic state in response to fluctuations in environment in such a way that it gives stable economic returns for place and year is termed as “well buffered”. Two basic concepts of phenotypic stability are distinguished: (i) the biological concept and (ii) the agronomical concept. The biological concept of stability is the constant performance of a genotype over a wide range of environments and the agronomical concept of stability implies that a stable genotype should always give high yield expected at the level of productivity of the respective environments, i.e., a variety with genotype-environments interaction as low as possible.

2.8.4 Combining ability

An important objective of maize breeding programmes is to develop and use inbred lines with superior combining ability for grain yield and other agronomic traits to form excellent hybrid combinations (Dhliwayo et al., 2009). Estimation of the breeding values and heterotic patterns of maize inbreds can be achieved with factorial mating designs such as the diallel and North Carolina Design II (Sprague and Tatum, 1942; Comstock and Robinson, 1948; Griffing, 1956).

Diallel mating designs are important in plant breeding programmes for the determination of general combining ability (GCA), specific combining ability (SCA) and for estimating heritability of quantitative traits (Hayman, 1954; Griffing, 1956; Fry, 2004). Griffing's (1956) diallel methods have been widely used in crop plants (Evans et al., 1966; Stuthman et al., 1971; Borges, 1987; Pixley and Bjarnason, 1993; Kang et al., 1995, 1999; Zhang et al., 1996). Diallel mating designs are used to obtain estimates of genetic effects for a fixed set of parental lines (fixed effects) or to estimate GCA and SCA variance components from a set of randomly chosen parental lines (random effects) from multi-environment experiments (Zhang and Kang 1997). The random model can be used to estimate GCA and SCA variances. The fixed model can be used to measure the GCA effects for each parent and SCA effects for each pair of parents (Bernardo, 2002). GCA and SCA effects indicate the potential value of inbred lines for use as parents in the formation of hybrids (Sprague and Tatum, 1942). Combining ability of inbred lines is the most important factor which determines the potential of lines for hybrid development (Hallauer and Miranda, 1988). Genetic variance, as determined by the concept of combining ability, is partitioned into two components: variance due to GCA and variance due to SCA (Hallauer and Miranda, 1988; Sughroue and Hallauer, 1997). The magnitude of GCA variance indicates the measure of additive gene action while that of SCA gives an estimate of non-additive gene action: dominance and epistasis (Sprague and Tatum, 1942; Rojas and Sprague, 1952; Gowen, 1964; Kambal and Webster, 1965).

Additive and non-additive effects in diallel crosses statistical analysis indicate the type of gene action important in controlling a particular trait under study (Baker, 1978). The proportion of additive and non-additive components of genetic variance is determined by the genetic structure of the crosses analysed and the environmental conditions where the genotypes were grown

(Khotyleva and Trutina, 1973). Younes and Andrew (1978) reported the importance of additive gene action versus non-additive gene action for the majority of traits in previously unselected material. Pixley and Bjarnason (1993) found that GCA was more important for parents that have been isolated from populations improved through recurrent selection for GCA and for parents that have not been grouped into heterotically complementary groups during their development. Betrán et al. (2003) found negative SCA for hybrids which were compiled from inbred lines with the same germplasm origin or which were related and greater SCA was found for hybrids formed from inbred lines of different source germplasm origin. Crossa et al. (1990b) reported highly significant GCA and SCA variance for grain yield, anthesis date and prolificacy, in a diallel cross among Mexican races of maize. GCA effects were more important in determining grain yield among the maize genotypes. Lee et al. (2005) reported the influence of both additive and non-additive genetic effects on grain yield of inbred line crosses but the additive genetic component was more important: contributing 74% of the total genetic variance for grain yield. Betrán et al. (1999) found that the importance of GCA and additive genetic effects increases as the level of drought stress increases. In the same study, non-additive gene effects were more important under low N stress. Betrán et al. (2003) reported the importance of additive genetic effects accounting for 84% of the genetic variance under severe drought, 60% under well-watered conditions and 61% across drought and low N stress and unstressed environments. Under low N, the non-additive gene effects were more important than the additive genetic effects.

2.8.5 Heterosis/hybrid vigour

The phenomenon of heterosis has been exploited extensively in maize breeding (Hallauer and Miranda, 1988). The term heterosis was first used by Shull in 1914. Heterosis may be defined as the superiority of an F_1 hybrid over either its parents in terms of yield or some other character (Singh, 2005). Falconer and Mackay (1996) defined heterosis as the difference between the performance of a cross for a trait and the average performance of the two parents for that trait. According to Miranda (1999), heterosis is the genetic expression of the superiority of a hybrid in relation to its parents. The two main types of heterosis are mid-parent and high-parent heterosis. Mid-parent or average heterosis is the increased vigour of the F_1 over the mean of two parents. High-parent or better parent heterosis is the increased vigour of the F_1 over the better parent

(Sinha and Khanna, 1975; Jinks, 1983). Many crops have benefit from the expression of heterosis but both the genetic and physiological mechanisms underlying this phenomenon are still elusive (Hallauer and Miranda, 1988; Tollenaar et al., 2004). Dominance, over-dominance and epistasis are the three major theories that explain the mechanisms underlying the phenomenon of heterosis (Hallauer and Miranda, 1988). However, heterosis is believed to result largely from dominance gene action (Singh, 2005).

Diallel cross analysis for a fixed set of open-pollinated varieties provides the foundation for the initial analysis of heterotic pattern among crosses (Hallauer and Miranda, 1988). Preliminary inferences are deduced when effects in the analysis of variance are significant. Thus, average heterosis indicates the superiority of variety crosses over mid-parent values. When variety heterosis is significant, it means the heterotic pattern of at least one of the varieties is different from the others when crossed with the remaining varieties. Specific heterosis results from specific crosses. Significant heterosis occurs when at least one cross differs from the others due to non-additive effects and differences in gene frequency of varieties (Hallauer and Miranda, 1988).

It is very expensive and time consuming to identify superior parental inbred lines that can be used to produce superior hybrids in the development of maize hybrids (Betrán et al., 2003). *Per se* performance of maize inbred lines does not predict the performance of maize hybrids for grain yield (Hallauer and Miranda, 1988). If single-cross hybrid performance or heterosis between parental inbred lines can be predicted, this could increase the efficiency of hybrid breeding programmes. The relationship between genetic distance and heterosis of two parental varieties was reported by Moll et al. (1965) and Hallauer and Miranda (1988). Higher levels of heterosis were observed with increased differences between parents up to a certain point, but heterosis declined when the differences were too large (Moll et al., 1965). On the other hand, low grain yield heterosis was observed for crosses among genetically similar germplasm and for crosses among broad genetic base germplasm (Hallauer and Miranda, 1988; Crossa et al., 1990b; Beck et al., 1991; Vasal et al., 1992a; b). In crosses among CIMMYT's subtropical and temperate maize germplasm, Beck et al. (1991) found high-parent heterosis for grain yield ranging from -14.8 to 9.9%. Glover et al. (2005) found high-parent heterosis of 48% in crosses among 10 Chinese and

US lines. They found that populations used in these crosses had narrow genetic base relative to those used in other exotic maize diallel studies. Vasal et al. (1992a) reported high-parent heterosis of 13% in diallel crosses among seven CIMMYT sub-tropical and temperate early-maturity maize lines. Tollenaar et al. (2004) reported an average heterosis of 167% for grain yield, 109% for kernels per plant and 12% for 1000-kernel weight.

2.8.6 Genetic distance versus hybrid performance

Environment can have a large influence on the performance of inbred lines and hybrids. It can change the relationship between genetic distance and heterosis. Betrán et al. (2003) evaluated inbred lines and hybrids in 12 stress and non-stress environments and found that heterosis was expressed more under drought stress and less under low N environments than under non-stress environments. Bruel et al. (2006) evaluated the genetic diversity of 16 maize lines and determined the correlation between genetic distance and hybrid performance using random amplified polymorphic DNA (RAPD) markers and observed a direct relationship between genetic divergence and productivity of the hybrids. Legesse et al. (2008) investigated the relationship of genetic distance with hybrid performance and midparent heterosis (MPH) in highland maize germplasm. They observed that genetic distances derived from the inbred line x all testers and from the population testers' sub-group were not positively correlated with hybrid performance and MPH for most traits: grain yield, plant height and days to silking. This implied that genetic distance could not be used to predict hybrid performance in that set of germplasm. However, genetic distance can effectively predict hybrid performance in cultivar development and this study evaluated SNP-based genetic diversity among *Fbr1* maize lines and its relationship with heterosis for grain yield of the testcross hybrids.

2.9 Inducing the few-branched-1 mutation in maize inbred lines

2.9.1 Ethyl methanesulfonate (EMS) and mutation breeding

The utilization of induced mutations for the improvement of crop plants has yielded several mutants which have been used directly as new cultivars (Gottschalk and Wolff, 1983). The basis for evolutionary change and all genetic variation that exist among various individuals emanate from mutations (Keightley et al., 2000). Many of the induced mutants, mainly the defective types

which are not usually found among plants of commonly grown varieties, may be useful as material for genetic studies.

Chemical mutagens like EMS have been used as mutagens for both mammalian and plant cells and have been used to generate mutants with desirable traits that can be used in mutation breeding (IARC, 1974). Mutation breeding makes wide use of deviations from the average to improve the characteristics of important crops (Kumar and Kumar Rai, 2007). Induced mutagenesis creates new variability within a short period of time (Akgun and Tosun, 2004). EMS is a very efficient mutagen for creating genetic variability in the natural gene pool of *Zea mays* L. (Kumar and Kumar Rai, 2007). EMS induces random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation. This typically produces only point mutations which are predominantly guanine (G)/cytosine(C) to adenine (A)/thymine (T) transitions (Anderson, 1995; Davies et al., 1999). EMS can induce mutations at a rate of $5x10^{-4}$ to $5x10^{-2}$ per gene without substantial killing. The ethyl group of EMS reacts with guanine in DNA, forming the abnormal base O-6-ethylguanine. During DNA replication, DNA polymerases that catalyse the process frequently place thymine (T), instead of cytosine (C), opposite O-6-ethylguanine. Following subsequent rounds of replication, the original G:C base pair can become an A:T pair, thus changing the genetic information and is usually detrimental to cells.

In common beans, EMS has been used to generate hypocotyls and flower colour mutations in the M_2 generation and several morphologic mutants were found. These were later used as markers for genetic studies (Barbosa et al., 1988). Davies et al. (1999) reported an EMS mutagenesis experiment in *Caenorhabditis elegans* in which they studied the effects of induced mutations on reproductive output. Most of the EMS-induced mutations characterised to date are point mutations. In other organisms some deletions and insertions caused by EMS have been reported, for example in Drosophila (Mogami et al., 1986).

2.10 Introduction of the *Fbr1* tassel mutation into CIMMYT elite maize lines

2.10.1 Recurrent backcrossing

One of the most important objectives of plant breeding is to introgress one or more genes from a donor into the background of an elite variety and to recover the original parent type as quickly as possible (Semagn et al., 2006a). That way, the best qualities of a good variety are recovered from unwanted recombination, when introducing desirable traits from either domesticated or wild germplasm sources. When a desirable trait has been introduced into the parent a number of backcrosses can be made to make the new plant as similar to the recurrent parent as possible: a process known as recurrent backcrossing. Recurrent backcross breeding in maize has facilitated the transfer of favourable alleles for monogenic traits from donor genotypes to elite inbred lines.

Recurrent backcrossing is a breeding method that is used to transfer alleles at one or more loci from a donor to an elite variety (Allard, 1960; Reyes-Valdés, 2000). The assumption made during backcrossing is that the proportion of the recurrent parent genome is recovered at a rate of $1-(1/2)^{t+1}$ for each of t generations of backcrossing (Babu et al., 2004). After six generations of backcrossing 99.2% of the recurrent parent genotype is expected to be recovered and the lines at this stage are said to be near-isogenic. Specific backcross progeny usually deviate from this expectation due to chance and/or linkage between a target gene from the donor parent and nearby genes (Ribaut and Hoisington, 1998). In a study of barley lines backcrossed for seven generations, the segments around the introgressed genes varied from about 1 centimorgan (cM) to 14 cM (Bjornstad et al., 2002) while Young and Tanksley (1989) found introgressed segments as large as 4 cM in tomato cultivars developed after 20 backcrosses, and one cultivar developed after 11 backcrosses still contained the entire chromosome arm carrying the gene from the donor parent. Semagn et al. (2006a) highlighted two main limitations of the backcrossing approach. One is the time needed to do the necessary number of backcrosses, to achieve the introgression objective, the second is that other genes flanking the gene of interest are often simultaneously transferred from the donor parent (linkage drag).

The level of recovery of the recurrent parent genotype can be tested using molecular markers. The relatedness between the recurrent parent and the ‘new’ line: with the added trait from the

donor parent, can be assessed using molecular markers such as simple sequence repeat (SSR) markers or single nucleotide polymorphisms (SNPs).

2.11 Genetic fingerprinting of maize using genetic markers

2.11.1 DNA based markers

DNA-based or molecular markers can be used to determine the amount of genetic diversity in many crop species. Their expression is not influenced by the environment; hence the results obtained after genetic characterisation of genotypes reflect the actual level of genetic difference existing between these genotypes. This is not the case in morphological markers (Smith and Smith, 1992; Westman and Kresovich, 1997). DNA-based marker applications in plant breeding are mostly DNA fingerprinting which is used for genetic diversity assessment and genetic markers for mapping and tagging traits of interest and for accelerated back-crossing.

2.11.2 Single Nucleotide Polymorphism (SNP) markers

A SNP marker is a single base change in a DNA sequence, with a usual alternative of two possible nucleotides at a given position (Semagn et al., 2006b). SNPs can be used as molecular markers in crop improvement for example in quantitative trait locus (QTL) discovery, genetic diversity assessment, association analysis and marker-assisted selection (Hyten et al., 2008). SNPs are abundant and uniformly distributed throughout the genomes of most plant species (Yan et al., 2009). SNPs are ideal for genetic research in many crops and several high throughput platforms have been developed that allow rapid and simultaneous genotyping of up to a million SNP markers (Yan et al., 2010). In crop improvement, SNPs can be used in the same way as other genetic markers are used. There are more than 30 different SNP detection methods that have been developed and applied in different plant species (Gupta et al., 2008).

Genomes of several crops have been sequenced and this has allowed the study of sequence variations between individuals, cultivars, and subspecies (Semagn et al., 2006b). These studies showed that SNPs and insertions and deletions (InDels) are highly abundant and evenly distributed throughout the genome in various species including plants (Garg et al., 1999; Drenkard et al., 2000; Nasu et al., 2002; Batley et al., 2003a). Yu et al. (2002) compared sequences from a japonica rice cultivar to those from an indica cultivar and identified, on

average one SNP every 170 base pair (bp) and one InDel every 540 bp. These polymorphisms are highly abundant in plant genomes, thus making the SNP marker system an attractive tool for mapping, marker-assisted breeding, map-based cloning and in genetic characterisation of crops (Gupta et al., 2001; Rafalski, 2002a; Batley et al., 2003b; Yan et al., 2009).

In other methods, allele discrimination is usually based on size differences on a gel, but this is not the case with SNP. Various SNP genotyping methods have been developed based on several methods of allelic discrimination and detection platforms (Rafalski, 2002b; Vignal et al., 2002; Sobrino et al., 2005; Tost and Gut, 2005). All methods for SNP genotyping involve the generation of an allele-specific product which is then analysed (Semagn et al., 2006a). SNP detection methods can be classified into direct hybridisation techniques and techniques that involve the generation and separation of an allele-specific product (e.g. restriction enzyme cutting, single strand DNA conformation and hetero-duplexes, primer extension, and oligonucleotide ligation assay) (Vignal et al., 2002).

There are four types of SNP genotyping assays which are divided based on molecular mechanism (Sobrino et al., 2005). The first is allele specific hybridisation. Hybridisation with allelic specific oligonucleotides (ASO) is done when two ASO probes are hybridised with the target DNA that contains the SNP. Under optimised conditions, only the perfectly matched probe-target hybrids are stable. ASO is based on distinguishing between two DNA targets differing at one nucleotide position by hybridisation. The second type is primer extension reactions, which involve mini-sequencing and allelic-specific extension. In mini-sequencing, a primer anneals to its target DNA immediately upstream to the SNP and its extension is done with a single nucleotide complementary to the polymorphic base. In allelic-specific extension, the 3' end of the primers is complementary to each allele of the SNP. The primer extends only when there is a perfect match. The third method is oligonucleotide ligation where for every one SNP, two allelic-specific probes and one common ligation probe are required. The common ligation probe is hybridised adjacent to the allelic-specific probe. When there is a perfect match of the allelic-specific probe, the ligase joins both allelic-specific and common probes. The last method is invasive cleavage where the oligonucleotides required (invader probe and allelic-specific probes) anneal to the target DNA with an overlap of one nucleotide. When the allelic-specific

probe is complementary to the polymorphic base, it overlaps the 3' end of the invader oligonucleotide, forming the structure that is recognised and cleaved by the Flap endonuclease, releasing the 5' arm of the allelic-specific probe.

The hybridisation techniques that are commonly used are derived from the Dot Blot. In the Dot Blot, DNA which is to be tested (either genomic, cDNA or a PCR reaction) is fixed on a membrane and hybridised with an oligonucleotide probe (Semagn et al., 2006a). These hybridization techniques need carefully designed probes and hybridisation protocols since they are prone to error (Pastinen et al., 1997). DNA chips (a collection of microscopic DNA spots attached to a solid surface e.g. glass, plastic or silicon chips) are the latest improvement of these techniques. For DNA chips, the probes are directly synthesized using a parallel procedure involving masks and photolithography (Pease et al., 1994).

Allele specific oligonucleotide ligation is a method for SNP typing based on the ability of ligase to covalently join two oligonucleotides when they hybridise next to one another on a DNA template (Semagn et al., 2006b). The invader assay is based on the specificity of recognition, and cleavage, by a flap endonuclease, of the three-dimensional structure formed when two overlapping oligonucleotides hybridise perfectly to a target DNA (Kaiser et al., 1999; Lyamichev et al., 1999).

Several detection methods are available for analysing the products of each type of allelic discrimination reaction: gel electrophoresis, fluorescence resonance energy transfer (FRET), fluorescence polarisation, arrays or chips, luminescence, mass spectrophotometry (Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or (MALDI-TOF), chromatography) (Semagn et al., 2006a). Many SNP typing protocols are available for use by researchers yet no single protocol is available that meets all the research needs. However, the best suitable technology can be selected considering aspects like sensitivity, reproducibility, accuracy, capability of multiplexing for high throughput analysis, cost effectiveness in terms of initial investment for equipment and cost per data-point, flexibility of the technology for uses other than SNP discovery, and time-consumption for analysis.

2.11.3 Genetic diversity studies using SNP markers

Maize is used as a model plant species for genetic research and over the past decades, more work has been done using various DNA marker technologies (Yan et al., 2010). Molecular markers that are extensively used have been classified as hybridisation-based markers e.g. restriction fragment length polymorphisms (RFLPs) (Helentjaris et al., 1986) and polymerase chain reaction (PCR)-based markers e.g. simple sequence repeats (SSRs) or microsatellites (Senior et al., 1993). RFLP and SSR markers possess several of the attributes an ideal marker system should have: high level of polymorphism, even distribution across the genome, co-dominance and production of accurate and reproducible data which can be generated in a high-throughput and cost-effective manner. SNP markers have become the marker system of choice since they meet all these criteria, including the potential for high throughput low cost genotyping (Yan et al., 2010).

Most genetic research and maize breeding work is based on inbred lines developed from hybrids, populations and landraces. Molecular markers such as RFLPs and SSRs or microsatellites are widely used to estimate the relationships among diverse lines (Yan et al., 2009). Marker-based relationships have been used in breeding programmes to estimate the coefficient of parentage and to establish heterotic groups and patterns for hybrid breeding (Reif et al., 2003; Xia et al., 2004; 2005); identify complex population structure and relative kinship (information necessary for association mapping studies) (Yu et al., 2006); and to identify core subsets of lines with the maximum diversity from a larger collection of analysed lines. Marker-based diversity studies have been done with focus on specific germplasm with limited sample size (generally less than 300 inbred lines), including U.S. Corn Belt lines (Lu and Bernardo, 2001; Gethi et al., 2002); European temperate lines (Reif et al., 2005), Chinese temperate lines (Xie et al., 2008), and tropical (Reif et al., 2003; Xia et al., 2004), and subtropical (Xia et al., 2005; Laborda et al., 2005) lines. A few studies also focused on more diverse mixes of germplasm (Reif et al., 2004; Tarter et al., 2004; Liu et al., 2003). For example, Liu et al. (2003) studied a wide collection of 260 lines from four major known subgroups (stiff-stalk, non-stiff-stalk, tropical and subtropical, and "mixed"). These lines are part of a diversity association mapping panel used in a number of studies (Yu et al., 2006; Flint-Garcia et al., 2005; Harjes et al., 2008).

Close to one million maize SNPs are currently available in public databases and many high throughput genotyping platforms have been developed for commercial use (Gupta et al., 2008). These genotyping platforms are valuable for speeding up research progress in large scale diversity analysis, high density linkage map construction, high resolution quantitative trait locus (QTL) mapping and are useful in genome-wide association studies (Yan et al., 2009). Hamblin et al. (2007) compared analyses based on 89 SSRs to analyses based on 847 SNPs in the same maize collection of 259 inbred lines and they found that the resolution in measuring genetic distance using SNPs based on allele-sharing was lower than the more polymorphic SSRs. There are greater possibilities of automating SNPs and this will allow a much higher number of them to be used cheaply in characterisation studies, overcoming the lower genetic information imparted by each SNP. Yan et al. (2009) applied a custom 1536 SNP GoldenGate assay to genotype a collection of lines chosen to represent the global maize diversity available in public maize breeding programmes. The collection included 351 lines selected from a tropical association mapping panel (containing CIMMYT and other public programme breeding lines), and 281 lines from a mostly temperate association mapping panel, well characterised in previous studies (Liu et al., 2003; Flint-Garcia et al., 2005; Harjes et al., 2008).

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Chapter 3

Genetic fingerprinting of 'few-branched-1' *(Fbr1)* and non-*Fbr1* CIMMYT maize lines using SNP markers to assess their relatedness and level of homozygosity

3.1 Abstract

Molecular marker systems such as single nucleotide polymorphisms (SNPs) are proving extremely useful in the characterisation of genetic diversity of maize (*Zea mays* L). The main objective of this study was to genetically fingerprint 12 'few-branched-1' (*Fbr1)* and 14 normal tasselled CIMMYT elite lines using SNP markers, to assess their relatedness and level of homozygosity. These 26 inbreds were assayed with 1074 SNPs. The polymorphic information content (PIC) of the 1074 SNP loci ranged from 0.015 to 0.50, with an average of 0.25. The small average PIC value indicated limited genetic diversity among inbred lines implying that most of these lines are related. Average residual heterozygosity ranged from 0.2 to 36.1% with an average of 8.2%, well above the expected ranges for residual heterozygosity found in maize inbred lines. Thus, some lines used in the study were still heterozygous and these need further selfing to reduce the residual heterozygosity. The modified Roger's distance (MRD) between pairs of inbreds averaged 0.30, with a range of 0.023 to 0.38.

A number of elite CIMMYT lines were successfully converted to *Fbr1*, and were homozygous for the 1074 SNP loci, thus could be used in breeding programmes involving these new tassel mutants. The unweighted paired group method using arithmetic averages (UPGMA) cluster analysis revealed two discrete clusters for the inbred lines, reflecting heterotic groups used by CIMMYT. In the principal component (PC) analysis, PC1 and PC2 explained 10.87 and 9.08% respectively, of the molecular variance in tassel size for the 1074 SNPs.The markers clearly separated maize lines according to tassel morphology. The results confirmed molecular markers as a powerful complement for genetic characterisation, assigning lines into defined heterotic groups and to examine the relationships among inbred lines at DNA level.

3.2 Introduction

The development and application of various DNA marker technologies has contributed significantly to genetic research in maize in the last decades (Yan et al., 2009). The development and use of molecular markers for the detection and exploitation of DNA polymorphism has made a significant contribution to the field of molecular genetics (Semagn et al., 2006). DNA-based or molecular markers are tools that can be used effectively for genetic diversity analysis of many crop species. Unlike morphological markers, these markers are not influenced by environmental factors (Smith and Smith, 1992; Westman and Kresovich, 1997); and they are a reflection of the actual level of genetic difference existing between genotypes.

SNP markers have been found to be abundant and evenly distributed throughout the genomes of most plant species. It is considered to be an ideal marker system for genetic research in many crops (Yan et al., 2009). Several high throughput platforms have been developed that allow rapid and simultaneous genotyping of up to a million SNP markers (Yan et al., 2009), and more than 30 different SNP detection methods have been developed and applied in different crop species (Gupta et al., 2008). Availability of genome sequences of several organisms has allowed the study of sequence variations between individuals, cultivars, and subspecies (Semagn et al., 2006). These studies showed that SNPs and insertions and deletions (InDels) are abundant and distributed throughout the genome in various plant species (Garg et al., 1999; Drenkard et al., 2000; Nasu et al., 2002; Batley et al., 2003a). By comparing sequences from a japonica rice cultivar to those from an indica cultivar, for example, Yu et al. (2002) identified, on average one SNP every 170 base pairs (bp) and one InDel every 540 bp. The abundance of these polymorphisms in plant genomes makes the SNP marker system an attractive tool for mapping, marker-assisted breeding, map-based cloning and in genetic diversity studies (Gupta et al., 2001; Rafalski, 2002; Batley et al., 2003b; Yan et al., 2009).

Hamblin et al. (2007) compared analyses based on 89 SSRs to analyses based on 847 SNPs in the same maize collection of 259 inbred lines and found that the resolution in measuring genetic distance using SNPs based on allele-sharing was lower than the more polymorphic SSRs. There is now the possibility of automating SNP analysis, which creates the opportunity to analyse a much higher number of them, and this has brought down the price of analysis. This also

overcomes the lower genetic information imparted by each SNP. Yan et al. (2009) applied a custom 1536 SNP GoldenGate assay to genotype a collection of lines chosen to represent the global maize diversity available in public maize breeding programmes. The collection included 351 lines selected from a tropical association mapping panel (containing CIMMYT and other public programme breeding lines), and 281 lines from a mostly temperate association mapping panel, well characterised in previous studies (Liu et al., 2003; Flint-Garcia et al., 2005; Harjes et al., 2008).

Recurrent backcrossing is a traditional breeding method which is used frequently to transfer alleles at one or more loci from a donor to an elite variety (Allard, 1960; Reyes-Valdés, 2000). It is assumed that the proportion of the recurrent parent genome is recovered at a rate of $1-(1/2)^{t+1}$ for each of t generations of backcrossing, and this is used for the planning of the traditional backcrosses (Babu et al., 2004). After six generations of backcrossing, the expected recovery of the recurrent parent genome would be 99.2%. These lines are then near-isogenic. Specific backcross progeny usually deviate from this expectation due to chance and/or linkage between a target gene from the donor parent and nearby genes (Ribaut and Hoisington, 1998).

The *Fbr1* tassel mutation is a new trait that has been introduced from a Mexican donor line into current elite CIMMYT maize lines by backcrossing. The usefulness of this trait as a contributing trait for drought tolerance has not yet been evaluated. Work is projected to be done with these new lines, particularly in projects to develop drought tolerant maize, and recommendations on the use of these lines in current breeding programmes are set to be developed. Little is known to date about the location of the *Fbr1* trait in the maize genome. Alongside the study of effects of the *Fbr1* trait under stress conditions, mapping of quantitative trait loci (QTL) associated with the *Fbr1* trait is work that still needs to be done. This will provide valuable information and insights on the usefulness of this new tassel mutation in potential marker-assisted selection (MAS) breeding programmes. The initial step, then, should be to genotype the *Fbr1* lines to assess their homozygosity levels. If the converted lines are not homozygous enough for breeding and molecular work, then more backcrossing/selfing may be required before more work can be done on the lines. Assessing the relatedness of these maize lines will help in future hybridisation

involving these lines. The main objective of this study was to fingerprint *Fbr1* and non-*Fbr1* CIMMYT maize lines using SNP markers to assess their relatedness and level of homozygosity.

3.3 Materials and methods

3.3.1 Germplasm

Twenty six CIMMYT maize inbred lines adapted to the mid-altitude, tropical and/or subtropical environment of southern Africa were used in this study: 12 are *Fbr1* genotypes and 14 have normal tassels (Table 3.1). The 12 are an arbitrary sample (within each CIMMYT maize line family, to make sure each family is represented in the sample), of the *Fbr1* genotypes produced after the tassel mutation was introgressed from a Mexican donor line into CIMMYT elite maize lines by backcrossing.

Table 3.1 CIMMYT maize inbred lines characterised by the 1074 known SNP markers

Inbred line	Pedigree	Heterotic group
CML395 TAS	[[CML395/TAS]BC2/[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]	B
CML443 TAS1	[CML443/TAS]BC2-2-5-2-1-B-B	A/B
CML444 TAS1	[[CML444/TAS]BC1/[CML444/CML395//DTPWC8F31-4-2-1-6]-2-1-1-1-B]-9-3-4-B	B
CML445 TAS1	[[CML445/TAS]BC3/[CML445/ZM621B]-2-1-2-3-1-B]-2-4-2-B	A/B
CML445 TAS2	[CML445/TAS]BC3-1-1-2-1-B	A/B
CML312 TAS	[[CML312/TAS]BC1/MAS[MSR/312]-117-2-2-1-B]-1-3-1-B-B	A
CML444 TAS2	[CML444/TAS]BC2-6-1-1-B-B	B
CML488 TAS	[CML488/TAS]BC2-6-4-2-B	A/B
CML442 TAS	[[CML442/TAS]BC1/ZM621A-10-1-1-1-2-BBBBBB]-2-1-B-B	A
CML445 TAS3	[CML445/TAS]BC3-1-1-2-2-B	A/B
CML443 TAS2	[CML443/TAS]BC2-2-9-1-2-B	A/B
CML444 TAS3	[CML444/TAS]BC2-5Y-3-1-B	B
CML443	CML443	A/B
CML444	CML444	B
CML488	CML488	A/B
CML445	CML445	A/B
CML395	CML395	B
CML312	CML312	A
CML442	CML442	A
LaPostaSeqC7-F180	LaPostaSeqC7-F180	B
LaPostaSeqC7-F18	LaPostaSeqC7-F18	B
CKL05005	CKL05005	B
G16BNSeqC4	G16BNSeqC4	A
LaPostaSeqC7-F71	LaPostaSeqC7-F71	B
CKL05003	CKL05003	B
CML144	CML144	A

The maize inbred lines were advanced by selfing during the 2009/2010 summer season at CIMMYT –Harare research station. After harvesting, 34 seeds per inbred line were packed in envelops and shipped to BioSciences east and central Africa (BecA) hub, Kenya, for the molecular marker analysis.

3.3.2 DNA extraction and SNP genotyping

Seedlings were raised in plastic seed trays for about two weeks until three to four leaf stage in a greenhouse at the BecA hub in Nairobi, Kenya. Equal amounts of leaf tissues were harvested from 10 plants per inbred line, and were bulked, cut into pieces with scissors, and transferred into 1.2 ml strip tubes that contained two 4 mm stainless steel grinding balls. The tissue was freeze-dried (lyophilised) for 3 days using a Labconco freeze dryer (http:// www.labconco.com), as described in the user's manual. The lyophilised leaf samples were ground into fine powder using GenoGrinder 2000 (Spex CertiPrep) at 1500 strokes per minute for 4 minutes at speed = 1x. Genomic DNA was extracted using a modified version of the high throughput mini-prep Cetyl Trimethyl Ammonium Bromide (CTAB) method (Mace et al., 2003).

3.3.2.1 DNA extraction protocol

A 65ºC water bath was turned on one for hour before starting the extraction procedure. Enough CTAB buffer for 100 samples was prepared (450 µl per sample plus 10% extra) and put into a disposable 50-ml Falcon tube. The buffer was incubated in a 65ºC water bath for 30 minutes. Warm CTAB buffer (450 µl) was added to each sample and was capped tightly with polyethylene (chloroform-resistant) strip caps. The samples were loaded onto the grinding device, GenoGrinder 2000, making sure the tubes balance across racks (each with 96 samples). The samples were processed in a GenoGrinder 2000 following the manufacturer's instructions, at 1 500 strokes per minute for 4 minutes. The samples were incubated for 30 minutes in a 65ºC water bath with occasional mixing. The tubes were removed from the water bath and 400 µl of chloroform-isoamyl alcohol (CIA) consisting of 24 parts chloroform: one part octa-1-ol (isoamylalcohol) was added to each sample. Samples were tightly capped and inverted two to three times to mix them well. The plates were centrifuged at 2250g for 20 minutes and about 300µl of the supernatant was transferred into fresh strip tubes without discarding the interface.

About 210µl of ice-cold isopropanol was added to the supernatant followed by mixing by inverting the tubes to precipitate the DNA. The plates were centrifuged at 2250g for 30 minutes. The supernatant was carefully poured out without disturbing the pellet, followed by air drying of pellet for 20 minutes. Low-salt TE buffer (200 µl) and 3 µl of RNase A were added per sample and left overnight at room temperature. A volume of 200 µl Phenol-chloroform-isoamylalcohol (PCI) was added to each sample followed by mixing well by inverting the tubes. The mixture was centrifuged at 2250g for 15 minutes.

The supernatant was transferred to freshly labelled strip tubes by use of multichannel pipettes and 200 µl of CIA was added. The mixture was mixed by inverting the tubes for two to three minutes. The mixture was centrifuged at 2250g for 15 minutes followed by transfer of the supernatant into freshly labelled strip tubes. Ethanol-acetate solution (315 µl) and 1.5 ml of 3 M sodium acetate (pH 5.2) was added to each sample and samples were placed at -20ºC for 10 minutes. The samples were centrifuged again at 2250g for 20 minutes and supernatant carefully discarded without disturbing the pellet. The pellets were washed with 200 µl of 70% ethanol and the plates were centrifuged at 2250g for 10 minutes. The supernatant was carefully poured off and the remaining pellet was air-dried for 1 hour. The air-dried pellet was re-suspended in 100 µl low-salt TE and stored at 4ºC awaiting DNA analysis.

The quality of the isolated DNA was verified after running the aliquots of DNA samples on a 0.8% agarose gel that contained 0.3µg/Ml SYBR safe DNA gel stain (Invitrogen). DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer.

3.3.2.2 SNP genotyping and allele calling

SNP genotyping and allele calling was made by KBiosciences (http://www.KBioscience.co.uk) using the KASPar system as described in the user's manual (http://www.kbioscience.co.uk/reagents/KASParSNP_Genotyping_System_Leafletv6.3.pdf). The genotyping process involved nine steps which were:

(i) Designing of assay by PrimerPicker

(ii) Arraying of samples in microtitre plate

(iii) Making assay mix from designed oligos

(iv) Making reaction mix from kit components and making assay mix

(v) Dispensing reaction mix over samples

(vi) Sealing of plates

(vii) The thermo-cycling process

(viii) Reading of plates in fluorescent plate reader, and

(ix) Plotting and scoring of data

Primer design for the KASPar chemistry was achieved using the PrimerPicker software found at http://www.kbioscience.co.uk/primer-picker/. DNA samples (4 µl) at ≥ 5ng/µl were arrayed in 96 well microtitre PCR plates. At least 24 samples were genotyped to ensure a sufficient number that will show clustering. A water sample was included per 96 well plate to act as a negative control. After arraying, samples were dried on the 96 well plates. Genotyping assays were designed and developed and these comprised of three unlabelled oligonucleotides, combined proportionately. The three constituent primers (allele specific primer 1, allele specific primer 2 and common or reverse primer) are stored together in one SNP-specific Assay Mix for ease of use. The assay mix was then combined with the reaction mix (4x) and added to the DNA samples to be genotyped. The 8µl of total reaction volumes for the 96 well genotyping comprised of 5 ng/ml DNA, 4X reaction mix, assay mix, KTaq polymerase, $MgCl_2$ (50 Mm) and H_2O. The combined assay mix and reaction mix were dispensed over DNA samples followed by plate sealing with the Fusion Laser welding system. The sealed plates were PCR cycled on a PCR thermal cycler using an initial hot-start activation followed by a two step cycling programme. The hot-start activation was at 94ºC for 15 minutes. The optimised cycling conditions were one cycle at 94ºC for 10 seconds, 57ºC for 5 seconds, 72ºC for 10 seconds followed by a second cycle (18x) at 94ºC for 10 seconds, 57ºC for 20 seconds and 72ºC for 40 seconds.

After the thermo-cycling process, the KASPar data were obtained from a Fluorescence Resonance Energy Transfer (FRET)-capable plate reader with relevant filters. Rhodamine X (ROX) is an internal standard dye which is used as a passive reference together with 5-Carboxyfluorescine (FAM) and 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC), which are allele specific dyes. ROX, as reference and fluorescence of either VIC or FAM

fluorophores were used to distinguish genotypes. The FAM and VIC data were plotted on the x- and y-axes, respectively and inclusion of the passive reference dye (ROX) allowed data to be normalised by dividing FAM and VIC values by the passive reference value for that particular well, thus removing the variable of liquid volume, leading to a tighter clustering, and hence, more accurate data.

3.3.3 Screening of SNP data

SNP markers (a total of 1250) were used for characterising the inbred lines of which 1242 SNPs had data that passed the quality control checks of KBiosciences. Eight SNP markers, BDIBC175, PHM2187_46, PZA01857_1, PZA03012_7, PZA02681_8, PZA00939_1, PHM4757_14 and PHM18705_23, when used to genotype the samples, did not return quality data.

One hundred and sixty one of the 1242 successful assays were monomorphic in all the lines, and seven markers had extremely high heterozygosity, therefore 1074 SNPs were used for final evaluation of the maize lines.

3.3.4 Statistical analysis

Summary statistics of genetic data such as minor allele frequencies, polymorphic information content (PIC), heterozygosity and number of alleles were computed with Powermarker version 3.25 (Liu and Muse, 2005). Modified Roger's genetic distance (MRD) (Wright, 1978; Goodman and Stuber, 1983) between each pair of inbred lines was computed as:

$$MRD_{ij} = \tfrac{1}{2} [\sum (X_{ai} - X_{aj})^2]^{\frac{1}{2}},$$

where X_{ai} is the frequency of the allele *a* for individual *i*, and X_{aj} is the frequency of the allele *a* for the individual *j*.

The PIC for each locus was determined as described by Smith et al. (1997). The genetic relationship among inbred lines was assessed using cluster analysis performed on the MRD distance matrix with UPGMA clustering.

Associations among genotypes were revealed with the Principal Component Analysis (PCA) algorithms (Gower, 1966) implemented in XLSTAT (2010), a statistical and multivariate analysis software (www.xlstat.com), based on MRD estimates between pairs of inbred lines.

Genotypes were grouped into two classes, according to tassel morphology: either normal tasselled (1) or *Fbr1* (2). Estimates of missing data were done using nearest neighbour analysis and the PCA type is Pearson (n).

3.4 Results and discussion

3.4.1 SNP performance and quality

Of the 1548 maize SNPs present in the oligo pool assay (OPA), 1250 known SNPs were called in the maize inbred lines with less than 4.7% missing data. Table 3.2 gives a summary of SNPs used in this study and their linkage groups.

Table 3.2 Summary of SNPs used in this study

Chromosome	SNP Number
1	264
2	172
3	169
4	163
5	194
6	130
7	101
8	142
9	115
10	98
Total SNPs	1548
Called SNPs	1250

Average residual heterozygosity ranged from 0.2 to 36.1%, with an average of 8.2%, which is however, well above the expected ranges for residual heterozygosity found in maize inbred lines. This could be because some of the inbred lines are still heterozygous and these need further selfing to reduce the residual heterozygosity. Yan et al. (2009) found heterozygosity ranging from 0 to 9.9%, with an average of 2.5% : in a highly diverse global maize collection of 632 inbred lines from temperate, tropical, and subtropical public breeding programmes, which were reported as within expected ranges. Xia et al. (2004) also found an average residual

heterozygosity of 4.8% among CIMMYT maize inbred lines investigated with SSR markers, which were in accordance with results reported by Heckenberger et al. (2002).

PIC demonstrates the informativeness of the SNP loci and their potential to detect differences among inbred lines based on their genetic relationships. The PIC values for the polymorphic 1074 SNP loci ranged from 0.015 to 0.50, with an average of 0.25 (data not shown). Dhliwayo et al. (2009) found PIC values for SSR loci, ranging from 0.00 to 0.77 with an average of 0.43, in elite CIMMYT and IITA tropical maize inbred lines, which was in turn lower than that reported for tropical (Betrán et al., 2003; Xia et al., 2004) and temperate inbred lines (Senior et al., 1998; Barata and Carena, 2006). The small PIC values indicate relatively little genetic diversity among the germplasm used in this study. Narrow genetic diversity is expected since the genotypes investigated are homozygous as confirmed in previous studies of CIMMYT inbreds (Warburton et al., 2002).

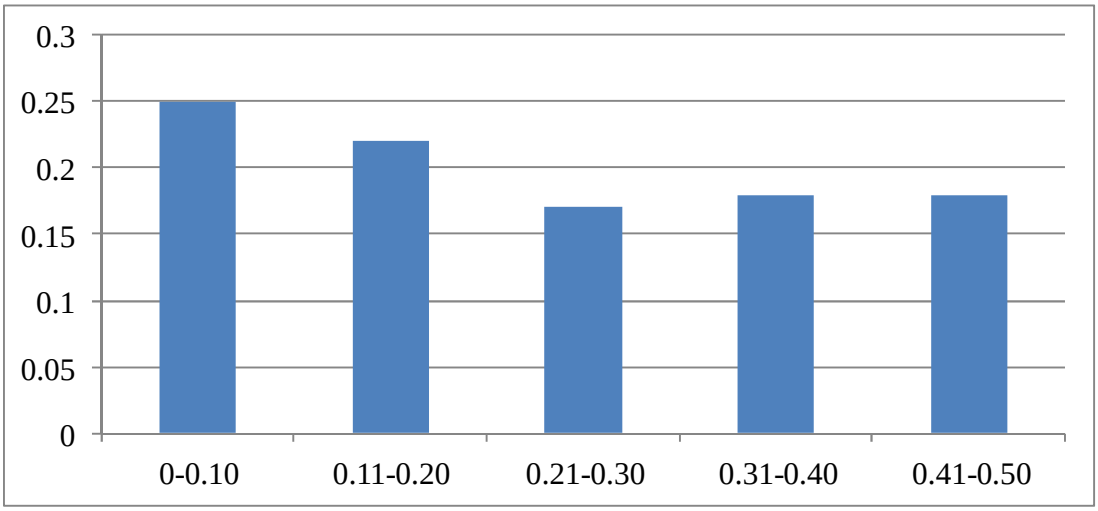

Figure 3.1 Minor SNP allele frequency distribution in the CIMMYT maize lines. Values on the x axis represent frequency of minor alleles and values on the y axis represents the proportion of the total SNP markers.

Figure 3.1 indicates a close-to-uniform distribution of minor allele frequencies in the 0-0.10, 0.11-0.20, 0.21-0.30, 0.31-0.40, and 0.41-0.50 classes. Twenty five percent of the SNP loci fall

into a class where the minor allele frequency was less than 0.10, implying that the genetic characterisation done with the 1074 SNP markers was reliable and informative.

Lu et al. (2010) in a study of quantitative trait loci underlying drought tolerance in maize, also found that 13.5% of the SNP markers had minor allele frequencies below 0.05. In a previous study on 96 Asian soybean landraces, Hyten et al. (2008) also found a uniform distribution of allele frequencies and 21% of SNP loci fell into a class with minor allele frequency less than 0.10. The uniform distribution of allele frequencies is normally expected, due to ascertainment bias, when SNPs used for molecular characterisation are initially discovered in a small sample of genotypes (Hartl and Clark, 2007). In the genetic characterisation and linkage disequilibrium estimation of a global maize collection using SNPs, Yan et al. (2009) observed a continuous allele frequency distribution for the 1229 SNP markers. The SNP haplotypes had a large number of alleles, but most were rare in the population: over half had allele frequencies <0.1 (Yan et al., 2009).

Minor allele frequency refers to the frequency at which the less common allele occurs in a given population. Information on distribution of minor allele frequency is crucial. Given that the number of individuals with a specific genotype can be very small: like in this study where population size is small, the effect of rare alleles on genetic characterisation of maize lines could go far beyond the effect of small population size. Allelles occurring at low frequency in a population are a major limiting factor in genetic characterisation and in the identification of markers associated with important traits (Lu et al., 2010). Also, the power to detect a given genetic effect with a given population size depends to a large extent on the minor allele frequency of the allele under test, thus, in genome-wide association studies for example, SNP arrays should include SNPs with a wide distribution of minor allele frequency. This distribution has an effect on the likelihood of obtaining undesirable false positive results (Tabangin et al., 2009). Genome-wide association studies using SNP arrays necessarily include SNPs with a wide distribution of minor allele frequencies, from nearly monomorphic (minor allele frequencies < 0.5%) to very common (minor allele frequencies ≈ 50%). Specifically, loci with a low minor allele frequency (< 10%) have significantly lower power to detect weak genotypic risk ratios than loci with a high minor allele frequency (> 40%) (Ardlie et al., 2002). Furthermore, previous

studies have demonstrated that rare genotypes are likely to result in spurious findings (Lam et al., 2007). Thus, many genome-wide association mapping have removed SNPs with minor allele frequencies of < 10% (Florez et al., 2007).

3.4.2 Homozygosity of the CIMMYT maize lines

Most CIMMYT lines are derived from the F5 and later generations of inbreeding and ≤ 6.25% heterozygosity is expected for the SNPs that are polymorphic between the two parents used for developing the line(s). However, the parents for inbred lines in this study were not genotyped, so they can be considered as fixed or pure lines if the proportion of heterozygote loci does not exceed 5% of the total markers used (Dr Semagn, Personal communication, 2010).

Table 3.3 show levels of homozygosity of the CIMMYT maize inbred lines characterised using the 1074 SNPs. Because genotyping of lines was done together with a number of other CIMMYT lines on the same SNP genotyping platform, six of these inbred lines were added to the analysis to increase the scope of information generated. The total number of lines used for the analysis became 34.

The proportion of homozygous loci for the total markers used for all inbred lines characterised ranged from 63.9% for CML445/TAS-BC3-source2 (the most heterozygous line), to 99.8% for LaPostaSeqC7-F180-source2 (the most homozygous line). Maize inbreds from CML488/TAS-BC2 in descending order, to LaPostaSeqC7-F180-Source2 (Table 3.3) were acceptably homozygous (≤ 5% heterozygosity for the 1074 SNPs used). It was surprising that CML443, CML312, CML444-Source1, and CML444-Source3 were heterozygous, as they are expected to be fixed. It was also unexpected that the same lines, though from different sources (for example, the four CML444's) had large differences in terms of homozygosity levels. CML444-Source1 was 76.6% homozygous, while CML444-Source4 had a homozygosity level of 99.3%. The reason could be that, while the greatest care is taken to maintain genetic purity during maintenance of these lines in breeding programmes, there are chances of contamination in the field during pollination, and seed mixes can occur during seed preparation. Consequently, these cause variation within lines that were originally fixed. Studies have already been performed to investigate the diversity of some selected CIMMYT inbred lines (Reif et al., 2003; 2004; Xia et

al., 2004; 2005) using SSR markers. More than 500 CIMMYT derived maize lines are used widely to develop new hybrid varieties in breeding programmes worldwide .

Table 3.3 Homozygosity levels of the maize inbred lines characterised using the 1074 SNPs

Inbred line	% missing data	% homozygosity
CML445/TAS-BC3-Source2	7.7%	63.9%
CML443	4.3%	66.2%
CML312	11.6%	71.2%
CML444-Source1	16.7%	76.6%
CML445/TAS-BC3-Source1	4.1%	77.6%
CML444/TAS-BC2-5Y	3.3%	80.9%
LaPostaSeqC7-F18	11.3%	82.1%
CML444-Source3	20.4%	85.4%
CKL05005	6.9%	87.6%
CML395/TAS	2.8%	90.8%
CML445/TAS	2.2%	91.4%
CML443/TAS-BC2	2.7%	92.1%
CML442/TAS	2.2%	94.2%
CML444/TAS	2.3%	94.5%
CML488/TAS-BC2	1.1%	96.5%
CML443/TASBC2-5Y	2.3%	96.7%
G16BNSeqC4	4.3%	96.7%
LaPostaSeqC7-F71	20.4%	96.8%
DTPWC9-F92	2.5%	96.8%
CML488	1.1%	97.0%
CKL05003	2.4%	97.2%
CML445	3.3%	98.1%
CML444-Source2	1.7%	98.3%
CML312/TAS	2.0%	98.4%
CML444/TAS-BC2	1.7%	98.8%
CML442/CML197/TAS	2.9%	99.1%
CML312/CML445/TAS	1.7%	99.2%
CML395	1.7%	99.2%
LaPostaSeqC7-F180-Source1	3.2%	99.3%
CML144	2.8%	99.3%
CML444-Source4	2.2%	99.3%
CML442	1.1%	99.4%
ZEWAc1F2-134	1.3%	99.5%
LaPostaSeqC7-F180-Source2	1.1%	99.8%

Yan et al. (2009) compared 21 CIMMYT maize inbred lines to lines with same name but maintained in different labs for more than 30 years and found that 81% of the lines were still genetically similar while 19% had become different. It is critical to assess homozygosity of CIMMYT lines, especially at molecular level to verify fixation of lines before embarking in critical actions like making test crosses for QTL analysis (where homozygosity of parental lines is crucial), as some of the lines would have become heterozygous during the maintenance course. Additionally, care should be taken in future exchange and conservation of germplasm for genetic research.

Fbr1 lines CML488/TAS-BC2, CML443/TASBC2-5Y, CML312/TAS, CML444/TAS-BC2, CML442/CML197/TAS and CML312/CML445/TAS, were also acceptably homozygous (≤ 5% heterozygosity for the 1074 SNPs used), indicating that most alleles from the recurrent parent have been retained after the introduction of the *Fbr1* tassel mutation into these genotypes. These *Fbr1* converted lines are fixed for the tassel mutation and these lines could be used as parental lines in the development of mapping populations for future marker assisted breeding work.

3.4.3 Genetic diversity

Most markers detected at least one allele for each of the 26 inbred lines; therefore, no loci or individuals were excluded from these analyses. Genetic distance ranged from 0.023 between LaPostaSeqC7-F18 and LaPostaSeqC7-F71, the most similar pair, to 0.38 between CML442 and CML444/TAS-BC2. The mean genetic distance for all pair wise comparisons was 0.30, which was lower than that reported in previous studies for tropical germplasm (Xia et al., 2004; 2005), and that reported among elite CIMMYT and IITA tropical maize inbred lines (Dhliwayo et al., 2009) with characterisation done using SSR markers. This means the germplasm in this study has a high level of relatedness. This is not unexpected since the 12 lines that were converted to *Fbr1* and the original parents (unconverted lines) are being analysed for diversity and as such, the single mutation should not be expected to cause much divergence (Figure 3.2 confirmed this). Reif et al. (2003), investigating the diversity among seven of CIMMYT's tropical maize populations with molecular markers, also identified low variance between populations. The lower average MRD also suggested a high average degree of relatedness among the CIMMYT maize lines used in the study. The high relatedness among the CIMMYT lines could be

attributed to sampling effects caused by different criteria used to choose the plant material for the study (Xia et al., 2004). Unlike lines studied by Enoki et al. (2002), Lu and Bernardo (2001), and Pejic et al. (1998), inbred lines in this study were not selected based on pedigree information, hence, sampling effects probably contributed to the decrease in average MRD.

UPGMA clustering showed two major clusters (Figure 3.2). One cluster (group in green colour) consisted of inbred lines in heterotic group A and A/B, while the other cluster (group in orange colour) consisted of inbred lines in heterotic group B and A/B, thus the lines were clustered according to heterotic grouping. This showed the efficiency of the SNP markers in characterising the inbred lines, thus placing them in their respective heterotic grouping. CML488 and CML488/TAS-BC2 were tightly clustered (MRD or dissimilarity = 0.025), indicating that the two lines are genetically similar. The case was similar for CML444 and CML444/TAS-BC2 with genetic dissimilarity of 0.05 (Figure 3.2). This confirmed recovery of the recurrent parent genotype in both cases, such that the *Fbr1* converted lines resembles the elite parental CIMMYT maize lines in all aspects except for few tassel branch number. CML444/TAS, CML444 and CML444/TAS-BC2 were clustered together, indicating that they are genetically similar. Genetic distance between CML395 and CML395/TAS, and CML445 and CML445/TAS-BC3-S1 was 0.12. Distance between the two *Fbr1* lines CML443/TAS-BC2 and CML443/TASBC2-5Y was also small (MRD = 0.15). This means lines in each of the three pairs are closely related. The tight clustering of CML312/TAS and CML442/TAS was surprising (2% dissimilar). Similar results were obtained for the pair CML444/TAS-BC2-5Y and CML445/TAS (2.5% dissimilar). This could be a result of seed mixes during seed preparations. The maize lines constituting each pair should be genetically different since they are not related by ancestry.

Genetic similarity between normal tasselled lines and their *Fbr1*-converted sister lines implied that the conversion from normal tasselled to *Fbr1* genotype by backcrossing was successful. Thus, the *Fbr1*-converted lines are expected to have all the elite characteristics of the recurrent parent, with the added *Fbr1* tassel trait. These lines would be useful in testcross evaluations designed to study genetic effects of this new *Fbr1* trait on grain yield under stress conditions. The homozygous *Fbr1* lines would be used in the development of mapping populations, for QTL studies, associated with the *Fbr1* trait.

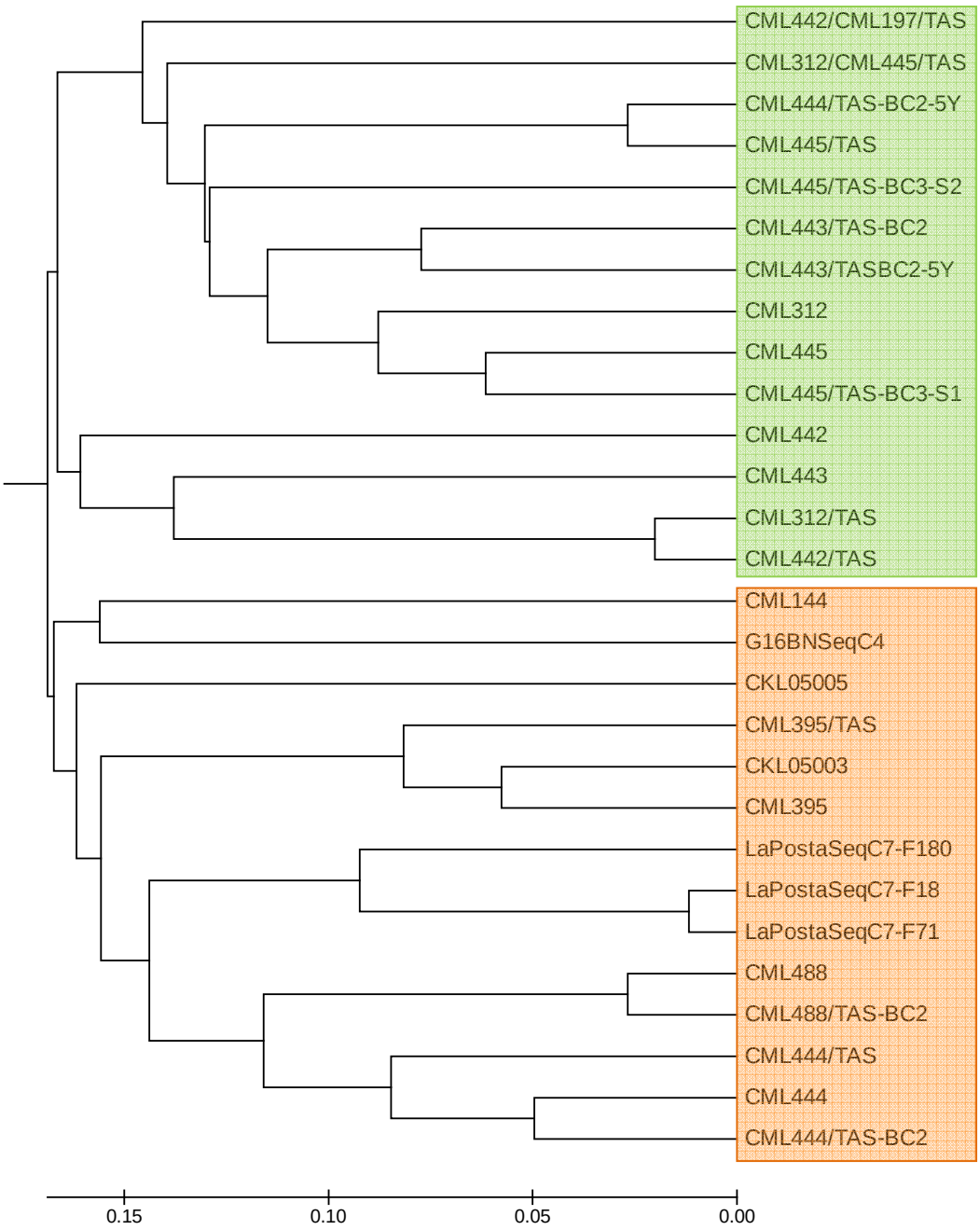

Figure 3.2 Dendrogram constructed using unweighted pair group method with arithmetic mean clustering of maize inbred lines from CIMMYT based on 1074 SNPs. The scale bar on the axis is expressed in Modified Roger's distance (1972) which shows percentage dissimilarity between or among genotypes.

This will provide valuable information and insights on the usefulness of this new tassel mutation in potential marker-assisted selection for the trait (a method designed to maximize genetic gains while reducing the time and cost of running the breeding programme).

3.4.4 Principal component analysis

Principal component analysis was carried out to determine the amount of genotypic variation for tassel size explained by the SNP markers. Figure 3.3 shows factors (F) or principal components (PC) plotted against the cumulative genetic variability explained by the 1074 SNP markers. The eigenvalue for PC1 was highest (114.04) and explained 10.87% of genetic variability for tassel size in the maize lines. PC2 and PC3 explained 9.08 and 8.20% of genetic variance for tassel size respectively, and consequently explained 19.95 and 28.15% respectively, of cumulative variability for tassel size. Of the 1074 SNPs, 7.3% did not contribute to the variation observed in PC1 with 69.8% of the SNPs contributing less than 0.1% variation in tassel size. SNP marker PZB00772_7 contributed most to variation observed in PC1 (0.77%). Eleven percent of the 1074 SNPs did not contribute to the variation observed in PC2 while marker PHM4066_11 made the highest contribution (0.57%) to variation observed for tassel size. Of the total SNP markers used, 68.6% contributed less than 0.1% of PC2 variation.

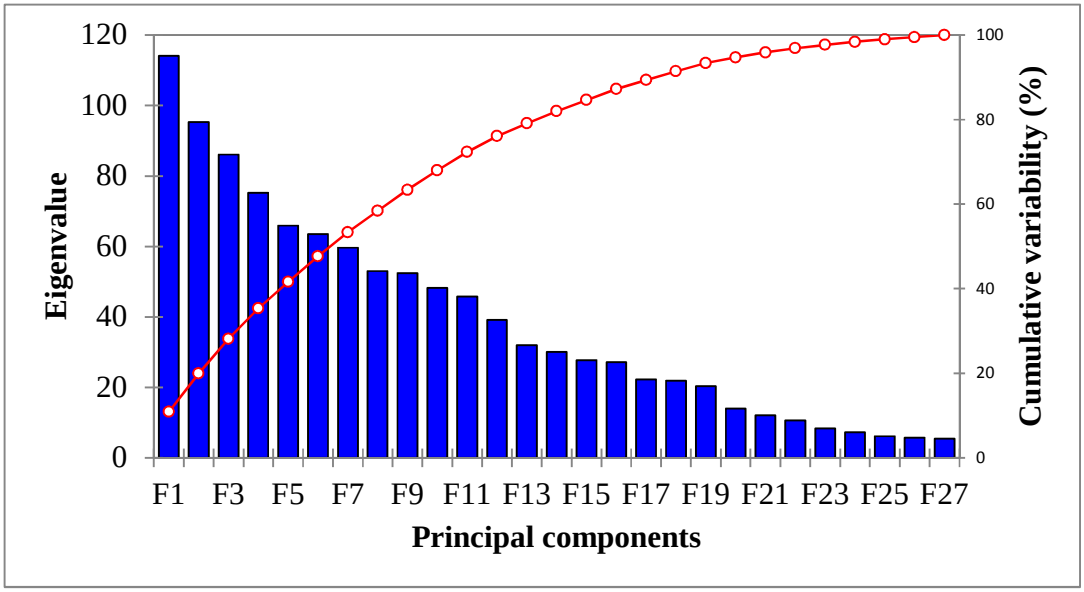

Figure 3.3 Scree plot of eigenvalues: corresponding proportion and cumulative variation for all the principal components for tassel size in the maize hybrids.

These results showed that there was variation in contribution made by different SNP markers to differences observed for tassel size among genotypes.

The relative magnitude of the coefficients (eigenvectors) (Table 3.4) reflects the relative contribution of each genotype to PC scores. Genotypes CML395/TAS, CML395, and CKL05003 made the highest contribution to PC1 together while genotypes CML445, CML445/TAS-BC3-S1, CML444TAS, CML443/TAS-BC2, CML442/CML197/TAS and CML312/CML445/TAS made high negative contribution to PC1. Inbred lines CML442/TAS and CML312/TAS contributed most to variation in PC2 while lines CML488 and CML488/TAS-BC2 made the highest negative contribution to PC2.

Figure 3.4 shows the genetic relationships between *Fbr1* and normal-tasselled maize lines (with respect to PC1 and PC2) based on the MRD estimates of all the maize lines. The first and second principle coordinate (PC) explained 10.87 and 9.08% of the molecular variance for the 1074 SNPs used. PC1 and PC2 for the most part separated two clusters, which were group 1 (composed of normal-tasselled genotypes), and group 2 (composed of few-branched genotypes) as indicated in the grouping by tassel size in Table 3.4. Although some of the SNP markers grouped some *Fbr1* lines as normal-tasselled genotypes, and vice versa, generally two main clusters are clear, one for normal tasselled genotypes (group 1), and another for *Fbr1* lines (group 2). The SNPs were therefore able to separate the maize inbred lines according to tassel morphology, which was in accordance with preliminary field morphological characterisation done.

Table 3.4 Eigenvectors for the three first principal components (PC) for tassel size for the maize inbred lines.

Name	Group by tassel size[†]	PC1	PC2	PC3
G16BNSeqC4	1	4.243	4.700	-4.927
CML442/CML197/TAS	2	-13.563	-2.597	3.504
CML312/CML445/TAS	2	-13.246	-1.032	5.288
CKL05005	1	4.967	4.264	-6.251
LaPostaSeqC7-F71	1	6.719	0.087	-3.155
LaPostaSeqC7-F18	1	6.607	1.344	-3.290
CKL05003	1	12.048	3.763	-18.802
CML144	1	5.926	-1.284	2.874
LaPostaSeqC7-F180	1	10.113	2.183	0.155
CML395/TAS	2	13.147	-2.485	-15.482
CML443/TAS-BC2	2	-13.620	1.255	-2.563
CML444/TAS	2	10.919	-12.911	3.461
CML445/TAS	2	-13.873	-4.211	-5.220
CML445/TAS-BC3-S1	2	-17.586	-1.710	-2.956
CML312/TAS	2	7.027	23.609	15.377
CML444/TAS-BC2	2	8.769	-8.650	0.600
CML488/TAS-BC2	2	8.305	-19.514	16.663
CML442/TAS	2	6.386	24.245	16.350
CML445/TAS-BC3-S2	2	-6.668	0.268	-2.199
CML443/TASBC2-5Y	2	-12.739	0.764	-4.900
CML444/TAS-BC2-5Y	2	-12.890	-4.354	-4.263
CML443	1	0.175	8.625	1.428
CML444	1	7.071	-11.170	6.119
CML488	1	8.755	-20.096	15.905
CML445	1	-21.364	-4.232	-2.973
CML395	1	13.028	2.261	-21.041
CML312	1	-8.868	5.999	3.928
CML442	1	0.211	10.878	6.373

[†] Genotypes were assigned to subgroups according to tassel morphology. Group 1 = normal tasselled genotypes, group 2 = *Fbr1* genotypes.

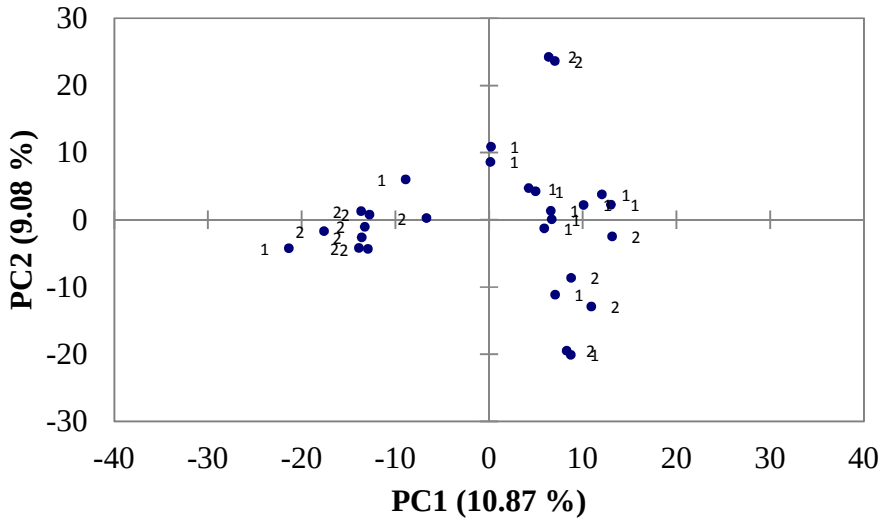

Figure 3.4 Principal component analysis of the maize inbred lines based on the modified Roger's distance calculated from 1074 SNPs marker loci. Genotypes were assigned to subgroups according to tassel morphology (whether *Fbr1* or normal tasselled). PC1 and PC2 are the first and second principal coordinates, respectively, and number in parentheses refers to the proportion of variance explained by the principal coordinates. Cumulatively PC1 and PC2 explained 19.95% of total variation in tassel size. The dots in the figure represents data points while the numbers represent tassel morphology (1 = normal-tasselled lines and 2 = *Fbr1* lines).

3.5 Conclusions

The low average MRD suggests a higher average degree of relatedness among the CIMMYT maize lines used in this study. This was because the 26 elite lines were related. The fact that genetic distances were able to effectively group the maize inbred lines according to their heterotic patterns used by CIMMYT, and that, in PCA, genetic distances separated the maize lines according to tassel morphology i.e. whether *Fbr1* or normal-tasselled, highlights the potential value of genetic distances for preliminary classification of poorly characterised germplasm. The results confirm molecular markers as a powerful complement to help assign lines into defined heterotic groups and to examine the relationships among inbred lines at DNA level. Molecular markers were useful to determine heterotic grouping in a short time. These results revealed the efficiency of backcrossing in converting elite normal-tasselled CIMMYT

maize lines to few-branched-lines since most of *Fbr1* lines were homozygous for the SNP loci used. The fact that many homozygous elite lines with the *Fbr1* trait were identified could open a new window in potential marker assisted selection (MAS) for the trait. Furthermore, more homozygous lines with the *Fbr1* trait could be used in breeding programmes aimed at unveiling the untapped potential of these new mutants in maize production.

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Chapter 4

Genetic analysis and yield evaluation of CIMMYT few-branched-1 (*Fbr1*) maize inbred lines and hybrids under stress and non-stress environments

4.1 Abstract

Inheritance of the *Fbr1* tassel mutation in maize (*Zea mays* L.), and effect of abiotic stress on yield of *Fbr1* genotypes is still elusive. The aim of this study was to evaluate yield performance of *Fbr1* maize lines and hybrids under optimum and stress environments, and to determine mode of gene action governing tassel size and other yield components. Variance was highly significant ($P \leq 0.001$) for all traits and GxE interaction effects were significant for all traits measured except for kernel row number and anthesis silking interval. Generally, *Fbr1 x Fbr1* hybrids had lower grain and pollen yields, and were less adapted to abiotic stress conditions, raising questions on the value of incorporating such trait in breeding programmes targeting stress tolerance. Although literature has shown that breeding for small tassels could improve grain yield under stress environments, the results of this study showed the contrary. Apparently, incorporation of the *Fbr1* tassel trait should accompany selection for other traits associated with stress tolerance under low N and drought conditions, e.g. the "stay green" trait, factors associated with premature senescence, synchrony between male and female flowering and decreased barrenness. Estimates of genetic components of variance revealed the importance of both additive and dominance components in determining inheritance of all traits. Dominance gene action was predominant in inheritance of grain yield, prolificacy and ear weight, thus heterosis breeding should be effective for these traits. Additive gene action was predominant in determining tassel size and pollen yield, thus progress can be made by selecting within segregating progenies when improving maize populations for the *Fbr1* trait.

4.2 Introduction

Maize (*Zea mays* L.) breeding programmes focus on developing and using inbred lines with superior combining ability for grain yield and other agronomic traits to form excellent hybrid combinations (Dhliwayo et al., 2009). The identification of parental inbred lines that form superior hybrids is the most costly and time-consuming phase in maize hybrid development

(Betrán et al., 2003). *Per se* performance of maize inbred lines does not predict the performance of maize hybrids for grain yield (Hallauer and Miranda, 1988).

There are very few studies on the maize tassel and on variation in pollen production (Vidal-Martínez et al., 2004) since work that is commonly done is based on grain yield (Vidal-Martínez et al ., 2001a), and apparently grain yield is based upon the female structures and not the male flowers.

The choice of an efficient breeding procedure depends to a large extent on knowledge of the genetic system controlling the character to be selected (Azizi et al., 2006). Whereas dominance gene action would favour the production of hybrids, additive gene action indicates that standard selection procedures would be effective in bringing about advantageous changes in character. Tassel branch number, tassel length and tassel weight are important tassel characters while ear height, ear length and ear diameter are important ear characters that affect maize plant yielding efficiency (Sofi, 2007). The tassel traits affect grain yield either physiologically by competing for photosynthates or physically by shading effect (Guei and Wasson, 1996). Plant breeders have generally selected for small tassels as large tassel size has been found to be negatively correlated with grain yield. In hybrid breeding programmes an ideal male parent is supposed to have large tassels that can produce large amounts of pollen whereas an ideal female should partition more assimilates towards big ears and hence should possess small tassels (Upadyayula et al., 2006).

The nature of gene action involved in inheritance of tassel traits help breeders to devise better selection strategies, to seek improvement in these traits in the desired direction (Sofi, 2007). Many studies have revealed that additive gene action is predominant in the inheritance of tassel and ear traits whereas few studies have found evidence for non-additive gene action such as dominance and epistasis (Schuetz and Mock, 1978; Guei and Wasson, 1996; Berke and Rocheford, 1999; Wolf and Hallauer, 1997; Hinze and Lamkey, 2003).

CIMMYT initiated hybrid maize breeding programmes in the mid-1980s and breeding programmes have invested resources in tropical maize germplasm development and improvement in Africa (Dhliwayo et al., 2009). The drought tolerant maize for Africa (DTMA)

initiative is one such programme that is focusing on developing maize germplasm tolerant to drought. Yield can be increased under drought stress by manipulating 'adaptive traits' that limit yield under such stress. Tassel size is one such trait, since tassels act as competitive sinks under stress (Ribaut et al., 2004), and the bigger tassel exerts high apical dominance at the expense of developing ears, thus reducing grain yield (Sangoi and Salvador, 1996). Working with the hypothesis of tassel size effect on yield under stress, CIMMYT breeders have successfully introduced an ethyl-methane sulfonate (EMS)-induced, few-branched-1602: designated as *Fbr1* by Neuffer (1989), tassel mutation from a Mexican donor line of tropical adaptation into elite CIMMYT maize lines by backcrossing. MacRobert (Personal communication, 2009) observed that the *Fbr1* is consistently a dominant mutation, which has demonstrated additive effects in certain genotypes. Information is therefore needed on genetic effects of the *Fbr1* trait on pollen and grain yield of maize genotypes as this would help breeders design appropriate breeding and selection strategies. Evaluation of *Fbr1* populations under drought and low N stress conditions allows the determination of effects of the tassel mutation under these conditions. These particular populations can be of interest if yield advantages over the normal tasselled types under stress outweigh presumed pollen reductions due to reduced tassel size.

Plant breeding goals have been attained through effective management of genetic variability using effective breeding methods for developing superior genotypes for target environments. Therefore information will be needed to determine the genetic variability of *Fbr1* maize populations and the relative importance of additive and non-additive genetic effects to develop high yielding hybrids or synthetics for the target production areas. Information about agronomic performance, presence of useful genetic variance and high combining abilities of populations are then desirable for planning the plant breeding programme (Beck et al., 1991; Melani and Carena, 2005).

Not all crosses among lines are highly productive. It is, therefore, necessary to cross lines and evaluate a large number of crosses to determine which crosses have superior performance, since heterosis is a function of the differences in allelic frequency between lines and the level of dominance of alleles influencing the trait (Shull, 1910). This may be done through conducting a diallel analysis. According to Yan and Kang (2003) the main purpose of conducting diallel

analysis is to obtain information on the parents about their genetics and potential of producing superior hybrids or inbred lines for synthetic varieties. The two components of the total variance of crosses are the variances for general and specific combining ability, which reflect additive and non-additive gene effects, respectively (Falconer, 1981). Analysis of combining ability allows the choice of parental populations with high combining ability to develop superior hybrids and segregating populations with large genetic variability. It also gives information on gene action and is frequently used to choose parents with a high GCA and hybrids with high SCA effects (Yingzhong, 1999). Besides gene effects, breeders would also like to know how much variation in a crop is genetic and to what extent this variation is heritable; because efficiency of selection mainly depends on additive genetic variance, influence of environment and interaction between genotype and environment (Novoselovic et al., 2004). Large GxE effects are a problem in breeding because of lack of a predictable response to selection (Dudley and Moll, 1969). Literature about maize suggests that additive gene effects with partial to complete dominance are more important than dominance effects in determining grain yield (Lamkey and Lee, 1993).

A genetic study on the effect of the *Fbr1* trait was conducted to determine mode of gene action governing the tassel mutation and grain yield. Evaluation of *Fbr1* and normal tasselled maize lines and hybrids under stress and non-stress conditions was conducted to assess whether the mutants offer a yield advantage over the normal tasselled maize, particularly under stress environments.

4.3 Materials and Methods

4.3.1 Germplasm and mating design

Six CIMMYT maize inbred lines adapted to the mid-altitude zones of southern Africa (altitudinal range of 850-1520 masl) were selected for this study i.e. three with the *Fbr1* tassel morphology and three with normal tassels (Table 4.1). These lines were selected to represent the few-branched mutants and the normal-tasselled elite CIMMYT maize lines. These inbred lines were crossed in a half diallel mating design with (n (n-1)/2) F_1 crosses (Griffing, 1956) during the off-season of 2009 under irrigation at Muzarabani in Zimbabwe to make 15 F_1 hybrids constituted of (*Fbr1* x *Fbr1*) and (*Fbr1* x normal) and (normal x normal) F_1 hybrids. Seed for the six parents was also produced during the same season for evaluation in replicated experiments.

Table 4.1 Pedigrees of the six maize inbred lines: three *Fbr1* and three normal-tasselled, crossed using diallel mating system to form the 15 F_1 hybrids

Line	Pedigree
L1	CML443
L2	CML488
L3	CML444
L4	[CML443/TAS]BC2-2-9-1-2-B
L5	[CML488/TAS]BC2-6-4-2-B
L6	[CML444/TAS]BC2-5Y-3-1-B

4.3.2 Agronomic management, environments and stress management of trials

Two sets of trials, the six inbred parents and the 15 hybrids plus five normal-tasselled hybrid checks, were grown adjacent to each other in three environments in Zimbabwe during 2010 and 2011. The three trial environments were CIMMYT- Harare Maize Research Station (17.80 S, 31.05 E, 1468 masl) (optimum conditions), CIMMYT-Harare Maize Research Station under low N during the summer wet season, and Chiredzi Research Station (21.03 S; 31.57 E, 392 masl) during the winter dry season (under managed drought).

Under optimum growing conditions in all sites, a basal application of 400 kg/ha of compound Z fertilizer (8% N: 14% P_2O_5: 7% K_2O: 0.8% Zn) was broadcast and disc–incorporated by a tractor. Ammonium nitrate (33% N) was split applied at 200 kg/ha. The first application of 100kg/ha was done at four weeks after crop emergence and the second split was given at six weeks after emergence. Trials were rain-fed, but a light irrigation was applied immediately after planting to facilitate seed germination and seedling emergence. Irrigation was also applied in the case of a long dry spell. Generally, an irrigation of 7mm/hr for six hours was applied just after planting to facilitate germination. Total water application per irrigation was 42 mm. Thereafter, the irrigation interval varied from 9 to 15 days depending on temperature and crop development stage. Average rainfall was 700-800 mm and 650-700mm potential evapotranspiration was experienced during the growing seasons for Harare in 2010 and 2011.

The experiments under low N were also conducted at Harare using, except for N management, the same crop management practices as under recommended agronomic management. Low N

experiments were grown in fields that were depleted of N by continuously cropping maize (main season) or irrigated wheat (winter dry season), removing all stover biomass after harvest and not applying any N fertilizer. No chemical N fertilizer was applied to the low N experiments. For trials under managed drought stress in Chiredzi, three to four irrigations totalling 250 mm of water were applied at the beginning of the season and irrigation stopped at 43 to 57 days after planting (about 50 days before anthesis). The crop completed its life cycle without any further irrigation or rain.

For all trials, seedbeds were kept weed-free throughout the season. A mixture of atrazine (Atrazine WP), dual (Metolachlor) and gramoxone (Paraquat), at 4.5, 1.8 and 1.0 l/ha, respectively was applied as a post-planting pre-emergence spray for weed control. Herbicides were applied using a 500 L spray tank with a 10 m boom width and 20 nozzles, mounted on a pick-up truck. After three weeks of crop emergence, basagram was applied at 3 l/ha to control nutsedge (*Cyperus spp*) and broadleaf weeds. At three to four weeks after crop emergence, Bentazon (Basagran) was applied to control all weeds. From seven weeks on, the field was kept weed-free by hand weeding.

For pest management in all trials, scouting for cutworm (*Agrotis ipsilon*) damage started immediately after crop emergence. Where cutworms were found to be a problem, a mixture of 60g dipterex (Trichlorfon WP), 20 kg of maize meal and 20 l of water was applied as bait along crop rows in the evening. At five weeks after crop emergence, scouting for the maize stalk borer (*Busseola fusca*) started and endosulfan 1% granules (thionex): at 2 kg/ha in a mixture of two parts sand and one part chemical, was applied by hand every 10 days alternating with dipterex at 2 kg/ha. Maize streak virus disease was controlled by applying carbofuran (curater) mixed with sand in a ratio of three parts chemical to four parts sand in the planting hole to kill the vectors of the disease, *Cicadulina* leafhoppers.

4.3.3 Experimental design and data collection

The experimental design was an alpha lattice (0,1) (Patterson et al., 1978) with two replications for hybrid and inbred trials in each environment. The 15 hybrids plus five hybrid checks were laid out as a 4 x 5 incomplete lattice design in all three environments in 2010 and 2011, for evaluation of tassel and ear traits, where pollen yield, pollen yield components, grain yield and grain yield components were recorded. A separate trial of six inbred parents, laid out as a 2x3 incomplete lattice design, with two replications was planted adjacent to the hybrid trial in the same field. Plot size at all locations was a single 4 m row with 0.75 m between rows and 0.25 m between plants within rows, giving final plant populations of ≈ 53 000 plants per hectare at all sites.

Pollen production/pollen yield was estimated by the bagging method, which is similar to the technique followed in maize controlled pollinations, where the tassels of four selected plants were sampled for pollen production (Vidal-Martínez et al., 2001b). Pollen samples were collected daily from the same plant throughout the entire shedding period (6 to 12 d). Anthers and insects were removed by sieving the collected pollen through a #35 U.S. standard testing sieve (500 μm opening) (Vidal-Martínez et al., 2004). The collected pollen was dried under normal day temperatures and favourable atmospheric shedding conditions, to a moisture content of less than 10% (Goss, 1968), prior to weighing with a precision balance to obtain the daily and total production of pollen during anthesis.

Four tassels, visually selected by comparing anthesis development in unbagged plant-tassels per plot were used for estimating tassel characteristics (pollen production components) – tassel branch number and total tassel length (Upadyayula et al., 2005). Total tassel length in centimetres was measured as the distance from the non-branching node present below the lowermost primary branch to the tip of the central spike. Average tassel branch number was the number of primary branches per plant-tassel. The amount of pollen produced by a maize plant depended on the number of staminate flowers per plant and the amount of pollen per anther (Goss, 1968). Therefore, plants had different amounts of collected pollen throughout the entire shedding period. Pollen yield in grams was measured on a sample of four plants per plot during the pollen shedding period.

Ear weight, kernels per ear, ear-row number, kernel-row number and ear length were measured and considered as grain yield components. Twelve ears per plot were used for estimating these grain yield components (Vidal-Martínez et al., 2001b). Grain yield (adjusted to 12.5% moisture content) was obtained considering harvested plot area and counting number of plants and harvested ears per plot. Days to anthesis (number of days from planting to 50% pollen shed), days to silking (number of days from planting to 50% silk emergence), and ASI were measured on plot basis for both inbred parent and hybrid trials.

4.3.4 Statistical analysis

All trials were first analysed individually (including checks) according to an alpha lattice (0,1) design (Patterson et al., 1978) using Proc Mixed in SAS (SAS Institute, 2003), to determine either individual or combined significant response of traits among genotypes. The combined analysis of variance of a response included the factors genotype (G), location (L) and incomplete block (replication) within location (B) and was done using the general linear model (GLM) procedure in SAS System for Windows, Version 9.1 (SAS, 2003). Analysis of variance (ANOVA) for grain yield, grain yield components, pollen yield and pollen yield components was done for each location and a combined ANOVA was computed across all locations using a GLM procedure, by considering locations as fixed, and genotypes (inbred lines or hybrids), replication and incomplete blocks as random factors.

The response $\mathbf{Y_{ijk}}$ of genotype **i** in location **j** and incomplete block (replication) **k** is:

$$\mathbf{Y_{ijkr} = \mu + G_i + L_j + B_k + GL_{ij} + \varepsilon_{ijk}}$$

Where:

μ = grand mean

G_i = effect of the i^{th} genotype

L_j = effect of the j^{th} location

B_k = effect of k^{th} incomplete block

GL_{ij} = interaction effect of the i^{th} genotype with j^{th} location

ε_{ijk} = random error

ASI data was normalised using the transformation of LN (ASI +10) (Bolaños and Edmeades, 1996), before ANOVA. Linear contrasts were performed using SAS to test linearity of performance in *Fbr1* x *Fbr1*, *Fbr1* x normal tasselled and normal tasselled x normal tasselled hybrids. Multiple comparisons of means (Tukey, 0.05) were carried out using SAS (2003).

Combining ability, mode of gene action governing pollen yield components, pollen yield, grain yield components and grain yield were estimated using the DIALLEL-SAS05 programme (Zhang et al., 2005), a comprehensive programme for Griffing's and Gardner-Eberhart analyses. The random-effects model of diallel method 4 was used in the analysis and provided estimates of GCA (σ^2_g) and SCA (σ^2_s) variances, which were used to estimate additive (σ^2_A) and dominance (σ^2_D) variance. The DIALLEL-SAS programme computed data for environmental effects, effects due to genotypes, block, and interactions between various effects. For a diallel mating from a set of inbred lines, the generation means (Y_{ijk}) observation in environment *k* of maternal line *i* and paternal line *j* can be partitioned as the model:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + l_k + (gl)_{ik} + (sl)_{ijk} + \varepsilon_{ijk}$$

where, Y_{ijk} = observation in environment *k* of parents *i* and *j*; μ = general mean; *gi* or *gj* = GCA effect of parents *i* and *j*; *sij* = SCA effect of the cross between parents *i* and *j*; l_k = effect of location k; $(gl)_{ik}$ or $(gl)_{jk}$ is interaction effect between GCA of parent *i* or parent *j* with location *k*; $(sl)_{ijk}$ is interaction effect between SCA of cross *ij* and location *k*; and ε_{ijk} = error associated with ij^{th} cross evaluated in *k* location. F values for testing combining abilities were computed as follows:

$\sigma^2_{scaEnv} = MS_{scaEnv}/MSe$

$\sigma^2_{gcaEnv} = MS_{gcaEnv}/MS_{scaEnv}$

$\sigma^2_{sca} = MS_{sca}/MS_{scaEnv}$

$\sigma^2_{gca} = (MS_{gca} + MS_{scaEnv})/ (MS_{sca} + MS_{gcaEnv})$

whereσ^2_{gca}, σ^2_{sca}, σ^2_{gcaEnv}, $\sigma^2_{scaEnv}$are variance due to GCA, SCA, GCA x environment and SCA x environment, respectively, and MS_{gca}, MS_{sca}, MS_{gcaEnv}, MS_{scaEnv} and MS_e are mean squares due to GCA, SCA, GCA x environment, SCA x environment and error, respectively.

Broad sense heritability (H) and narrow sense heritability (h^2) for mean values over environments were calculated following the components of variance (Teklewold and Becker, 2005):

$$H = \frac{2\sigma^2_{gca} + \sigma^2_{sca}}{2\sigma^2_{gca} + \sigma^2_{sca} + [2\sigma^2_{gca}/Env] + [\sigma^2_{sca}/Env] + [\sigma^2_{e}/REnv]}$$

$$h^2 = \frac{2\sigma^2_{gca}}{2\sigma^2_{gca} + \sigma^2_{sca} + [2\sigma^2_{gca}/Env] + [\sigma^2_{sca}/Env] + [\sigma^2_{e}/REnv]}$$

Genetic ratio was estimated as a ratio of combining ability variance components:

$$Genetic\ ratio = \frac{2\sigma^2_{gca}}{2\sigma^2_{gca} + \sigma^2_{sca}}$$

as suggested by Baker (1978) for prediction of progeny performances. The relative importance of GCA and SCA on progeny performance, with a theoretical maximum of unity, was estimated as the ratio:

$\sigma^2_{gca}/\sigma^2_{sca}$, where σ^2_{gca} and σ^2_{sca} are variance components for GCA and SCA.

A GGE biplot analysis was done to evaluate mean performance and stability of hybrids for yield using Genstat version 14 (Genstat, 2011). A GGE biplot, graphically displays GxE interaction in a two way table (Yan et al., 2000).

4.4 Results and discussion

4.4.1 Evaluation of maize lines and hybrids for grain and pollen yield

Results of the combined ANOVA across sites (environments) and years for the inbred lines revealed significant differences among lines for all traits measured (Table 4.2). For the maize hybrids, combined ANOVA across sites and years also showed highly significant differences ($P \leq 0.001$) for all traits measured (Table 4.3). Environmental effects for grain yield, 1000-kernel

weight, ear weight, kernels per ear, total tassel length, tassel branch number, pollen yield, anthesis silking interval, days to anthesis, and days to silking were all significant, for both inbred lines and hybrid progenies indicating that these traits are influenced by environmental conditions. Variation due to environment was, however, not significant for kernel row number for inbred lines, indicating that the trait is not affected by environment. Other authors have found that environmental effects were significant for days from emergence to silking (Mickelson et al., 2001), kernel row number (Soengas et al., 2003), and grain yield (Doerksen et al., 2003; Soengas et al., 2003; Mickelson et al., 2001; Vidal-Martínez et al., 2001a).

GxE interaction effects were highly significant ($P \leq 0.001$) for pollen yield, and significant for 1000-kernel weight, ear weight, days to anthesis: $P \leq 0.01$, tassel branch number, and days to silking: $P \leq 0.05$. This means inbred lines did not respond to the environment similarly for these traits. For the hybrid progenies, GxE interaction was significant for all traits except for kernel row number and anthesis silking interval.

Non-significant GxE interaction for kernel row number and anthesis silking interval suggests the genotypes maintained their rank for these traits across environments and selection for the traits in one environment might be effective when selecting for a broad range of environments. Other researchers have reported that GxE interaction effects were significant for kernel row number (Zare et al., 2011), days to silking (Mickelson et al., 2001), kernel row number and grain yield (Doerksen et al., 2003; Soengas et al., 2003; Vidal-Martínez et al., 2001a; Welcker et al., 2005).

For maize inbred lines, orthogonal contrasts were highly significant ($P \leq 0.001$) for tassel branch number and were significant ($P \leq 0.05$) for pollen yield and days to silking (Table 4.2). For the maize hybrids, the contrasts were highly significant ($P \leq 0.001$) for tassel branch number, pollen yield and were significant at $P \leq 0.05$ for anthesis silking interval (Table 4.3). This indicates that there was a significant linear trend for tassel branch number in *Fbr1* versus normal tasselled inbred lines. Inbred lines with the *Fbr1* mutation had lower mean tassel branch numbers compared to normal tasselled lines (Table 4.4). Pollen yield in both inbred lines and hybrids also showed a decreasing trend from normal x normal, *Fbr1* x normal, and *Fbr1* x *Fbr1* tasselled hybrids, which is indicative of additive gene action governing pollen yield. It is apparent that

inbred lines and hybrids that had many tassel branches produce more pollen than those with few branches. This has raised questions on the viability of breeding programmes targeting tassel reduction as a way of increasing grain yield, since pollen production is heavily compromised and the effects are significant under stress conditions and when the lines are used as males in hybrid seed production (Monneveux et al., 2006).

For the hybrids, there was a linear trend in tassel branch number of *Fbr1* x *Fbr1*, *Fbr1* x normal and normal x normal hybrids. *Fbr1* x *Fbr1* hybrids had the lowest tassel branch number, followed by *Fbr1* x normal, then normal x normal tasselled hybrids which had the highest number of tassel branches (Table 4.4). Indirectly this is indicative of the predominance of additive gene action governing the tassel branch number trait. Mean grain yields for hybrids ranged from 0.72 kg/plot under low N stress to 2.92 kg/plot under optimum conditions. For the parental inbred lines mean grain yield ranged from 0.32 kg/plot under low N to 1.19 kg/plot under drought stress. Hybrid vigour was expressed for total tassel length as maize hybrids had higher mean tassel lengths compared to parental inbred lines (Table 4.4).

The significant GxE interaction for most traits in the maize hybrids means ranking of hybrids changed with change in environmental conditions. Determining hybrids that are stable with high mean yield across environments is crucial. Figure 4.1 and 4.2 show that normal x normal tasselled hybrids NN13, NN14, and NN15 were high yielding and highly stable under drought, and low N conditions. This could be because normal x normal hybrids had bigger tassels with many branches, which supplied large amounts of pollen for maximum seed set. *Fbr1* x normal crosses i.e. hybrids TN10, TN7, TN8, and TN11, were adapted to all three environments and were quite stable for pollen production under these environments. Hybrids TN7, TN8, and TN10 were more adapted to drought and low N stress than TN11. However, all *Fbr1* x *Fbr1* hybrids (hybrids TT1, TT2 and TT6) had low pollen yields, although stable under drought, low N stress and optimum conditions. These hybrids had smaller tassels with few branches, hence the reduced pollen yield. Breeding for few branches could pose a challenge in increasing grain yield under stress since number of tassel branches is considered as a vital pollen yield component (Vidal-Martínez et al., 2001b).

Table 4.2 Analysis of variance of grain yield, grain yield components, pollen yield and pollen yield components for the six maize inbred lines for experiments conducted in 2010-2011 across the three environments (low N, drought stress and optimum conditions)

		Mean squares										
Source	df	GYD[†]	1000-kw	Ear weight	KRN[‡]	KPE[§]	TTL[¶]	TBN[#]	PYD[††]	ASI[‡‡]	DTA[§§]	DTS[¶¶]
Replication	1	0.046	204.84	0.17	0.28	2110.02	15.65	7.95	0.0040	0.045	0.28	1.1
Entry	5	0.56**	13913.01***	2368.37***	12.98***	4343.46**	123.87***	199.04***	0.54***	0.13*	227.64***	225.70***
Site	2	5.42***	50562.92***	8946.84***	0.44	5784.22*	153.53***	19.69*	0.095**	0.40**	2779.83***	3357.54***
Year	1	0.006	12679.00***	20.9	0.74	139093.18***	706.50***	0.47	0.027	0.45**	9320.69***	10490.03***
Entry x site	10	0.13	2823.48**	601.45**	0.44	905.38	17.14	9.39*	0.11***	0.051	14.23**	13.67*
Entry x year	5	0.076	1321.21	238.9	0.27	3419.21*	17.22	2.46	0.0022	0.045	10.95*	5.93
Entry(site x year)	6	0.055	6435.43***	401.89*	0.14	33355.47***	94.66***	13.37**	0.015	0.069	1073.24***	973.36***
Fbr1 vs Norm	1	0.098	877.24	167.95	0.39	1069.39	78.45	438.96***	0.68*	0.3	189.87	310.92*
Error	28	0.098	877.24	167.95	0.39	1069.39	13.24	4.44	0.016	0.049	3.99	5.71

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, [†] Grain yield in kilograms, measured on plot basis, adjusted to 12.5% grain moisture content and to number of plants per plot

[‡] Mean kernel row number per each cob measured across all the three environments

[§] Mean number of kernels per ear

[¶]Total tassel length in centimeters, measured as the distance from the non-branching node present below the lowermost primary branch to the tip of the central spike

[#]Average tassel branch number is the number of primary branches per plant-tassel

[††]Pollen yield in grams measured on a sample of four plants per plot

[§§] DTA = mean number of days to anthesis across all the environments

[¶¶] DTS = mean days to silking across all the environments

Table 4.3 Analysis of variance of grain yield, grain yield components, pollen yield and pollen yield components for the 15 maize hybrids plus five hybrid checks for experiments conducted in 2010-2011 across the three environments (low N, drought stress and optimum conditions)

		Mean squares										
Source	df	GYD[†]	1000-kw	ear weight	KRN[‡]	KPE[§]	TTL[¶]	TBN[#]	PYD[††]	ASI[‡‡]	DTA[§§]	DTS[¶¶]
Replication	1	0.0020	9842.74***	141.74	0.43	9238.48	7.64	3.40	0.086	0.033	4.29	1.81
Entry	19	1.72***	12151.02***	5880.56***	2.20***	15504.93***	226.01***	417.90***	4.30***	0.11***	176.97***	136.61***
Site	2	103.75***	341826.9***	26134.39**	11.20***	397327.94***	1882.27***	1131.6***	21.68***	2.82***	15575.6***	11640.7***
Year	1	1.93***	1813.92	606.13	14.47***	5652.68	3059.88***	0.34	0.094	0.29***	31088.8***	29484.9***
Entry x site	37	0.56***	2207.24***	2328.19***	0.58	10402.77***	39.08**	16.97***	0.62***	0.038	14.78**	8.89***
Entry x year	19	0.27	917.53	527.71	0.65	2858.71	23.64	14.25*	0.28	0.042	5.04	7.76***
Entry(site x year)	19	0.59***	4523.23***	2386.66***	0.67	13981.83***	122.77***	32.59***	0.20	0.064**	1236.18***	1161.64***
Contrast (hybrids)	1	0.049	13.38	89.57	0.0069	939.93	0.43	480.03***	9.84***	0.18*	145.83	76.80
Error	76	0.18	805.30	591.61	0.61	3451.82	19.22	7.73	0.23	0.028	4.57	3.037

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, [†] Grain yield in kilograms, measured on plot basis, adjusted to 12.5% grain moisture content and to number of plants per plot, [‡] Mean kernel row number per each cob measured across all the three environments, [§] Mean number of kernels per ear, [¶]Total tassel length in centimeters, measured as the distance from the non-branching node present below the lowermost primary branch to the tip of the central spike, [#]Average tassel branch number is the number of primary branches per plant-tassel, [††]Pollen yield in grams measured on a sample of four plants per plot, [§§] DTA = mean number of days to anthesis across all the environments, [¶¶] DTS = mean days to silking across all the environments

Table 4.4 Mean grain yield, total tassel length, tassel branch number, and pollen yield for the inbred lines and hybrids, measured in 2010 and 2011 under optimum, low N, and drought stress conditions

	Grain yield (kg/plot)			Total tassel length (cm)			Tassel branch number			Pollen yield (g/plant)		
Inbred lines												
	Optimum	Low N	Drought	Optimum	Low N	Drought	Optimum	Low N	Drought	Optimum	Low N	Drought
[CML443/TAS]	0.93	0.31	1.51	43.78	39.74	34.63	3.25	2.82	2.69	0.09	0.11	0.04
[CML444/TAS]	1.48	0.69	2.02	35.04	36.57	27.57	4.19	2.83	3.25	0.14	0.10	0.11
[CML488/TAS]	0.76	0.23	1.13	33.05	33.84	33.06	2.07	4.38	5.65	0.14	0.24	0.10
CML443	1.09	0.24	0.85	42.64	41.11	38.49	10.25	8.25	14.69	0.17	0.31	0.14
CML444	1.65	0.28	1.66	35.77	36.15	34.51	10.88	11.25	13.07	0.25	0.27	0.88
CML488	0.88	0.30	1.52	37.08	38.79	35.22	10.69	9.00	9.82	0.69	0.44	0.94
Mean	1.13	0.34	1.45	37.89	37.70	33.91	6.89	6.42	8.19	0.25	0.25	0.37
$LSD_{0.05}$	0.45	0.32	1.19	10.85	5.52	5.46	6.12	3.12	1.58	0.28	0.10	0.17
Hybrid progeny												
Fbr1 x *Fbr1*	2.73	0.73	1.18	50.25	46.67	42.42	3.40	3.02	6.34	0.62	0.35	0.78
Fbr1 x Normal	2.74	0.70	1.39	50.29	44.80	41.25	7.76	6.77	12.74	0.98	0.51	1.39
Normal x Normal	3.28	0.72	1.57	55.01	47.53	44.37	16.63	12.98	24.15	1.94	1.13	3.49
Check 1[†]	4.24	0.71	0.75	61.82	49.90	45.58	20.82	18.38	27.69	1.91	1.60	3.13
Check 2	1.24	.	0.17	50.88	34.80	37.33	11.19	9.69	17.07	0.47	0.30	0.64
Mean	2.92	0.72	1.38	51.85	46.33	42.68	9.26	7.59	14.41	1.18	0.66	1.89
LSD0.05	1.06	0.57	0.10	9.98	9.69	6.37	5.00	5.40	6.14	1.59	0.58	0.90

[†] Check1: best performing hybrid check and check 2: poorest performing hybrid check. Only two out of five checks were selected for comparison with the hybrid.

Chinwuba et al. (1961) and Schwanke (1965) indicated that reduction in tassel size decreases apical dominance and consequently improves grain yield under stress environments, particularly at high plant populations. In this study, reduction in tassel size caused a reduction in pollen and grain yield and the effects of this reduction were pronounced under drought and low N stress.

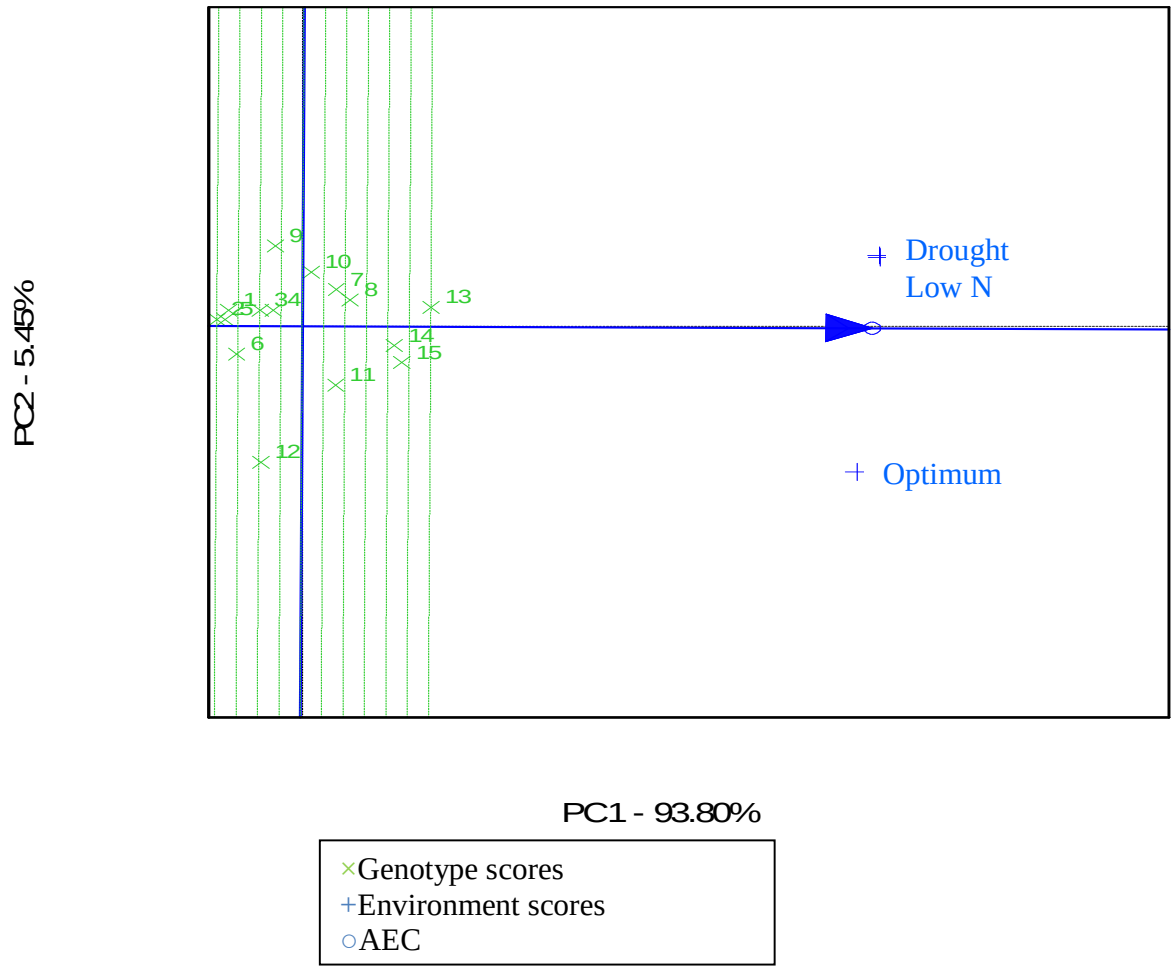

Figure 4.1 GGE biplot based on genotype-focused scaling for ranking of the 15 maize hybrids on basis of both mean pollen yield and stability. AEC is the average environment axis, which is defined by the average PC1 and PC2 scores of all environments. PC1 and PC2 explained 99.25% of total variation in pollen yield.

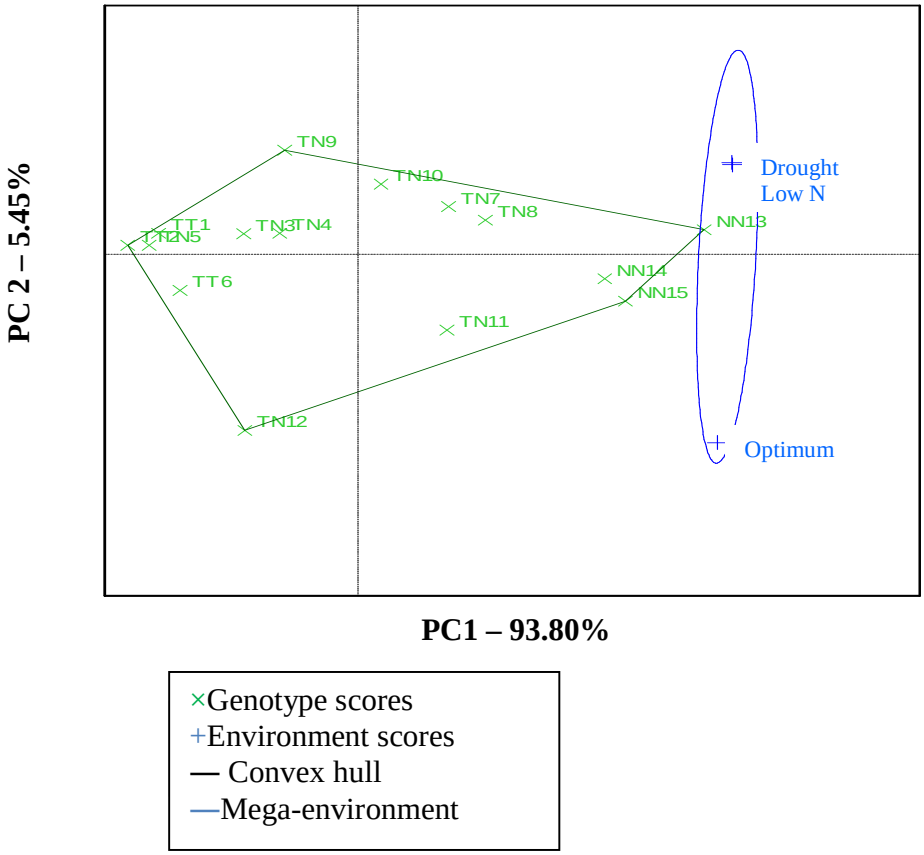

Figure 4.2 GGE biplot based on genotype-focused scaling for grouping of the 15 maize hybrids on basis of both mean pollen yield and stability.

4.4.2 Genetic analysis for tassel size, pollen and grain yield in *Fbr1* maize hybrids

Highly significant (P≤ 0.001) differences were observed among genotypes for all traits (Table 4.4). Thus, genotypic variance was partitioned into GCA and SCA variance for all traits measured. GCA mean squares were highly significant (P≤ 0.001) for all traits while SCA variance was significant for most traits except for total tassel length and kernel row number. Significant GCA variance for all traits measured indicated the importance of additive gene action in inheritance of the traits. Except for total tassel length and kernel row number, significant SCA variance indicated the importance of non-additive gene action in governing the traits.

GxE interaction was significant for grain yield, pollen yield, tassel branch number, and ear weight only (Table 4.5). Thus, the interaction was partitioned into GCA x Environment and SCA

x Environment variance. Significant GxE interaction effects indicate that tassel size, grain yield, and pollen yield reacted differently in the three environments. GCA x Environment interaction effects were significant for grain yield, pollen yield, and tassel branch number. Thus, the expression and magnitude of the additive genetic variance for these traits depended upon the environmental conditions. No SCA x Environment interaction was found for any of the traits; hence environmental conditions did not affect the SCA of lines used in formation of the hybrids.

Estimates of genetic parameters are shown in Table 4.6. The magnitude of GCA and SCA variance for different quantitative traits revealed that both additive and non-additive gene actions were important in the inheritance of tassel size, grain yield, pollen yield and other quantitative traits in the maize hybrids. GCA was more important than SCA in determining the inheritance of pollen yield, total tassel length, tassel branch number, 1000-kernel weight and kernel row number, hence these traits can be improved effectively through selection. SCA effects were more important than GCA effects in determining the inheritance of grain yield, ears per plant/ prolificacy, and ear weight. Similar results were reported by Crossa et al. (1990) who found highly significant GCA and SCA variance for grain yield, days to anthesis and ears per plant in a diallel cross among Mexican races of maize. In their study, GCA effects were predominant in determining grain yield among the maize genotypes.

However, Lee et al. (2005) reported that although both additive and non-additive genetic effects influence grain yield in inbred line crosses, 74% of the total genetic variance for grain yield was contributed by the additive genetic component. Guei and Wassom (1996) found significant additive genetic effects in the inheritance of tassel characteristics. Mock and Schuetz (1974) found that additive, dominance, and epistatic gene action, all influenced the inheritance of tassel branch number, but additive gene action was most important. Schuetz and Mock (1978), Guei and Wasson (1996), Berke and Rocheford (1999), Wolf and Hallauer (1997) and Hinze and Lamkey (2003) found evidence for non-additive gene action in inheritance of tassel traits. Results from this study are contrary to findings by Neuffer (1989) who pointed out that the few-branched-1 tassel mutation is a dominant mutation. MacRobert (Personal communication, 2009) also observed that the *Fbr1* is consistently a dominant mutation which has demonstrated additive effects in certain genotypes.

Table 4.5 Combined analysis of variance for grain yield and yield related traits in diallel cross of six inbred lines evaluated under optimum and stress environments.

	Mean squares[†]									
	DF[‡]	GYD	PYD	TTL	TBN	1000-kw	EPP	EW	KRN	EL
Environment	2	69.62***	13.78***	1125.50***	648.43***	212967.27***	0.11	140334.16***	2.58*	342.57***
Rep (Env) [§]	2	0.032	0.25	38.58	14.94	317.91	0.34**	204.69	0.22	1.24
Entry	14	1.18***	3.97***	262.19***	347.17***	9416.86***	0.27***	3705.72***	2.33***	13.36***
GCA	5	1.73***	9.69***	626.74***	802.86***	20619.72***	0.32***	11106.84***	3.81***	30.26***
SCA	9	0.67***	0.89***	110.66	48.18**	5075.78***	0.23***	4574.94***	0.48	13.91***
Entry x Env	28	0.46***	0.73***	38.35	20.78*	1518.97	0.01	1076.55*	0.38	2.16
GCA x Env	5	0.41*	2.55***	58.59	85.88***	4376.67**	0.01	1240.90	0.72	3.09
SCA x Env	9	0.15	0.34	27.98	20.28	1540.75	0.0073	414.18	0.82	3.25
Error	82	0.14	0.17	58.74	10.96	958.04	0.033	561.46	0.65	1.63

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$,[†] Variances for grain yield (GYD), pollen yield (PYD), total tassel length (TTL), tassel branch number (TBN), 1000-kernel weight (1000-kw), ears per plant (EPP), ear weight (EW), kernel row number (KRN) and ear length (EL), measured across sites and years, [‡]GCA, SCA, GCA x Env, SCA x Env degrees of freedom are 4, 5, 4, 5 for grain yield, pollen yield, total tassel length, tassel branch number, 1000-kw, ears per plant, ear weight, kernel row number, and ear length, [§] Rep(Env) - Replication within environment, Entry x Env: Entry x Environment, GCA x Env: GCA x Environment, SCA x Env: SCA x Environment

Table 4.6 Estimation of genetic parameters for grain yield (GYD), pollen yield (PYD), total tassel length (TTL), tassel branch number (TBN), 1000-kernel weight (1000-kw), ears per plant (EPP), ear weight (EW), kernel row number (KRN) and ear length (EL), of maize hybrids measured across sites and years

Genetic parameters[†]	**GYD**	**PYD**	**TTL**	**TBN**	**1000-kw**	**EPP**	**EW**	**KRN**	**EL**
σ^2_D[‡]	0.23	0.14	20.67	6.97	883.76	0.056	1040.19	0.085	2.67
σ^2_A[§]	0.12	1.10	80.92	114.84	2118.00	0.014	950.86	0.58	2.76
$\sigma^2_{gca}/\sigma^2_{sca}$	0.26	3.93	1.96	8.24	1.20	0.13	0.46	3.41	0.52
Genetic ratio[¶]	0.84	0.96	0.92	0.97	0.89	0.74	0.83	0.94	0.81
H	0.71	0.73	0.70	0.74	0.72	0.70	0.72	0.67	0.72
h^2	0.24	0.65	0.56	0.70	0.51	0.10	0.35	0.58	0.37
(σ^2_{gca})	0.060	0.55	40.46	57.42	1059.00	0.0072	475.43	0.29	1.38
(σ^2_{sca})	0.23	0.14	20.67	6.97	883.76	0.056	1040.19	0.085	2.67

[†]σ^2_D = dominance variance, σ^2_A = additive variance, σ^2_{gca} = GCA variance,σ^2_{sca}= SCA variance, H = broad sense heritability, h^2 = narrow sense heritability, [‡]$\sigma^2_D = \sigma^2_{sca}$ when inbreeding coefficient (F) of parents = 1 (100% inbreeding) and [§] $\sigma^2_A = 2 \times \sigma^2_{gca.}$ (Zhang et al., 2005), [¶]Genetic ratio or predictability ratio as calculated from Baker (1978).

The relative magnitude of two variances computed by predictability ratio or genetic ratio as suggested by Baker (1978) (Table 4.6) revealed the predominant role of non-additive gene action for all the traits. A genetic ratio closer to unity implies predictability of performance based on GCA alone (Zare, 2011). Traits with the highest GCA/SCA ratios had the highest predictability ratios (i.e. above 90%), and these are pollen yield, total tassel length, tassel branch number, 1000-kernel weight and kernel row number.

Narrow sense heritability is the proportion of additive genetic variance to total phenotypic variance. It reflects the fixable component of variance through selection leading to increased magnitude of a quantitative trait (Chakraborty et al., 2010). Narrow sense heritability was low: 0.10 for prolificacy, 0.24 for grain yield, 0.35 for ear weight and 0.37 for ear length, and moderate for pollen yield, total tassel length, tassel branch number, 1000-kernel weight and kernel row number (Table 4.6). Very low to moderate estimates of narrow sense heritability for all traits indicated further, the predominance of non-additive genetic variance in their expression.

4.5 Conclusions and recommendations

Fbr1 x *Fbr1* hybrids were generally low yielding under drought and low N stress environments. Although literature has shown that breeding for small tassels could improve grain yield under stress conditions, it remains elusive considering these results. Grain yield improvement and stress tolerance can be determined by multiple factors, which, when put together can additively contribute to increased yield performance. Reduction in tassel size could be one of these many factors that contribute to improved grain yield under stress conditions, but the factor cannot bring significant improvement on its own. Secondary traits like stay green, synchrony between male and female flowering, factors associated with premature senescence, and decreased barrenness can affect yield and should be selected for, together with small tassel size, as they are mechanisms associated with tolerance in water and low N limiting environments.

The genetic analysis results suggest that breeding methods such as recurrent selection or bi-parental mating followed by selection would be ideal to exploit both additive and non-additive gene action for the quantitative traits measured in *Fbr1* hybrids. Since non-additive gene action was important in the inheritance of grain yield, prolificacy, and ear weight, heterosis breeding could be used to harness dominance gene effects by producing and marketing high yielding *Fbr1* hybrids. Additive genetic variance was predominant in determining tassel size components and

pollen yield in the *Fbr1* hybrids. Thus breeding strategies aimed at incorporating and improving maize populations for the tassel mutation should target methods involving selection in segregating progeny populations. Narrow sense heritability values for tassel size determinants were moderately large, thus further confirming that effective progress can be made through selection for the *Fbr1* trait in maize.

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Chapter 5

Phenotypic relationships between grain yield and tassel size in CIMMYT few-branched-1 (*Fbr1*) maize genotypes under abiotic stress and optimal conditions

5.1 Abstract

Tassel size affects yielding efficiency in maize. The objectives of this study were to identify associations between tassel size (tassel branch number; total tassel length) and grain yield under stress and optimal environments. A correlation analysis was performed to examine relationships among grain yield, grain yield components, pollen yield and pollen yield components. Biplots of the first and second principal components showed negative association between pollen yield components and ASI and between tassel branch number and prolificacy under drought stress, which could be a consequence of apical dominance exerted by a larger tassel on the ear, under stress environments. Significant and positive associations were found between grain yield and grain yield components indicating that indirect selection for grain yield can be effectively achieved using grain yield components. Positive relationships between grain yield components and pollen yield components were found except for association of prolificacy with tassel branch number and total tassel length under drought stress and optimum conditions. A negative association between pollen yield components and grain yield components was expected particularly under stress, due to apical dominance exerted on the developing ear by the larger tassel. Hence, selection for upright tassel branches and shorter and lighter tassels may increase yield under stress as tassel branch number is not compromised, thus ensuring sufficient pollen availability for good seed set.

5.2 Introduction

In maize breeding, more attention is currently being given to the selection of crop features that can improve grain yield with high regulation of energy conversion. Among such phenomena are tassel characteristics, which influence plant performance and productivity significantly, particularly under stress environments.

Morphological characters and yield components occur sequentially during plant development and relationships among these characteristics may indicate processes determining yield (Ledent, 1984). Coordinated improvement in all yield components would result in long-term yield

improvement, and not just engaging single character selection to improve yield (McNeal et al., 1978). Studies of yield components in maize have been more common for grain yield than for pollen characteristics (Vidal-Martínez et al., 2001a). Individual tassel traits have been regularly related to grain yield but not to pollen yield components (Hallauer and Miranda, 1988). Sharma and Dhawan (1968) have pointed out the importance of considering certain tassel and ear characters simultaneously when developing new inbred lines. Changes and relationships between inherent physiological, biochemical and morphological characteristics provide an approach to identify traits associated with grain yield and make selection more accurate and reliable (Wilson, 1984). Modifications of morphological (e.g., size and number of sink organs) and developmental characteristics (e.g., duration of the silking-filling period) appear to have contributed more to increase the yield potential in maize than have the improvements of basic physiological processes such as photosynthesis and respiration (Miedema, 1984).

Most research for improvement of maize has focused on ear traits because of their agronomic importance, and few studies have targeted tassel traits and variation in pollen production and pollen production components (Vidal-Martínez et al., 2004). In an experiment to determine tassel morphologies that could be indicators of potential pollen production in maize, Fonseca et al. (2003) found that tassel weight loss, main stem length, tassel branch number, total branch length and main stem diameter were not accurate measures of pollen production per tassel in maize hybrids and inbred lines, since none of these characteristics captured all the genetic and environmental variation for pollen production per tassel.

A plant's efficiency can be measured by its ability to allocate most of the photosynthate produced toward the formation of grain (Guei and Wassom, 1996). Traits such as plant height, ear height, leaf area, and leaf number can affect photosynthetic efficiency of maize plants (Moss and Musgrave, 1971). Tassel size and leaf senescence of a plant are other important traits related to productiveness of maize, especially during grain filling. Tassel size affects grain yield, either physiologically by competition for assimilates, as available photosynthates are diverted away from the grain or physiologically by interception of radiation to the leaf canopy (Ribaut et al., 2004). Studies have shown that low yielding plants partition more photosynthates towards the formation of big and heavy tassels, than producing big ears (Hunter et al., 1969). Selecting for smaller tassel size should result in increased yield (Guei and Wassom, 1996). The negative effect of the tassels on yield was demonstrated when de-tasselled plants yielded 19% more grain than

plants that had not been de-tasselled or had tassels removed and then rejoined. This yield increase was attributed to interception of radiation by the tassels. Other studies have shown a correlation between detasselling and reducing the number of tassel branches with a positive effect on yield (Lambert and Johnson, 1977; Geraldi et al., 1985). In tropical maize, unlike in temperate maize, the indirect pressure of selection for reduced tassel size by selecting for increased grain production has had relatively modest effects on tassel size.

Genetic and phenotypic correlations are of interest to determine the degree of association between traits and how they may enhance selection (Hallauer and Miranda, 1988). Thus, these correlations are useful if indirect selection gives greater response to selection for a trait than direct selection for the same trait. Indirect selection for a complex trait, such as yield, is not simple. Since yield is an expression of fitness, drastic changes in one component of yield are accompanied by adjustments in other components, implying the existence of correlated changes of gene frequencies (Hallauer and Miranda, 1988). Effective selection is achieved on secondary traits with greater heritability than primary traits, and success in selection depends on association between traits. Although many authors have referred to a negative association between tassel size and yield potential (Hunter et al., 1973; Fakorede and Mock, 1978; Geraldi et al., 1985), positive association of these traits have also been reported (Neto and Miranda Filho, 2001). There is evidence that the correlation between yield and tassel size tends to be higher and negative under stress environments (Neto and Miranda Filho, 2001). It is expected since under stress, as resources become limited, tassel size influences the development of ears and silks, thus limiting grain yield by three different mechanisms: shading of the upper leaves, acting as a competitive sink and modifying the supply of growth regulators. The improvement of stress tolerance relies on manipulation of the traits ('adaptive traits') that limit yield under the particular stress environment.

Several researchers studied relationships between pollen and tassel components (Vidal-Martínez et al., 2001a; b; 2004; Fonseca et al. 2003; Rácz et al., 2006; Hidvegi et al., 2005; 2006) and a number of authors examined the inheritance of tassel characteristics (Mock and Schuetz, 1974; Geraldi et al., 1978; Berke and Rocheford, 1999). Tassel branch number was negatively related to grain yield (Geraldi et al., 1978, 1985; Vidal- Martínez et al., 2001b; Gyenesne Hegyi et al., 2001; Hegyi, 2003) and the results indicated that selection targeted on decreased tassel branch number and tassel size may have an indirect influence on increased grain yield. Selection for

smaller tassels decreases the energy of plant consumed for tassel development and the shading of flag and upper leaves by the tassel (Lambert and Johnson, 1977).

With this perspective, a 'few-branched-1' (*Fbr1*) tassel mutation in maize has been discovered by breeders in CIMMYT, and it seems that this mutation has great potential to reduce resources channelled by the plants to the tassels, and rather using this to fill the seeds, without any detrimental effect on pollen production and pollination. This mutation has been sucessfully introduced into current elite CIMMYT maize lines by backcrossing. The effect of the *Fbr1* tassel mutation on grain yield and likely response of genotypes to drought and low N stress has not been investigated. The effect of the small tassel morphology on maize grain yield, especially under stress conditions, is worthwhile investigating. With the knowledge of correlations between characteristics one can predict changes in features related to each other (Bódi et al., 2008). These correlations between traits may be useful as a means to simplify selection, if the correlation is consistent across genotypes and environment. Thus, the objective of this study was to determine association between grain yield and tassel size under optimal, low N and drought stress conditions in *Fbr1* maize genotypes.

5.3 Materials and methods

5.3.1 Germplasm and mating design

Details of the germplasm used and mating design are as described in materials and methods section in Chapter 4. Six CIMMYT maize inbred lines were selected for this study and these inbred lines were crossed in a half diallel mating design with ($n(n-1)/2$) F_1 crosses (Griffing, 1956) during the off-season of 2009 under irrigation at Muzarabani to make 15 F_1 hybrids, which were evaluated in yield trials.

5.3.2 Field evaluation procedures and data collection

Details on agronomic management, environments, stress management and experimental design are as described in Chapter 4 in the materials and methods section.

In this study, the hybrid trial, with 15 hybrids plus five hybrid checks were grown in three environments (optimum, low N, and drought stress conditions) in Zimbabwe during 2010 and 2011. The experimental design was a 4 x 5 incomplete alpha lattice (0,1) (Patterson et al., 1978)

with two replications, and tassel and ear traits: pollen yield, pollen yield components, grain yield and grain yield components, were evaluated.

Pollen yield was estimated by the bagging method, which is similar to the technique followed in maize controlled pollinations: when fresh anthers begin to extrude, the tassels of four selected plants were covered with brown paper bags placed as flat as possible in order to present less resistance to wind, with the bag pulled down past the first flag leaf and secured with a paper clip (Vidal-Martínez et al., 2001a). Pollen samples were collected daily from the same plant, taking off the bag with the sample and covering the sampled shedding tassels again throughout the entire shedding period (6 to 12 d). Anthers and insects were removed by sieving the collected pollen through a #35 U.S. standard testing sieve (500 μm opening) (Vidal-Martínez et al ., 2004). The collected pollen was dried under normal day temperatures and favourable atmospheric-shedding conditions, since fresh maize pollen had, at the time of collection, a water content between 50 to 65% and dries out rapidly with low relative humidity (Goss, 1968). The dried pollen with moisture content lower than 10% was weighed with a precision balance to obtain the daily and total production of pollen during anthesis. Shed duration (d) was determined by a direct method (a visual and percent calculation of the amount and duration of pollen shed until anthesis is complete).

Four tassels were used for estimating tassel characteristics. Total tassel length was measured from the non-branching node present below the lowermost primary branch to the tip of the central spike and tassel branch number is the number of primary branches per plant tassel (Upadyayula et al., 2005). The amount of pollen produced by a maize plant depended upon the number of staminate flowers per plant and the amount of pollen per anther (Goss, 1968). Therefore, plants had different amounts of collected pollen throughout the entire shedding period.

Ear weight, kernels per ear, ear row number, kernel row number, and ear length were measured and considered as grain yield components. Twelve ears per plot were used for estimating grain yield components (Vidal-Martínez et al., 2001a). Grain yield (adjusted to 12.5% moisture content) was obtained considering harvested plot area and counting number of plants and harvested ears per plot. Days to anthesis (number of days from planting to 50% pollen shed),

days to silking (number of days from planting to 50% silk emergence), and ASI were measured on plot basis for both inbred parent and hybrid trials.

5.3.3 Statistical analysis

Trials were analysed individually according to an alpha lattice (0,1) design (Patterson et al., 1978) using the GLM (general linear model) procedure of SAS (SAS Institute, 2003). Both replications and incomplete blocks were considered random effects, while genotypic effects were considered fixed. ASI data was normalised using the transformation of LN (ASI +10) (Bolaños and Edmeades, 1996), before analysis of variance together with other traits measured. Pearson's phenotypic correlation coefficients were computed to estimate associations among traits – pollen yield components, grain yield components, pollen yield and grain yield, using the least squares means for parameters measured in hybrid trials.

Pollen yield components and grain yield components data were analysed using the multivariate analysis (SAS, 2003) i.e. the principal component analysis. The relationship between pollen yield components and grain yield components were displayed by means of Gabriel's Biplot (Rawling, 1988). A biplot illustrates relationships among the independent variables, the relative similarities of the individual data points, and the relative values of the observations for each independent variable.

5.4 Results and discussion

5.4.1 Pollen and grain yield components variation

Variation in pollen yield components and grain yield components of genotypes was found (Table 5.1). Similarly, Vidal-Martínez (2001a) reported significant genetic variation in Mexican and Corn Belt genotypes of maize for similar quantitative traits. Differences due to environment and GxE interaction were significant for all pollen yield components. For grain yield components, environmental variation was found for most traits except for ear length, and GxE interaction was significant for most components except for kernel row number and cob circumference. Thus, for all pollen yield components and most grain yield components, there was differential genotypic response to the different environmental conditions (optimum, low N, and drought stress conditions). This GxE interaction is normally associated with changes in genotypic rankings and limits the identification of superior, stable hybrids for yield performance (Epinat-Le Signor et al.,

Table 5.1 Mean squares for pollen and grain yield components for genotypes and environments

		Pollen yield components							
Variable	PYLD[†]	TBN	TTL	ASI	AD				
Genotype	4.30***	417.90***	226.010***	15.79***	136.61***				
Environment	21.68***	1131.60***	1882.27***	321.48***	11640.66***				
Genotype x E	0.62***	16.97***	39.08**	5.63***	8.89***				
		Grain yield components							
	GYLD[§]	1000-KW	KPE	EW	KRN	EL	CC	CW	SD
Genotype	1.72***	10466.03****	15504.93***	5880.56***	2.20***	16.20***	7.10***	83782.77***	176.97***
Environment	103.75***	329160.85***	397327.94***	261349.39***	11.20***	602.15	213.56***	3092845.29***	15575.60***
Genotype x E	0.56***	2215.19***	10402.77***	2328.19***	0.58	3.29**	1.19	29560.51***	3.24***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

[†] PYLD = Pollen yield (g plant^{-1}) Grain yield in kilograms, measured on plot basis, adjusted to 12.5% grain moisture content and to number of plants per plot

[‡] TBN = Tassel branch number; TTL = Total tassel length (cm); ASI = Anthesis silking interval and DTA = mean number of days to anthesis.

[§] GYLD = Grain yield (kg plot^{-1}); 1000-KW = 1000- kernel weight, KPE = Kernels per ear; EW = Ear weight; KRN = Kernel row number; EL = Ear length

2001). Although a substantial amount of variation among entries for pollen yield components and grain yield components was genotypic, environmental variation explained the larger part of differences among entries for pollen yield components and grain yield components (Table 5.1).

5.4.2 Association among pollen yield, pollen yield components, grain yield, and grain yield components

A positive relationship between pollen yield and grain yield was found for analysis done across the three sites (r = 0.37) and for data collected under optimum conditions (r = 0.48) and in both cases the relationship was significant at $P \leq 0.01$ (Table 5.2). Vidal-Martínez (2001b) also found a moderate and positive relationship between pollen yield and grain yield. Under low N and drought stress conditions, there was no significant association between pollen yield and grain yield, and pollen yield explained only 0.41% of the variation in grain yield: $r^2 = 0.0041$. Under drought conditions pollen yield explained only 5.3% of variation in grain yield. Under stress conditions, pollen yield is reduced, but the major contributor to yield reduction are effects imposed by stress on the plants' metabolic and physiological processes that in turn affect grain yield (Bänziger et al., 2000). Highly significant ($P \leq 0.001$) and positive relationships between pollen yield and tassel branch number were obtained across sites, under optimum, low N, and under drought stress conditions. These ranged from r = 0.64 under optimum conditions to r = 0.79 under drought stress. Under stress conditions, tassel branch number explained a considerable amount of variation in pollen yield ($r^2 = 0.45$ under low N and $r^2 = 0.62$ under drought stress). This is true because the bigger the tassel: with many primary branches, the more the pollen yield, especially because of extended pollen availability, as the many branches shed pollen at different times. That is one reason why breeders have conflicting interests in selection of tassel traits. From the standpoint of yielding efficiency and shading effect, a smaller tassel is ideal but in case of certain situations such as hybrid breeding and stress environments, larger tassels are selected for to ensure sufficient and extended pollen availability (Sofi, 2007). This is especially crucial for stress environments where pollen production is drastically reduced. Across sites and under optimum conditions, significant and positive relationships of pollen yield with grain yield components were found. Their correlation values ranged from 0.19 ($P \leq 0.05$) to 0.42 ($P \leq 0.001$) across site and 0.34 ($P \leq 0.05$) to 0.55 ($P \leq 0.001$) under optimum conditions, suggesting a moderate relationship between these traits. Significant and small association was found for pollen yield with grain yield components under low N and drought stress conditions with pollen yield explaining not more than 3.6% and 7.3% of variation in kernel row number

under low N and drought stress respectively. Although under stress pollen yield is drastically reduced, the effects on grain yield could be a result of different genotypes responding and adjusting for yield differently in reaction to stress.

Table 5.2 Phenotypic correlations among pollen yield (PYLD), pollen yield components (PYC), grain yield (GYLD) and grain yield components (GYC) in maize hybrids grown in different environments

	Across	**Optimum**	**Low-N**	**Drought**
PYLD – GYLD	0.37**	0.48**	0.06	0.23
PYLD – PYC				
Pollen yield - Total tassel length	0.01	0.02	0.13	0.32**
Pollen yield - Tassel branch number	0.78***	0.64***	0.67***	0.79***
Pollen yield - Anthesis silking interval	-0.37***	-	-0.20	0.09
Pollen yield - Anthesis date	-0.22**	-	0.01	0.32**
Pollen yield - Silking date	-0.26**	-	-0.02	0.37**
PYLD – GYC				
Pollen yield - 1000-kw	0.30***	0.50**	0.08	0.21
Pollen yield - Ear weight	0.30***	0.53***	0.09	0.04
Pollen yield - Kernel row number	0.29***	0.34*	0.19	0.27
Pollen yield - Ear length	0.29***	0.35*	0.01	0.08
Pollen yield - Kernels per ear	0.19*	0.42**	0.004	0.01
Pollen yield - Cob circumference	0.32***	0.55***	0.16	0.12
Pollen yield - cob weight	0.42***	0.51***	0.15	0.07
PYC – GYLD				
Total tassel length - Grain yield	0.37***	0.24*	0.27*	0.65**
Tassel branch number - Grain yield	0.22**	0.30**	0.08	0.29
Anthesis silking interval - Grain yield	-0.62***	-0.33**	-0.46***	-0.42
Anthesis date - Grain yield	-0.28**	-0.04	-0.16	-0.36
Silking date - Grain yield	-0.33***	-0.20	-0.19	-0.48*
GYC – GYLD				
1000-kernel weight - Grain yield	0.86***	0.77***	0.46***	0.49***
Ears per plant - Grain yield	-0.50***	-0.63***	-	-0.70***
Ear weight - Grain yield	0.92***	0.81***	0.86***	0.86***
Kernel row number - Grain yield	0.37***	0.21*	0.29*	-0.20*
Ear length - Grain yield	0.88***	0.73***	0.76***	-0.77***
Cob circumference - Grain yield	0.82***	0.69***	0.59***	0.72***
Cob weight - Grain yield	0.95***	0.89**	0.87**	0.97***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

The association of grain yield with pollen yield components: total tassel length, was positive, moderate and significant and ranged from 0.24 under optimum to 0.65 under drought stress conditions. The relationship was also significant ($P \leq 0.01$) and positive between tassel branch number and grain yield across sites and under optimum conditions, and a significant and very small association was found between tassel branch number and grain yield under low N and drought stress conditions. Tassel branch number explained not more than 0.6% and 8.4% of variation in grain yield under low N and drought stress respectively. Afzal et al. (1997) also found positive correlations between grain yield and tassel branch number. However, Vidal Martínez (2001b) found negative associations between pollen yield components and grain yield. Many authors have referred to a negative association between tassel size and yield potential (Hunter et al., 1973; Fakorede and Mock, 1978; Geraldi et al., 1985). There is evidence that the correlation between yield and tassel size tends to be higher and negative under stress caused by unfavourable environments (Neto and Miranda Filho, 2001). They emphasized that environmental factors such as photoperiod, solar radiation and rainfall affect the yield potential of maize and consequently the association between traits may change if there is differentiated variety response to the environmental factors.

The relationship of grain yield with ASI, days to anthesis, and days to silking were negative under all environments. Altenbas and Algan (1993) and Rather et al. (1999) also found positive correlations between grain yield and days to silking and tasseling. Some other published results were contrary to this, however (Umakanth et al., 2000). These results showed the importance of synchrony between female and male flowering dates. High yielding hybrids had a small or even negative ASI, showing that the female flowered earlier than the male plants, thus increasing the chances of complete pollination and consequently increasing grain yield. Grain yield showed a linear trend with ear traits, which suggests that each of the grain yield components may contribute significantly to grain yield. Kumar and Mishra (1995) and Iqbal and Chuhan (2003) reported a positive correlation of grain yield with kernel row number and 100-kernel weight. So selection for these traits can help improve maize grain yield per unit area. However, association between grain yield and ears per plant (prolificacy) were moderate to high and negative across all sites. Thus grain yield was considerably reduced as number of ears per plant increased. This is plausible under stress because of competition of ears for assimilates. Grain yield is determined by the degree to which structures such as ears and kernels, which serve as repositories, or *sinks*, for assimilates, have been established.

Except for prolificacy, association between grain yield components with pollen yield components was mostly positive and low to moderate in magnitude (Table 5.3).

Table 5.3 Phenotypic correlations between pollen yield components (PYC) and grain yield components (GYC) in maize hybrids under optimal, low N, drought stress and across all conditions

	TTL[†]	**TBN**	**ASI**	**DTA**	**DTS**
			r : Across sites		
1000-KW	0.45***	0.20**	-0.53***	-0.34***	-0.38***
Ears per plant	0.46***	-0.02	0.43***	0.10	0.20
Ear weight	0.44***	0.21**	-0.58***	-0.32***	-0.37***
Kernel raw number	0.29***	0.28***	-0.38***	-0.40***	-0.42***
Ear length	0.47***	0.16*	-0.70***	-0.44***	-0.49***
Cob circumference	0.55***	0.28***	-0.58***	-0.46***	-0.50***
Cob weight	0.28***	0.25***	-0.59***	-0.23**	-0.28***
Kernels per ear	0.42***	0.12	-0.58***	-0.43***	-0.47***
			r : Optimum conditions		
1000-KW	0.17	0.28*	-0.08	-0.09	-0.12
Ears per plant	-0.24	-0.03	0.40**	0.21	0.40**
Ear weight	0.21	0.32**	-0.22	-0.05	-0.16
Kernel raw number	0.08	0.53***	0.08	0.42**	0.43**
Ear length	0.28*	0.16	-0.27	-0.17	-0.29*
Cob circumference	0.33**	0.37***	-0.08	-0.08	-0.12
Cob weight	0.10	0.30**	-0.30*	0.12	-0.04
Kernels per ear	0.03	0.21	-0.33*	-0.17	-0.33*
			r : Low-N stress conditions		
1000-KW	0.52***	0.15	-0.34**	-0.38***	-0.39***
Ear weight	0.53***	0.12	-0.56***	-0.43***	-0.46***
Kernel raw number	0.32**	0.09	-0.43***	-0.47***	-0.48***
Ear length	0.51***	0.06	-0.59***	-0.45***	-0.48***
Cob circumference	0.60***	0.31**	-0.43***	-0.50***	-0.51***
Cob weight	0.11	0.16	-0.30**	0.05	0.02
Kernels per ear	0.51***	0.02	-0.57***	-0.53***	-0.55***
			r : Drought stress conditions		
1000-KW	0.65**	0.29	-0.36	-0.20	-0.16
Ears per plant	-0.77***	-0.29	0.52*	0.37	0.47*
Ear weight	0.76***	0.08	-0.62**	-0.54*	-0.60**
Kernel raw number	-0.29	-0.02	-0.31	0.50*	0.35
Ear length	0.71***	0.12	-0.55*	-0.55*	-0.59**
Cob circumference	0.78***	0.22	-0.43	-0.30	-0.28
Cob weight	0.67**	0.16	-0.48*	-0.59**	-0.61**
Kernels per ear	0.54*	0.03	-0.09	-0.39	-0.31

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

[†] TTL = Total tassel length (cm), TBN = Tassel branch number; ASI = Anthesis silking interval, DTA = mean number of days to anthesis, and DTS = mean number of days to silking.

Relationships between tassel and ear traits have been previously reported (Sharma and Dhawan, 1968; Vidal Martínez et al., 2001a).

Vidal Martínez et al. (2001a) found negative phenotypic correlations between pollen yield components and grain yield components. Negative associations between pollen yield components and grain yield components were expected in this study, especially under stress environments, as a consequence of either a trade-off phenomenon between male and female functions which are in competition for resources; or apical dominance which provides a negative effect of the tassel on the development of the female ear (Devlin, 1989; Garnier et al., 1993). These results would indicate physiological agreement with those models when more than one trait is involved in expressing pollen yield components-grain yield components relationships (Vidal Martínez, 2001b). The general negative association of prolificacy with tassel branch number and total tassel length (tassel size) was also found by Souza Junior et al. (1985) who reported on a negative correlation between tassel size and prolificacy which was explained by a large amount of indol-acetic-acid (IAA) produced by larger tassels and causing inhibition of prolificacy, or vice versa (Anderson, 1967).

5.4.3 Correlation matrix biplots of pollen production and grain yield components

Maize hybrids evaluated under drought stress conditions accounted for 64.83% of phenotypic variability according to the first two principal components (PC) (Figure 5.1). Under low N stress, hybrids accounted for 65.98% of phenotypic variation, where PC1 accounted for 45.53% and PC2 accounted for 20.44% of the phenotypic variation in the hybrids (Figure 5.2).

The contribution of traits to the eigenvectors for the principal components can be represented as a biplot, where X and Y-axes represent the proportion of variation attributable by PC1 and PC2 (Figure 5.1 and 5.2). The lengths of these vectors reflect the magnitude of variation for each trait, whereas the angle between vectors reflects the degree of association between the traits (Gabriel, 1971). Thus, Figure 5.1 shows kernel row number and days to silking/female flowering (FF) having larger variation than the other traits under drought stress conditions, while anthesis silking interval shows greater variation under low N stress conditions (Figure 5.2). Under drought stress, anthesis silking interval and pollen yield are closely and positively correlated but inversely correlated with ears per plant/prolificacy. DuPlessis and Dijkhuis (1967) and Edmeades et al. (2000a) observed that when maize flowers are under drought stress, there is

delay of silking in relation to pollen shed resulting in increased ASI, whose duration is highly correlated with kernel set. Kernels per ear are closely and positively associated with grain yield under drought conditions. Most grain yield components under drought stress are inversely correlated with days to male and female flowering. Days to male and female flowering are, in turn, inversely correlated with grain yield under drought stress. Delayed silking lengthens the anthesis silking interval and causes a reduction in yield (Bruce et al., 2001). Under drought conditions, pollen can arrive after it has desiccated, when silks are withered or senesced (Basseti and Westgate 1993a; b) or after ovaries have exhausted their starch reserves (Saini and Westgate, 2000; Zinselmeier et al., 2000).

Bolaños and Edmeades (1993a; b) reported a short anthesis silking interval as a key trait for obtaining high grain yield in maize. They found an increase in the interval from -0.4 days (when silking date anticipates anthesis date) to 10 days, promoting a decline in yield of 8.7% per day. Hall et al. (1981, 1982) suggested that an increased anthesis silking interval under drought stress conditions reduced kernel number because of lack of pollen for late-appearing silks. A shorter anthesis silking interval should thus contribute to the pollination of a large number of differentiated florets (Uribelarrea et al., 2002). Tassel branch number is closely associated but inversely correlated with prolificacy under drought stress conditions. Under stress, larger tassels produce large amounts of indol-acetic-acid (IAA) which inhibits prolificacy, or vice versa (Anderson, 1967). Under drought stress, tassel branch number was not closely correlated with grain yield and most of the grain yield components (Figure 5.1). However, phenotypic variation for tassel branch number, anthesis silking interval and pollen yield under drought stress was larger than that of grain yield components. Under low N stress conditions, grain yield and cob weight, ear weight and ear length, tassel branch number and cob weight were positively and closely associated but were all inversely associated with anthesis silking interval (Figure 5.2). This showed that delayed silking has a large effect on grain yield under low N stress. All vectors showed equally large variation under low N stress. Tassel branch number had positive association with most grain yield components under low N stress unlike under drought stress conditions.

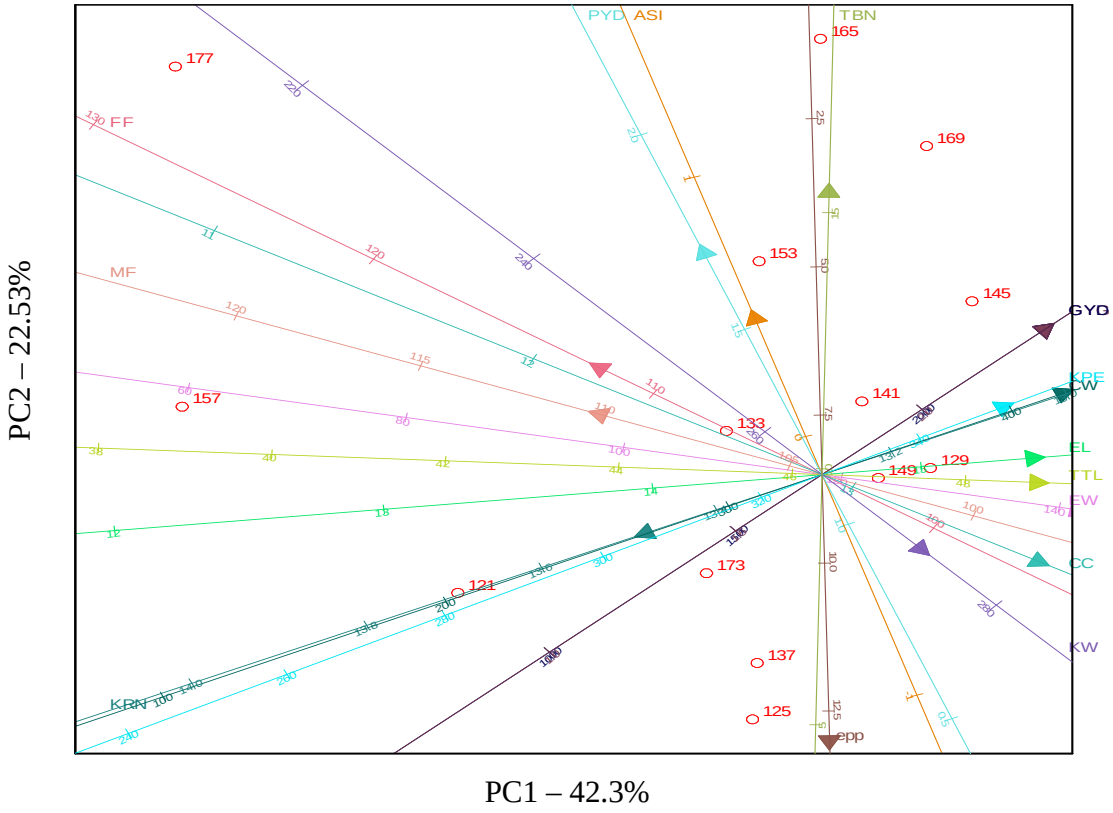

Figure 5.1 Biplot of first (PC1) and second (PC2) principal components expressing the proportion of variation derived from grain yield components and pollen production components (vectors) in the maize hybrids under drought stress conditions. MF = days to anthesis, FF = days to silking, PYD = pollen yield, ASI = anthesis silking interval, TBN = tassel branch number, GYG = grain yield, KPE = kernels per ear, CW = cob weight, EL = ear length, TTL = total tassel length, EW = ear weight, CC = cob circumference, KW = kernel weight, and EPP = ears per plant. PC1 and PC2 cumulatively explained 64.83% of total variation in yield components.

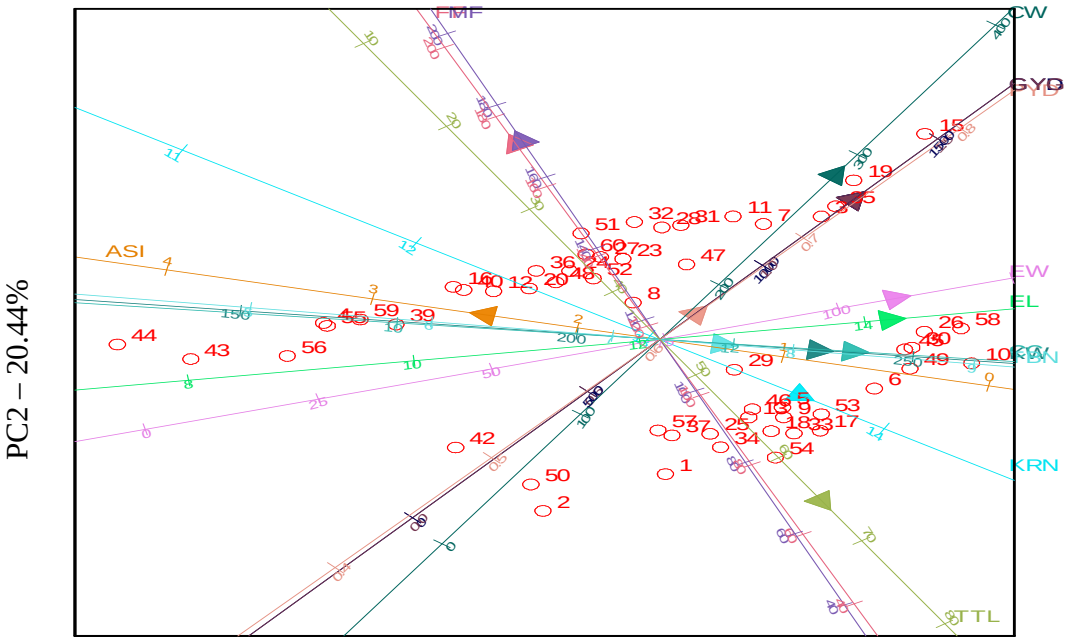

Figure 5.2 Biplot of first (PC1) and second (PC2) principal components expressing the proportion of variation derived from grain yield components and pollen production components (vectors) in the maize hybrids under low N stress conditions. MF = days to anthesis, FF = days to silking, PYD = pollen yield, ASI = anthesis silking interval, TBN = tassel branch number, GYG = grain yield, KPE = kernels per ear, CW = cob weight, EL = ear length, TTL = total tassel length, EW = ear weight, CC = cob circumference, KW = kernel weight, and EPP = ears per plant. PC1 and PC2 cumulatively explained 65.98% of total variation in yield components.

The lack of consistency of PC scores and the different magnitude of variation, as well as the different degrees of associations among traits measured under drought and low N stress, may be due to the presence of phenotypic plasticity which is the amount of change in the expression of traits in different environments (Bradshaw, 1965; Vidal-Martínez et al., 2004). This plastic response of genotypes to different environments is also shown in an analysis of variance in Table 5.1 where environmental and GxE interaction mean squares were significant for most traits. The presence of genotypic variation for all the measured traits suggests genetic differences between genotypes and indicates that phenotypic plasticity could itself be under genetic control and

would, therefore, be subject to selection pressures (Bradshaw, 1965; Vidal-Martínez et al., 2001b).

Edmeades et al. (2001) reported that although grain yield is usually the primary trait for selection under stressed environments, suitable secondary traits can improve selection progress especially if the secondary traits are (1) genetically associated with grain yield under the stress environment, (2) highly heritable, (3) stable and feasible to measure, and (4) not associated with yield loss under ideal growing conditions. Using selection theory, Bänziger and Lafitte (1997) showed that the use of secondary traits plus yield, improved selection gains for maize yield under low N by 20% versus selection for yield alone, with the gains increasing as N deficiency intensified.

One way of improving performance of maize under stress that is centered on mechanisms that improve partitioning of assimilates to the ear at flowering, at the expense of tassel and stem growth, is reducing tassel weight. Reports showed that reduction of tassel weight was much greater than the reduction in primary branch number as reported by Bolaños et al. (1993), who found reduction of tassel weight by 2.6% per cycle of recurrent selection. Chapman and Edmeades (1999) also reported a reduction in tassel weight of 5.9% per cycle of recurrent selection, suggesting that selection for reduced tassel weight may be performed without decreasing tassel branch number and pollen production at the same time (Monneveux et al., 2006). Thus, selecting for reduced tassel weight rather than few tassel branch number can increase maize grain yield thus reducing complications of reduced pollen amount in few branched genotypes. Sofi (2007) found negative correlations for tassel length with ear weight and ear length and also concluded that selecting for upright tassel branches may compensate for yield reduction without compromising tassel size to ensure sufficient pollen availability.

5.5 Conclusions and recommendations

Appropriate selection indices help to achieve desired change in phenotypic expression of particular traits. As such, correlation studies of tassel components may be of significant value as indirect selection criteria in breeding and seed production.

Reduced tassel size appears to be a relevant breeding objective under stress, especially in tropical germplasm and also considering the fact that this trait can be easily altered by selection, and is

highly heritable. Biplots of PC1 and PC2 showed negative association between ASI and grain yield components and prolificacy with tassel branch number under drought stress. This could be a consequence of apical dominance exerted by a larger tassel on the ear, under stress environments. We were anticipating that the *Fbr1* genotypes could potentially increase grain yield under drought stress. However, reduced pollen production in genotypes with few tassel branches, can result in reduced kernel set. The desiccating drought conditions can exacerbate the problem as pollen and silk viability is reduced. Selecting for shorter and lighter tassels may result in higher yields without compromising on tassel size to ensure sufficient pollen availability, especially under stress environments and in hybrid seed production.

Positive associations among grain yield components and grain yield across all environments indicated the importance of the grain yield components for indirect selection for grain yield, especially considering that heritability for grain yield was lower than that of grain yield components in all cases across all three sites. Indirect selection is effective to a greater extent when heritability of secondary traits is greater than that of primary traits.

Genotypic variation and environmental differences on tassel morphological traits and grain yield components indicated that pollen production and grain yield components rely on phenotypic plasticity and genetic variation. Thus, selection of genotypes with plastic response to different environments effectively improves breeding progress and grain yield.

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CHAPTER 6

Determination of yield stability of few-branched-1 (*Fbr1)* maize lines and hybrids across optimal and stress environments using AMMI and GGE biplot analysis

6.1 Abstract

Genotype x environment (GxE) interaction was investigated for grain yield of few-branched-1 (*Fbr1*) maize (*Zea mays* L.) lines and hybrids. Additive main effect and multiplicative interaction (AMMI) and genotype main effect plus genotype x environment interaction (GGE) models were used to determine yield stability and adaptation of genotypes across optimal and stress environments. The AMMI model explained 83.25% of the *Fbr1* hybrid variation and 84.19% of inbred-line variation. The GGE biplot captured 68.3% of GxE variation among hybrids. Generally, IPCA1 and IPCA2 for both models captured much of the GxE interaction sum of squares; hence, best predicted yield and stability of genotypes. IPCA scores of genotype and environment revealed a disproportionate genotype response (crossover GxE interaction) in AMMI analysis. Significant variation in stability of *Fbr1* lines and hybrids as measured by mean yield and AMMI Stability Value (ASV) was observed. Mean grain yield ranged from 0.84-2.18 and 0.38-0.92 kg/plot for the maize hybrids and lines respectively and ASV ranged from 0.08-20.87 for the maize hybrids, and from 0.14-9.00 for the maize lines. Based on the ASV scores, hybrid H38 followed by H26 were most stable while hybrid H36 followed by H69 were unstable. According to the Genotype Selection Index (GSI), the ideal genotype for selection based on both stability and grain yield was hybrid H26 and line L6. However, the AMMI biplot revealed hybrid H36 as the ideal genotype (highest yielding with IPCA score close to zero, therefore stable) and hybrid H69 as the most undesirable genotype (lowest yielding). The GGE biplot also ranked hybrid H36 as the vertex genotype, ideally suited to drought, low N and optimal environments, while H69 was the poorest genotype with no specific adaptation. Thus, AMMI and GGE classification models could be used simultaneously to make selection of genotypes more precise and refined.

6.2 Introduction

Maize production in sub-Saharan Africa has historically been constrained by a number of biotic and abiotic factors, including drought, low soil fertility, insects, disease, and weeds. However, plants vary tremendously in their ability to withstand abiotic stresses, both between species and

within populations of a single species (Ribaut et al., 2002). Abiotic stresses limit crop productivity in every season and in every crop worldwide, yet the nature of tolerance is not well characterised. Among abiotic stresses found in developing countries, drought and low soil fertility are paramount (Beck et al., 1996) and considering on-going climatic changes attributable primarily to global warming (Curry et al., 1995), the pressure on food production in water-limited environments will probably increase in the future. Because of its genetic complexity, drought tolerance is probably the most difficult trait to improve through conventional breeding, the challenge being even greater for developing drought tolerant plants for water-limited environments where occurrence, timing, and severity of drought may fluctuate from year to year.

Even though the challenge of developing abiotic-stress-tolerant crop varieties has been undertaken, most practical breeding efforts remain focused on increasing productivity under favourable conditions where genetic variance, heritability and therefore breeding progress for grain yield are greatest (Bänziger et al., 2004). Plant breeders invariably encounter GxE interactions when testing varieties across environments and depending on the magnitude of the interactions or differential genotypic responses to environments, the varietal rankings can differ greatly across environments (Kaya et al., 2002). GxE interactions are common under drought and make breeding progress difficult. GxE interactions may originate from environmental variation in the timing and severity of water deficit, genetic variation in flowering time and nutrient deficiencies and toxicities whose occurrence and severity interact with water deficits (Bänziger and Cooper, 2001; Cooper et al., 1999). GxE interactions in southern African maize-growing environments result from factors related to maximum temperature, seasonal rainfall, season length, within season drought, subsoil pH and socio-economic factors that result in sub-optimal input application (Bänziger et al., 2004). However, there is extensive evidence that selection under target stresses may accelerate breeding gains for stress environments (Atlin and Frey, 1990; Bänziger et al., 1997; Ceccarelli et al., 1992; Pederson and Rathjen, 1981; Ud-Din et al., 1992).

Numerous methods have been developed to reveal patterns of GxE interaction for total grain yields, such as joint regression (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Perkins and Jinks, 1968), AMMI (Gauch, 1992) and type B genetic correlation (Burdon, 1977; Yamada, 1962). The AMMI model offers a more appropriate first statistical model of choice when main effects and interaction are both important (Zobel et al., 1988; Crossa et al., 1990; Gauch and

Zobel, 1997). The AMMI model combines analysis of variance for the genotype and environment main effects with principal component analysis of the GxE interactions (Gauch and Zobel, 1996). AMMI increases the precision of yield estimation and selection of higher yielding genotypes than treatment means (Crossa et al., 1990) and has no specific experimental design requirements except for a two-way data structure (Zobel et al., 1988). The yield-stability statistic (YS_i) (Kang, 1993) and the GGE distance (i.e., the distance from the markers of individual genotype to the ideal genotype) (ideal genotype has the highest yield and is absolutely stable) in GGE biplot analysis (Yan, 2001; Yan and Kang, 2003) help select for yield and stability. The GGE biplot analysis is based on singular-value decomposition and principal component analysis (Yan and Kang, 2003). GGE biplot methodology has been used to evaluate test environments in maize (Vivek et al., 2010), for analysing multi-environment cultivar trials and studying GxE interactions (Crossa and Cornelius, 1997; Yan et al., 2000). Biplots have also been used for studying response patterns of entries when crossed with testers, that is, line x tester interactions (Narro et al., 2003) and diallel crosses (Yan and Hunt, 2002; Bhatnagar et al., 2004).

Plant breeding goals have been attained through effective management of genetic variability using effective breeding methods for developing superior genotypes for target environments. As such, information on yield performance and stability of *Fbr1* maize lines and hybrids is required to develop high yielding hybrids or synthetics for the target production areas. The objectives of this study were to analyse GxE interaction and stability of *Fbr1* single-cross hybrids and parental lines for grain yield across stress and optimal environments using AMMI and GGE biplot models.

6.3 Materials and methods

6.3.1 Plant materials

Nine *Fbr1* CIMMYT maize lines (CMLs) were used as parental lines in this study (Table 6.1). These lines were crossed in a half-diallel mating design with (n(n-1)/2) F_1 crosses (Griffing, 1956) during the off-season of 2009 under irrigation at Muzarabani (Zimbabwe) to form single cross hybrids that were evaluated herein together with the parental lines. The hybrids were named according to the names of parental lines involved in the cross, for example, hybrid H12 was a single cross between lines L1 and L2.

Table 6.1 Pedigrees of the nine *Fbr1* maize inbred lines used to form the F_1 hybrids

Line	Pedigree
L1	[[CML395/TAS]BC2/[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-B]-8
L2	[CML443/TAS]BC2-2-5-3-1-B
L3	[[CML444/TAS]BC1/[CML444/CML395//DTPWC8F31-4-2-1-6]-2-1-1-1-B]-9-3-4-B
L4	[[CML445/TAS]BC3/[CML445/ZM621B]-2-1-2-3-1-B]-2-4-2-B
L5	[CML445/TAS]BC3-1-1-2-1-B
L6	[[CML312/TAS]BC1/MAS[MSR/312]-117-2-2-1-B]-1-3-1-B
L7	[CML444/TAS]BC2-6-1-1-B
L8	[CML488/TAS]BC2-6-4-2-B
L9	[[CML442/TAS]BC1/ZM621A-10-1-1-1-2-BBBBBB]-2-1-B

6.3.2 Field experiment

Details on agronomic management, environments and stress management are described in detail in Chapter 3 in the materials and method section.

Trials of the 36 crosses plus four hybrid checks were grown using one row plots, two replications and the design was a 4 x 10 incomplete alpha lattice (0,1) (Patterson et al., 1978) in all testing environments (optimum, low N and drought stress conditions). Trials of the nine inbred parents were planted close to the hybrid trials. Two seeds were planted per hill spaced 75 cm between rows and 25 cm between hills and were thinned three weeks after emergence for both trials. The experiments under optimum and low N stress conditions were conducted at the CIMMYT research station in Harare (17.80 S, 31.05 E, 1468 masl), with 700-800 mm rainfall and 650-700 mm potential evapo-transpiration during the season. Low N experiments were grown in fields that were depleted of N by continuously cropping maize (main season) or irrigated wheat (winter dry season) and removing all stover biomass after harvest and not applying any N fertilizer. The experiments under managed drought stress were conducted during the winter dry season in Chiredzi (21.03 S, 31.57 E, 392 masl) and at Nanga research station in Zambia. The trials in the four locations were conducted for two years (2010 and 2011). Plot size at all locations was a single 4m row with 0.75 m between rows and 0.25 m between plants within a rows, giving final plant populations of ≈ 53 000 plants per hectare at all sites. Grain yield, adjusted to 12.5% grain moisture content, was measured on single plot basis from each inbred line and hybrid across sites and years.

6.3.3 Statistical analysis

The General Linear Model (GLM) procedure of SAS (SAS, 2003), was used to carry out the combined analysis of variance of a response and included the factors genotype (G), environment (E), their interaction (GxE) and incomplete block (replication) within environment (B).

The response $\mathbf{Y_{ijk}}$ of genotype **i** in location **j** and incomplete block (replication) **k** is:

$$\mathbf{Y_{ijkr} = \mu + G_i + E_j + B_k + GE_{ij} + \varepsilon_{ijk}}$$

Where:

μ = grand mean

G_i = effect of the i^{th} genotype

E_j = effect of the j^{th} environment

B_k = effect of k^{th} incomplete block

GE_{ij} = interaction effect of the i^{th} genotype with j^{th} environment

ε_{ijk} = random error

6.3.3.1 Biplot analysis

Two types of biplots, AMMI biplot (Zobel et al., 1988) and GGE biplot (Yan et al., 2000), were used to visualize the GxE two-way data for the *Fbr1* maize lines and hybrids. Both types of biplots display treatment x environment interactions, but each has its unique functions (Ma et al., 2004). The AMMI biplot allows visualisation of the main effects of treatments and of the environments, in addition to the most important GxE interactions. The GGE biplot allows visualisation of any crossover treatment x environment interactions, relationships among treatments, and relationships among environments. In this regard, the joint use of both types of biplot should allow a comprehensive understanding of yield performance of the *Fbr1* lines and hybrids.

6.3.3.1.1 AMMI biplot

The AMMI model was used to investigate the agronomic nature of GxE interaction. The AMMI model first fits additive effects for the main effects of genotypes and environments, using the additive usual transpose analysis of variance procedure. The programme then fits multiplicative effects for GxE by principal component analysis (Zobel et al., 1988; Gauch and Zobel, 1996; Gauch and Zobel, 1997). The model was proposed by Zobel et al. (1988) as:

$Y_{ge}=\mu+ \alpha_g+\beta_e+\sum_{n=1}^{n} \lambda_n \gamma_{gn} \eta_{en} +\theta_{ge}$,

where Y_{ge} is the yield of genotype, g, in environment, e; μ is the grand mean; α_g is the genotype mean deviation; β_e is the environment mean deviation; λ_n is the eigenvalue of the principal component (PCA) axis, n; γ_{gn} and η_{en} are the genotype and environment PCA scores for the PCA axis and θ_{ge} is the residual.

The AMMI1 analyses for the parental lines and hybrids were computed using Genstat version 14 (Genstat, 2011) statistical package. The AMMI1 biplot was constructed by plotting the main effects of treatments and environments against their respective interaction scores, which are symmetrically scaled scores of the first-interaction principal component (IPC1) resulting from subjecting the double-centered data (i.e., the interaction matrix) to singular-value decomposition (Yan, 2002). The biplots were used to reveal relationships among genotypes, environments, and between genotype and environments. Environments are represented as vectors and genotypes are represented as points, such that genotypes and environments that are close together are similar. The angle between two environment vectors indicates degree of association and small angles indicate similarity, 90º angles indicate orthogonality and no association and angles > 90º indicate a negative association of genotype performance between environments (Zobel et al., 1988). Orthogonal projections of genotypes on environment vectors indicate relative performance of genotypes in given environments.

6.3.3.1.1.1 AMMI Stability Value (ASV)

The AMMI model does not make provision for a quantitative stability measure, yet such measures are essential in order to quantify and rank genotypes according to their yield stability (Sabaghnia et al., 2008). The AMMI stability value (ASV) was calculated using the following formula, as suggested by Purchase (1997) to rank genotypes:

$\text{ASV} =\sqrt{[[(\overline{SSPC1}/SSPC2)\ (PC1)]square + (PC2)square}$,

where ASV = AMMI Stability Value, SS = sum of squares, PC1 = interaction of PCA1 and PC2 = interaction of PCA2. The genotypes with the highest ASV value were considered the most

stable. SSPC1/SSPC2 is the weight given to the IPCA1-value by dividing the IPCA1 sum of squares by the IPCA2 sum of squares. The larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller IPCA scores indicate a more stable genotype across environments (Farshadfar, 2008).

6.3.3.1.1.2 Genotype Selection Index (GSI)

Based on the rank of mean grain yield of genotypes (RY_i) across environments and rank of AMMI Stability Value ($RASV_i$), a selection index called GSI was calculated for both *Fbr1* parental lines and hybrids using the formula:

$GSI_i = RASV_i + RY_{i,}$ (Farshadfar, 2008).

The least GSI was considered as the most stable with high grain yield.

6.3.3.1.2 GGE biplot

A GGE biplot was constructed using the first two principal components (IPCA1 and IPCA2) derived from subjecting the environment-centered data to singular-value decomposition (Yan, 2002; Yan and Tinker, 2006). A GGE biplot does not display the main effects of the environments but has many visual interpretations that an AMMI1 biplot does not have: (i) the polygon view of a GGE biplot allows visualisation of the which-won-where pattern (which genotype or treatment had the highest yield in which environment), (ii) the average environment coordination view allows simultaneous visualisation of the mean performance and stability of the treatments, the discriminating ability vs. representativeness of the environments; and (iii) the environment vector view allows visualisation of the interrelationship among environments (Yan, 2001; 2002; Yan and Kang, 2003). For appropriate visualisation of both the relationship among the environments and the crossover treatment x environment interactions, the singular values were entirely partitioned into environment eigenvectors (Yan, 2002).

The GGE biplot analysis for grain yield of the hybrids in optimum, low N and drought stress conditions, the average tester coordination for entry evaluation, and which hybrid is best for which character, was done using the GGE biplot package that runs in a Windows environment, an earlier version of which was described in Yan (2001). Up-to-date information on GGE biplots is available at http://www.ggebiplot.com.

6.4 Results and discussion

6.4.1 Analysis of variance

The combined analysis of variance across environments showed significant differences for grain yield for hybrids (or inbred lines), environments and GxE interaction (Table 6.2 and 6.3). The significant GxE interaction effects demonstrated that genotypes responded differently to variations in environmental conditions. These variations could be attributed to different climatic and edaphic conditions at the different locations. The ANOVA showed that, out of the total sum of squares for hybrids, 81.33, 8.86 and 9.79% was attributable to environment, genotype and GxE interaction effects, respectively (Table 6.2). For the parental lines 78.31, 12.37 and 9.30% of the total sum of squares were due to environment, genotype and GxE interaction respectively (Table 6.3). A large contribution of environment to total variation indicated that environments were diverse, with large differences among environmental means causing most of the variation in grain yield. In both inbred lines and hybrid progenies, the large proportion of variation due to environment did not, however, reduce the importance of the differences due to genotypes or GxE interactions. The size of genotype sum of squares in relation to the GxE sum of squares indicated substantial differences in genotype response in different environments.

Table 6.2 Analysis of variance for the AMMI model for grain yield of the 36 maize hybrids evaluated under optimum, low N and drought stress environments

Source of variation†	Degrees of freedom	Sum of squares	Mean squares	Total variation explained (%)	G x E explained (%)	G x E cumulative (%)
Block(environ)	4	2.8	0.69	0.36	-	-
Treatments	143	646.6	4.52***	82.89	-	-
Hybrids	35	57.3	1.64***	8.86	-	-
Environments	3	525.9	175.31***	81.33	-	-
Hybrids x env	105	63.3	0.60*	9.79	-	-
IPCA1	37	37.5	1.01***	-	59.24	59.24
IPCA2	35	16.5	0.47*	-	26.07	85.31
G x E residuals	33	9.4	0.28	-	-	-
Pooled Error	280	130.7	0.47	16.75	-	-

* $P \leq 0.05$, *** $P \leq 0.001$ † IPCA is the interaction principal component axis

Table 6.3 Analysis of variance for the AMMI model for grain yield of the nine maize inbred lines evaluated under optimum, low N and drought stress environments

Source of variation†	Degrees of freedom	Sum of squares	Mean squares	Total variation explained (%)	G x E explained (%)	G x E cumulative (%)
Block(environ)	4	0.17	0.043	0.36	-	-
Treatments	35	40.02	1.143***	83.83	-	-
Lines	8	4.95	0.619***	12.37	-	-
Environments	3	31.34	10.45***	78.31	-	-
Line x env	24	3.72	0.16*	9.30	-	-
IPCA1	10	2.75	0.28**	-	73.92	73.92
IPCA2	8	0.86	0.11*	-	23.12	97.04
G x E residuals	6	0.12	0.020	-	-	-
Pooled Error	66	7.55	0.11	15.81	-	-

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, †IPCA is the interaction principal component axis

6.4.2 AMMI model and pattern analysis

In the AMMI model, principal component analysis is based on the matrix of deviation from additivity or residual, while pattern analysis employs both classification and ordination techniques. In this respect, both the results of AMMI analysis, the genotype and environment were grouped based on their similar responses (Gauch, 1992; Wade et al., 1995; Pourdad and Mohammadi, 2008).

6.4.2.1 ANOVA for AMMI

The GxE interaction was further analysed with the aid of the AMMI model for grain yield stability of the *Fbr1* maize lines and hybrids. For the hybrids (Table 6.2), the first interaction principal component axis (IPCA1) captured 59.24% of the interaction sum of squares in 35.24% of the interaction degrees of freedom. Similarly, the second interaction principal component axis (IPCA2) explained a further 26.07% of the GxE sum of squares. For the parental inbred lines (Table 6.3), IPCA1 accounted for 73.92% of the interaction sum of squares in 41.67% of the interaction degrees of freedom, while IPCA2 captured an additional 23.12% of the GxE sum of squares. For the hybrids, the mean squares for the IPCA1 and IPCA 2 were significant at $P \leq 0.001$ and $P \leq 0.05$ respectively, while for the parental lines; IPCA1 and IPCA2 were significant at $P \leq 0.01$ and $P \leq 0.05$. Cumulatively, IPCA1 and IPCA2 contributed to 85.31 and 97.04% of

the total GxE variation in *Fbr1* maize hybrids and lines respectively. Therefore the post-dictive evaluation using an F-test at P≤ 0.05 suggests that two IPCA1 and IPCA2 were significant for the model with 72 degrees of freedom for hybrids and 18 degrees of freedom for the parental lines. The AMMI model contained 83.25% of the treatment sum of squares, while residual contained 16.75% for hybrids. Similarly, the AMMI model explained 84.19% of treatment variation, while the residual explained 15.81% for inbred lines. These results indicate that the AMMI model fitted the data well and justifies the use of AMMI in this analysis.

In general, IPCA1 and IPCA2 captured much of the GxE interaction sum of squares. Thus, these two IPCAs best predicted yield performance of the maize lines and hybrids across the four environments and consequently facilitated graphical visualisation of the genotypes in low dimension. Gauch and Zobel (1996) and Yan and Rajcan (2002) indicated that the most accurate model for AMMI can be predicted by using the first two IPCAs. Conversely, Sivapalan et al. (2000) recommended a predictive AMMI model with the first four IPCAs. These results indicate that the number of the terms to be included in an AMMI model cannot be specified *a priori* without first trying AMMI predictive assessment. Generally, factors like type of crop, diversity of the germplasm, and the range of environmental conditions can affect the degree of complexity of the best predictive model (Crossa et al., 1990).

6.4.2.2 IPCA, crossover (qualitative) and non-cross over interaction (quantitative)

IPCA scores of genotype and environment assumed both positive and negative values (Tables 6.4, 6.5, and 6.6). Consequently, a genotype that has a large positive IPCA score within some environments can have large negative interactions with some other environments (Farshadfar, 2008). Thus, these scores presented a disproportionate genotype response (Yan and Hunt, 2001; Mohammadi et al., 2007), which was the major source of variation for any crossover interaction.

The disproportionate genotype response is referred to as crossover GxE interaction, while scores with the same sign or near zero represent a non-crossover GxE interaction or a proportionate genotype response (Mohammadi et al., 2007; Mohammadi and Amri, 2008).

Table 6.4 Mean grain yield for hybrids (Y_i), scores for the interaction principal component axis (IPCA) 1 and 2, AMMI stability value (ASV), rank of hybrids based on ASV, and the Genotype Selection Index (GSI_i) for the 36 maize hybrids

Hybrid[†]	Mean yield (Y_i)	RankY_i	IPCA1	IPCA2	ASV	RankASV_i	GSI_i
H12	1.52	24	0.14	0.21	0.23	6	30
H13	1.55	22	-0.18	-0.17	0.26	10	32
H14	1.36	29	0.15	0.48	0.48	22	51
H15	1.71	14	0.04	-0.20	0.20	5	19
H16	1.95	6	-0.17	-0.35	0.36	15	21
H17	1.91	7	0.09	0.24	0.24	7	14
H18	1.33	30	-0.10	-0.27	0.27	11	41
H19	1.70	15	0.22	-0.02	2.27	34	49
H23	2.02	3	0.45	-0.41	0.65	25	28
H24	1.55	22	0.08	0.15	0.16	3	25
H25	1.03	34	-0.25	0.04	1.66	30	64
H26	1.99	5	0.09	0.10	0.13	2	7
H27	1.69	17	-0.63	0.02	20.87	36	53
H28	1.77	11	0.02	-0.27	0.27	11	22
H29	1.59	21	0.02	0.18	0.18	4	25
H34	1.15	33	-0.20	-0.22	0.28	13	46
H35	1.85	10	0.09	-0.44	0.44	20	30
H36	2.18	1	0.60	-0.21	1.73	31	32
H37	0.94	35	-0.52	0.19	1.44	29	64
H38	1.25	31	-0.05	-0.03	0.08	1	32
H39	1.42	28	-0.58	0.15	2.24	33	61
H45	1.61	19	0.22	0.12	0.41	19	38
H46	1.68	18	0.47	0.22	1.05	27	45
H47	1.51	25	0.04	-0.23	0.24	7	32
H48	1.50	26	-0.32	-0.33	0.46	21	47
H49	1.25	31	-0.44	0.17	1.12	28	59
H56	2.00	4	0.50	-0.06	4.39	35	39
H57	1.87	9	0.19	0.14	0.28	13	22
H58	1.75	12	-0.17	0.06	0.49	23	35
H59	1.44	27	0.27	0.11	0.69	26	53
H67	1.74	13	0.00	0.37	0.37	16	29
H68	1.89	8	0.01	-0.37	0.37	16	24
H69	0.84	36	-0.43	-0.09	2.03	32	68
H78	1.70	15	0.06	0.39	0.39	18	33
H79	2.04	2	0.07	0.24	0.24	7	9
H89	1.61	19	0.21	0.07	0.61	24	43

[†]Hybrid H12 for example, is a cross of line 1 and line 2; mean grain yield in kg/plot. Rank Yi = rank of hybrids based on mean yield, Rank ASV_i = rank of hybrids based on AMMI stability value, and GSIi = Genotype Selection Index.

Table 6.5 Mean grain yield for parental lines (Y_i), scores for the interaction principal component axis (IPCA) 1 and 2, AMMI stability value (ASV), rank of lines based on ASV, and the Genotype Selection Index (GSI_i) for the nine inbred lines

Line	Mean yield (Y_i)	RankY_i	IPCA1	IPCA2	ASV	RankASV_i	GSI_i
L1	0.48	7	0.25	0.03	2.21	7	14
L2	0.38	9	0.33	-0.21	0.54	6	15
L3	0.48	7	0.17	0.00	9.00	9	16
L4	0.79	4	-0.13	-0.41	0.41	5	9
L5	0.83	3	0.13	0.38	0.39	4	7
L6	0.89	2	0.00	0.14	0.14	1	3
L7	0.92	1	-0.73	0.15	3.60	8	9
L8	0.65	6	-0.19	-0.21	0.27	2	8
L9	0.69	5	0.19	0.13	0.30	3	8

†Mean grain yield in kg $plot^{-1}$. Rank Yi = rank of inbred lines based on mean yield, Rank ASV_i = rank of lines based on AMMI stability value, and GSI_i = Genotype Selection Index

Table 6.6 Environment means, interaction principal component axis (IPCA) 1 and 2 scores, and environmental variance for maize hybrids and inbred lines

Environment	Mean grain†yield	IPCA[1]	IPCA[2]	Environmental variance
		Hybrids		
Drought Zambia	0.99	-0.63	0.92	0.20
Drought Zim	2.24	1.51	0.067	0.60
Low N	0.40	-0.40	0.095	0.082
Optimum	2.80	-0.48	-1.08	0.89
Margin	1.61			1.36
		Inbreds		
Drought Zambia	0.19	0.47	-0.13	0.022
Drought Zim	0.79	-0.060	0.59	0.081
Low N	0.34	0.30	-0.22	0.028
Optimum	1.39	-0.71	-0.23	0.34
Margin	0.68			0.33

†Grain yield is in kg/plot adjusted to 12.5% grain moisture content and to the number of plants per plot. Plot size is the same across locations.

6.4.2.3 The AMMI Stability Value (ASV)

ASV is the distance from zero in a two dimensional scattergram of IPCA1 scores against IPCA2 scores. Since the IPCA1 score contributes more to GxE sum of squares, it has to be weighed by the proportional difference between IPCA1 and IPCA2 scores to compensate for the relative contribution of IPCA1 and IPCA2 to total GxE sum of squares (Farshadfar, 2008). The distance from zero is then determined using the theory of Pythagoras (Purchase et al., 2000).

Stability analysis conducted to assess the grain yield performance of the *Fbr1* hybrids and parental lines across stress and optimal conditions showed a significant variation in stability of hybrids and lines as measured by mean grain yield and ASV (Table 6.4 and 6.5). Mean yield ranged from 0.84-2.18 and 0.38-0.92 kg/plot for the maize hybrids and lines respectively. ASV ranged from 0.08-20.87 for the maize hybrids, and from 0.14-9.00 for the maize lines. In the ASV method, a genotype with the lowest ASV score is the most stable, thus, hybrids H38, followed by H26, H24, H29, H15 and H12 were the most stable, while hybrids H36, H69, H39, H19, H56 and H27 were undesirable (Table 6.4). Parental inbred lines L6 followed by L8, L9 and L5 were stable while L3 was undesirable (Table 6.5).

AMMI biplot analysis and ordination techniques revealed significant differences for IPCA1 and IPCA2 and these explained 85.31% and 97.04% of variability in the GxE interaction for hybrids and inbred lines respectively. Generally, the AMMI model is important in reducing the 'noise' even if the principal components do not cover much of GxE sum of squares (Gauch and Zobel, 1989; Gauch, 1992). The biplot analysis for hybrid and environmental means on IPCA1 (Figure 6.1) displayed that hybrid H36 was the ideal genotype under drought (Zimbabwe) and optimum environments while hybrid H69 was an undesirable hybrid (lowest yielding). Although mean yield for hybrid H69 was lowest, it had specific adaption to low N environments. Similarly, inbred lines L6 and L7 were high yielding and stable while line L5 and environments drought-Zimbabwe and optimum showed the greatest effect in the GxE interaction (Figure 6.2). Most hybrids are located close to the centre of the biplot indicating stability of these entries across environments (Manrique and Hermann, 2000). Whatever the direction is, the greater the IPCA scores, the more specifically adapted these genotypes are to specific environments (Zobel et al., 1988; Crossa et al., 1990, 1997).

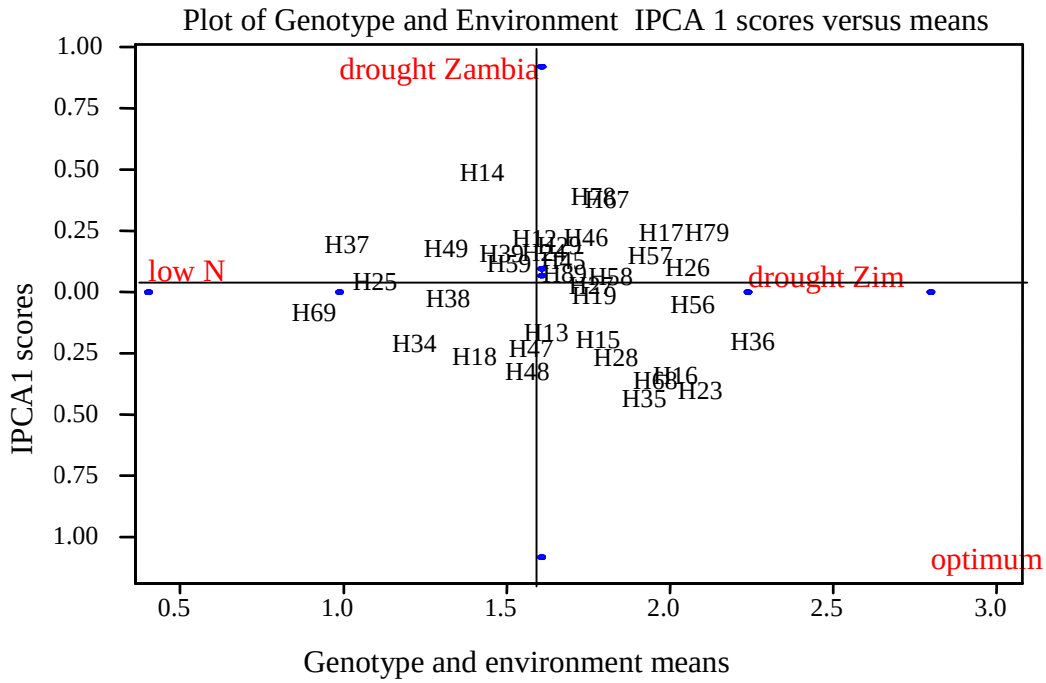


Figure 6.1 Yield of the 36 hybrids modelled as a function of the score on the first GxE interaction principal component axis of the four locations.

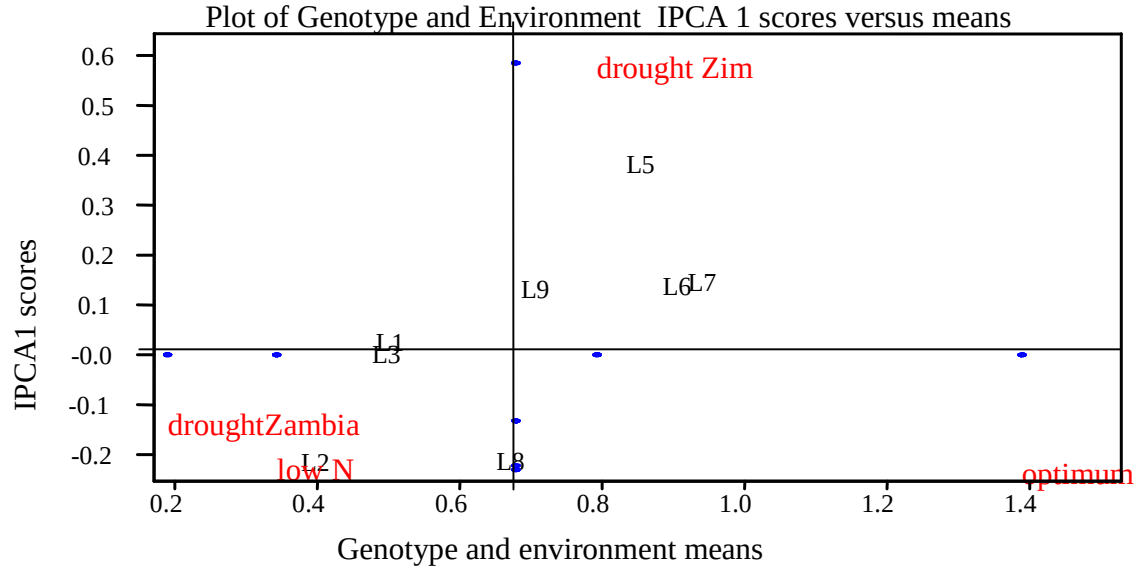


Figure 6.2 Yield of the nine inbred lines modelled as a function of the score on the first GxE interaction principal component axis of the four locations.

Genotypes further from the centre of the biplot show specific adaptation, thus, hybrid H14 has specific adaption to drought (Zambia), while hybrids H69 and H37 show specific adaption to low N conditions. Hybrids H35, H23, H16 and H68 have positive interaction with optimum growing conditions, but as the length of the vectors for hybrid H35 and H23 are more on the optimum environment, these hybrids have specific adaptability to optimum environments. Most hybrids are clustered at the centre of the biplot, thus they show no specific adaptation and are stable. Inbred lines L2 showed specific adaptability with low N and drought (Zambia) environments because their angle is less than 90% and their GxE interaction is positive. Line L5 is specifically adapted to drought (Zimbabwe). However, inbred lines L1 and L3 are on the zero line of the biplot and thus are most stable.

For both hybrid and inbred line evaluation, the biplots (Figures 6.1 and 6.2) characterised drought (Zimbabwe) and optimum as high potential environments, while drought (Zambia) and low N were low potential environments.

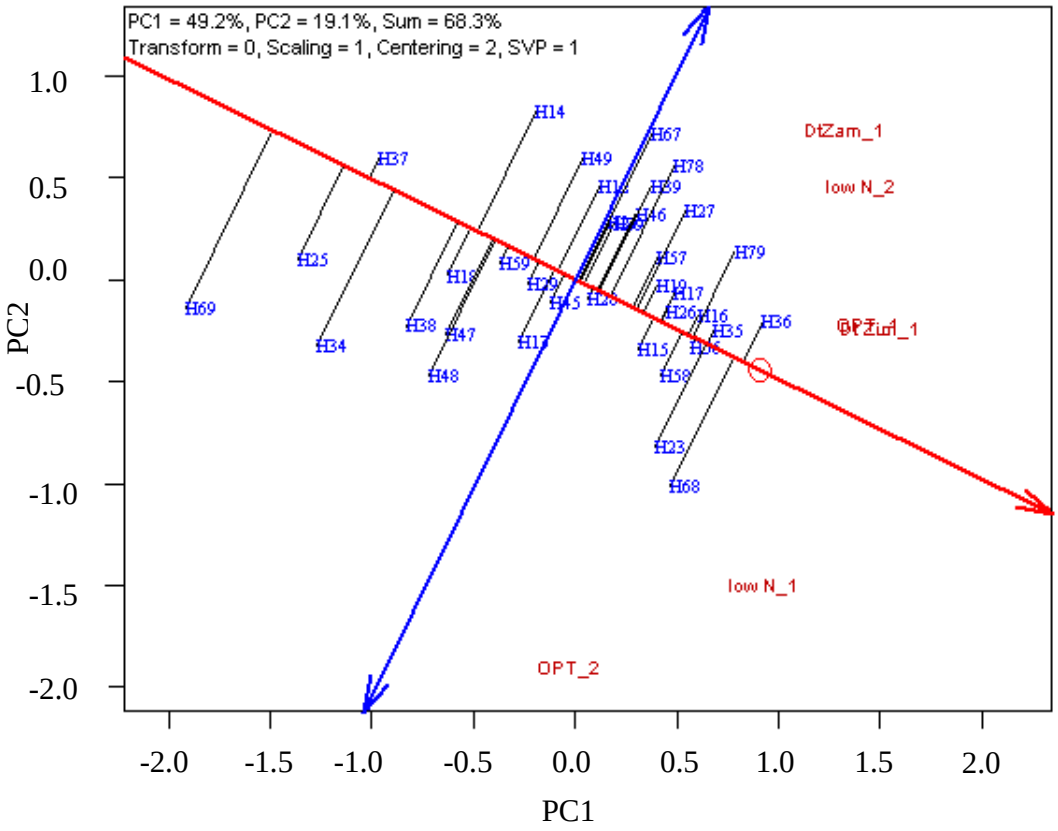
6.4.2.4 Genotype Selection Index (GSI)

Stability *per se* should, however, not be the only parameter for selection, because the most stable genotypes would not necessarily give the best yield performance (Mohammadi et al., 2007). The ASV approach incorporates both mean grain yield and stability in a single criterion and simultaneously selects desirable genotypes based on the two criteria. In this regard, since ASV takes into account both IPCA1 and IPCA2 it justifies most of the variation of GxE interaction. Based on the GSI (Table 6.4 and 6.5), the most desirable genotype for selection based on both stability and high grain yield was hybrid H26 followed by hybrid H79; and line L6 followed by L5. From the AMMI biplot, it could be seen that these lines and hybrids are quite stable and high yielding.

6.4.3 GGE biplot analysis

Results on yield performance and stability of the 36 *Fbr1* maize hybrids across environments in the two years (2010 and 2011 i.e., year 1 and year 2 respectively) are shown in Figures 6.3 and 6.4. In the GGE biplot analysis, IPCA1 explained 49.2% of GxE variation, while IPCA2 contained 19.1% of the GxE variation. The total GxE variation explained by the GGE biplot was 68.3%. The average-environment axis (AEA), which is the single-arrowed line, points to higher average grain yield; while the double arrowed line (AEC: average-environment coordinate)

points to greater variability (smaller stability) in either direction (Jandong et al., 2011). Hybrid H36 (cross of L3 x L6) recorded the highest average grain yield followed by hybrid H68, while hybrid H69 had the lowest mean grain yield (Figure 6.3). The results also indicated that the highest yielding H36 had a short AEC value implying that it is stable across drought, low N and optimum conditions.



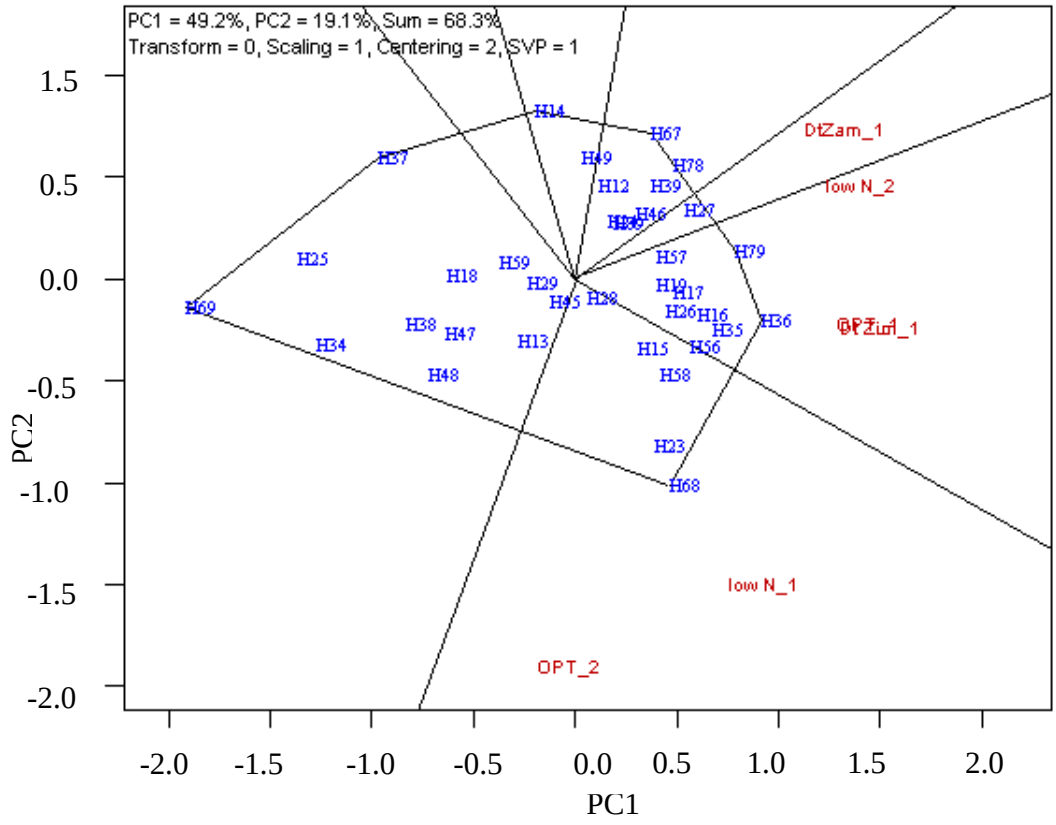
The average tester coordination for entry evaluation

Figure 6.3 Ranking of the *Fbr1* maize hybrids based on both mean yield performance and stability## refers to the hybrids, where for example H25 is the hybrid of lines L2 and L5 (Table 6.1). Environment_#, for example, low N_1 is low N environment in year 1.

Although hybrid H68 had the highest mean yield, it had a long AEC, indicating that it was less stable across all environments. H68 was, however, specifically adapted to low N and optimum conditions. Hybrids H78, H67, H14, H48 and H34 had long AEC, thus were unstable across all

environments. The lowest yielding hybrid, H69 also had the longest AEC indicating that was unstable and as a result, was the most undesirable of all the *Fbr1* hybrids evaluated.

Plotting genotypes against environments revealed environment(s) where each genotype was best suited (Figure 6.4). The biplot divided the four environments x two years, into seven sectors. In the polygon view of grain yield performance, the winning genotypes for each environment are located on the vertex of the polygon.



Which wins where or which is best for what

Figure 6.4 The polygon view of the GxE biplot showing the grain yield performance of maize hybrids in each environment and year (the "which wins where" concept). H## refers to the hybrids, where for example H25 is the hybrid of lines L2 and L5 (Table 6.1). Environment_#, for example low N_1 is low N environment in year 1.

Hybrid H36 was the vertex genotype followed by hybrid H79, under low N_2, drought-Zimbabwe, and optimum_1 conditions. Hybrid H27 produced the highest grain yield under drought-Zambia, and hybrid H68 produced the highest yield under low N_1 and optimum_2 while hybrid H69 followed by H37 were the poorest performers across all the environments evaluated. Hybrid H36 was, to the approximation of the biplot, the best hybrid followed by H79 for drought, low N and optimum environments. The two hybrids were close to the ideal genotype described by Yan and Tinker (2006) because they had high mean yields (large IPCA1) and were stable across environments (IPCA2 near zero).

6.5 Conclusions and recommendations

In breeding programmes, genotypes are tested in a number of environments and thus environmental variations are important in determining yield performance and adaptation, thus, genotype evaluation based on several years and locations is strategic in breeding programmes. There is huge climatic variation across sub-Saharan Africa and drought occurs frequently. As such, given the expanse of the region, it would be best to develop cultivars with specific adaptation to specific environments and those with broad adaptation. Stability analysis can help to characterise the response of lines and hybrid cultivars to changing environments and to determine the best locations representative of the environmental diversity in major maize growing regions. Stability analysis for genotypes in this study identified *Fbr1* lines and hybrids with general and specific adaptation to particular environments. Results of this study showed that both yield and stability should be considered simultaneously to exploit the useful effect of GxE interaction and to make selection of the genotypes more effective. The results also demonstrated that biplots are useful tools for understanding complex agronomic data. The AMMI1 biplots allowed visual assessment of the genotypes and the environment main effects. These biplots also displayed the GxE interactions, but GGE biplots were more effective in revealing the relationship among genotypes in terms of their responses to the environment, and the crossover treatment x environment interactions.

The AMMI and GGE biplots similarly ranked hybrid H36 as the ideal genotype while hybrid H69 was classified as the poorest performer. These two classification models could be used simultaneously to make selection of genotypes more precise and refined. Various methods exist for statistical analyses of GxE interaction data and one should use different approaches to effectively interpret the results.

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Chapter 7

SNP-based genetic diversity among few-branched-1 (*Fbr1*) maize lines and its relationship with heterosis, combining ability and grain yield of testcross hybrids

7.1 Abstract

SNP markers are regarded as efficient, compared with other marker types, in genetic characterisation of maize (*Zea mays* L.) germplasm because of their vast coverage of the maize genome. The objectives of this study were to determine SNP-based genetic distance among *Fbr1* maize lines and to find association of these distances with SCA, mid-parent heterosis (MPH), high-parent heterosis (HPH) and mean grain yield of the hybrids. Twenty six CIMMYT maize lines (12 with the *Fbr1* gene, and 14 normal-tasselled) were genotyped using 1051 SNP marker loci. Fifteen of these lines were used in two separate diallel mating designs: a 9x9 and 6x6 crossing set-up, to make hybrids for yield evaluation. Average residual heterozygosity of SNP loci ranged from 0-32%, with an average of 8.65%. The polymorphic information content (PIC) for the SNP loci ranged from 0-0.38 with an average of 0.25. Mean genetic distance for all pair wise comparisons of lines was lower (0.30) suggesting a high level of relatedness among lines. SNP-based genetic distances were effective in grouping CIMMYT maize lines into predefined heterotic groups. Marker-based genetic distances were positively correlated with hybrid performance, SCA and heterosis indicating that they could accurately predict hybrid performance in this set of germplasm. Grain yield for the hybrids ranged from 0.49-2.48 kg/plot, with an average of 1.80 kg/plot. Hybrids constituted of closely related parental lines (according to SNP-based genetic distances) had the lowest mean grain yield, lowest SCA effects for grain yield, and had the lowest heterosis values. Thus, SNP-based genetic distance information would be useful for effective selection by avoiding genetically similar lines when selecting parents for breeding programmes that require genetically diverse lines as parents.

7.2 Introduction

The identification of parental inbred lines that form superior hybrids is the most costly and time-consuming phase in maize breeding (Betrán et al., 2003). *Per se* performance of maize inbred lines does not predict the performance of maize hybrids for grain yield (Hallauer and Miranda, 1988), thus, predictors of single-cross hybrid value or heterosis between parental inbred lines could therefore increase the efficiency of hybrid breeding programmes. The level of genetic

variation between two inbred lines has an influence on the general performance or heterosis in the resulting hybrid (Hinze and Lamkey, 2003). Hence, molecular markers which reflect such genetic variation can hasten the selection of parental inbred lines (Qi et al., 2010). Previous methods have included diallel crossing, multivariate analyses (Aydin et al., 2007) and several studies have shown that a multifaceted approach which includes morphological, biochemical and intense molecular trait evaluation of candidate inbred lines can be more reliable in heterotic breeding (Rencher, 1995).

The pre-selection of parents is an essential step in the prediction of hybrid performance (Munhoz et al., 2009). The traditionally applied methodology for this purpose is the formation of heterotic groups, based on the evaluation of the pedigree data and its relation with the heterosis values based on morphological traits of interest (Franco et al., 2001; Mohammadi and Prasanna, 2003; Miranda et al., 2008). Molecular markers have been used to detect the variation in the DNA sequence underlying the analysis of existing genetic dissimilarity of the parents (Munhoz et al., 2009), and markers have the advantage of simplifying the screening of parents, which is done through DNA evaluation (Mohammadi and Prasanna, 2003; Crossa and Franco, 2004; Legesse et al., 2008; Balestre et al., 2008; Dandolini et al., 2008; Silva et al., 2009). Several molecular marker platforms have been employed in analysing genetic diversity, quantitative trait loci (QTL) identification and in predicting heterosis in maize, although results on the latter aspect have been inconsistent (Smith et al., 1997; Ajmone-Marsan et al., 1998; Pejic et al., 1998; Melchinger, 1999; Phumichai et al., 2008; Dhliwayo et al., 2009). This inconsistency might have been due to differences in approach when dealing with QTLs, which do not normally follow the Mendelian pattern of inheritance (Qi et al., 2010).

The relationship between genetic distance and heterosis was reported before the development of genetic markers (Moll et al., 1965). The theory of quantitative genetics describes a correlation between parental divergence and the heterosis estimates (Falconer and Mackay, 1996). Thus, heterosis is a function of the square of the differences between the allele frequencies in the parents, that is, the genetic divergence and also the dominance effect of the alleles controlling the traits in question (Falconer, 1981). However, for maize, the results available for use of molecular markers to predict heterosis cannot be considered conclusive. Dudley et al. (1991) found no significant correlation between these variables in maize. Lanza et al. (1997) obtained a significant correlation between grain yield data and random amplified polymorphic DNA

(RAPD) based genetic distances. Amorim et al. (2006) found high correlation between grain yield and genetic divergence for interpopulation hybrids, but this correlation was low for intrapopulation hybrids, showing that markers would be efficient in predicting hybrids derived from different heterotic groups. However, Melchinger (1999) found that when genetic distance was used to predict hybrid performance, the efficiency of prediction was greater with crosses between inbred lines from the same heterotic group than in crosses between inbred lines from different heterotic groups.

Molecular markers have been used to analyse the genetic relationships among maize inbred lines and to examine the relationship between DNA marker-based genetic distance and single-cross grain yields in temperate maize (Stuber, 1989; Lee et al., 1989; Smith et al., 1990; Godshalk et al., 1990; Boppenmaier et al., 1992; Melchinger, 1993). Linkage disequilibrium between DNA markers and genes involved in the expression of target traits is required for genetic distance and hybrid performance to be correlated (Betrán et al., 2003). Charcosset and Essioux (1994) described a lack of correlation between heterosis and heterozygosity of marker loci as explained by differences in linkage disequilibrium among markers and QTLs between heterotic groups.

The *Fbr1* tassel trait is a new trait that has been introduced into CIMMYT elite germplasm, and there is great potential of using lines with the *Fbr1* trait in breeding programmes. Allele-based estimates of genetic distances between lines by use of molecular markers will allow the substitution of heterotic grouping based on phenotypic divergence of lines, and will facilitate early identification of contrasting parents for making crosses, hence reducing time required to conclude breeding programmes.

The objectives of this study were to determine SNP-based genetic distance estimates among *Fbr1* maize lines and to find correlation of genetic distance with SCA, heterosis and grain yield of the hybrids.

7.3 Materials and methods

7.3.1 Germplasm for SNP and diallel analyses

Twenty six CIMMYT maize inbred lines adapted to the mid-altitude, tropical and/or subtropical environments of southern Africa were used in this study: 12 are *Fbr1* and 14 have normal tassels

(Table 7.1). The *Fbr1* genotypes were produced after the tassel mutation was introduced from a Mexican donor line into CIMMYT elite maize lines by backcrossing.

Table 7.1 The CIMMYT maize inbred lines characterised by the 1051 known SNP markers

Line	Code	Pedigree	Heterotic group
L1	CML443 TAS2	[CML443/TAS]BC2-2-9-1-2-B	A/B
L2	CML444 TAS3	[CML444/TAS]BC2-5Y-3-1-B	B
L3	CML488 TAS	[CML488/TAS]BC2-6-4-2-B	A/B
L4	CML443	CML443	A/B
L5	CML444	CML444	B
L6	CML488	CML488	A/B
L7	CML395 TAS	[[CML395/TAS]BC2/[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-B]-8-2-2-B	B
L8	CML443TAS1	[CML443/TAS]BC2-2-5-3-1-B	A/B
L9	CML444 TAS1	[[CML444/TAS]BC1/[CML444/CML395//DTPWC8F31-4-2-1-6]-2-1-1-1-B]-9-3-4-B	B
L10	CML445 TAS1	[[CML445/TAS]BC3/[CML445/ZM621B]-2-1-2-3-1-B]-2-4-2-B	A/B
L11	CML445 TAS2	[CML445/TAS]BC3-1-1-2-1-B	A/B
L12	CML312 TAS	[[CML312/TAS]BC1/MAS[MSR/312]-117-2-2-1-B]-1-3-1-B	A
L13	CML444 TAS2	[CML444/TAS]BC2-6-1-1-B	B
L14	CML442 TAS	[[CML442/TAS]BC1/ZM621A-10-1-1-1-2-BBBBBB]-2-1-B	A
L15	CML445 TAS3	[CML445/TAS]BC3-1-1-2-2-B	A/B
L16	CML445	CML445	A/B
L17	CML395	CML395	B
L18	CML312	CML312	A
L19	CML442	CML442	A
L20	LaPostaSeqC7-F180	LaPostaSeqC7-F180	B
L21	LaPostaSeqC7-F18	LaPostaSeqC7-F18	B
L22	CKL05005	CKL05005	B
L23	G16BNSeqC4	G16BNSeqC4	A
L24	LaPostaSeqC7-F71	LaPostaSeqC7-F71	B
L25	CKL05003	CKL05003	B
L26	CML144	CML144	A

7.3.1.1 SNP genotyping of maize lines

The maize inbred lines were advanced by selfing during the 2009/2010 summer season at CIMMYT –Harare research station. After harvesting, 34 seeds per inbred line were packed in envelops and shipped to BecA hub, Kenya, for the molecular marker analysis.

7.3.1.1.1 DNA extraction and SNP genotyping

Details for DNA extraction and SNP genotyping are as explained in materials and methods in Chapter 3, section 3.3.2.1 and 3.3.2.2.

Generally, SNP genotyping and allele calling was made by KBiosciences (http://www.KBioscience.co.uk) [2010, November 30] using the KASPar system as described in the user's manual (http://www.kbioscience.co.uk/reagents/KASParSNP Genotyping System Leafletv6.3.pdf) [2010, November 30].

The design for the KASPar was achieved using the PrimerPicker software found at http://www.kbioscience.co.uk/primer-picker/ [2010, November 30].

7.3.1.1.2 Screening for SNP data

Screening for the SNP markers was the same as described in Chapter 3 section 3.3.3 under materials and methods, except that 1051 SNP loci were polymorphic and acceptably heterozygous, thus were used for final assaying of the maize lines.

7.3.1.2 Diallel analysis and field data collection.

The number of lines that are included in a diallel cross are usually at most 10, since the number of hybrids become larger with increase in number of parental lines included. In this regards, two half diallel crosses were done at Muzarabani (Zimbabwe) to make ($n(n-1)/2$) F_1 crosses (Griffing, 1956), that were evaluated in trials (2010 and 2011) under optimum, low N and drought conditions. The first half diallel crossing set-up involved inbred lines L1-L6 (Table 7.1), and produced 15 F_1 hybrids that were evaluated in yield trials in 2010 and 2011. The second half diallel crossing set-up involved nine inbred lines: L7-L14 and L3, and produced 36 F_1 hybrids that were, similarly, evaluated for grain yield in 2010 and 2011 under optimum, low N and drought conditions

7.3.1.2.1 Field evaluation procedures

Details on agronomic management, environments and stress management of trials are given in Chapter 4; section 4.3.2 and details on experimental design and data collection are explained in section 4.3.3.

In this case, four sets of trials, the six inbred parents and the 15 hybrids plus five hybrid checks; the nine inbred lines and 36 hybrids plus four hybrid checks, were grown adjacent to each other in three environments in Zimbabwe during 2010 and 2011. The experimental design was an alpha lattice (0,1) (Patterson et al., 1978) with two replications for hybrids and inbreds in each environment. The 15 crosses plus five hybrid checks were grown using one-row plots, two

replications and 4 x 5 incomplete lattice designs in all the three environments in 2010 and 2011, while the 36 hybrids plus six hybrid checks for the second trial were laid out in a 6 x 7 alpha lattice design, for grain yield evaluation. Two trials of inbred parents (for the two hybrid trials) were grown side by side with the hybrids to facilitate estimation of heterosis. Plot size at all locations was a single 4 m row with 0.75 m between rows and 0.25 m between plants within a row, giving final plant populations of ≈ 53 000 plants per hectare at all sites. Grain yield (adjusted to 12.5% moisture content) was obtained considering harvested plot area and counting number of plants and harvested ears per plot.

7.3.2 SNP data analysis

Summary statistics of genetic data such as minor allele frequencies, PIC, heterozygosity and number of alleles were computed with Powermarker version 3.25 (Liu and Muse, 2005). Roger's modified genetic distance (MRD) indicating genetic dissimilarity, (Wright, 1978; Goodman and Stuber, 1983) between each pair of inbred lines was computed using the formula in section 3.3.4; in materials and methods of Chapter 3.

The PIC for each locus was determined as described by Smith et al. (1997) while genetic relationship among inbred lines was assessed using cluster analysis performed on the MRD distance matrix with UPGMA clustering.

7.3.3 Statistical analysis

Individual analyses of variance were performed for each experiment with the general linear model procedure (PROC GLM) from SAS (SAS, 2003). The adjusted means were used to make subsequent calculations to estimate SCA. SCA was estimated using the Line x tester analysis programme in SAS (SAS, 2003). The fixed-effects model of diallel method 4 was used in the analysis and provided estimates of SCA effects for the hybrids across all environments. Midparent heterosis was calculated as:

$$\text{MPH} = \frac{\text{F1}-\text{MP}}{\text{MP}} \text{ x } 100$$

where, F_1 is the mean of the F_1 hybrid performance and $MP = (P_1 + P_2)/2$ in which P_1 and P_2 are the means of the inbred parents, respectively.

High-parent heterosis was calculated as:

$$HPH = \frac{F1-HP}{HP} \times 100$$

where HP is the mean of the best parent.

Pearson correlation coefficient (r) between genetic distance (GD) and single cross grain yield (F_1), MP, HP, MPH, HPH, and SCA were calculated from the means across environments. Statistical computations were performed with SAS statistical package (SAS, 2003). Broad sense heritability for grain yield for the hybrid sets was estimated using the formula (1- $1/F_{value}$). The F value was computed in the ANOVA across sites and years.

7.4 Results and discussion

7.4.1 Genetic analysis of maize lines and hybrids

The analysis of variance for grain yield of inbred lines and hybrids showed that genotypic and environmental variations were highly significant ($P \leq 0.001$) for both hybrids and parental inbred lines (Table 7.2 and 7.3). GCA_{female} and GCA_{male} were significant for the two sets of hybrids while SCA was also highly significant in the two hybrid sets. SCA ranged from -0.75 (hybrid 3x6 i.e. CML488TAS x CML488) to 0.507 (hybrid 9x11 i.e. CML444TAS1 x CML445TAS2).

Table 7.2 Combined analysis of variance across sites and years for grain yield for the two sets of hybrids formed from the two diallel mating designs

	Hybrid set 1		Hybrid set 2	
Source of variation	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Environment	2	214.38***	2	69.023***
Rep (Env)	3	0.59	3	0.38
Entry	34	1.15***	14	2.32***
GCA_{female}	7	0.82*	4	2.44***
GCA_{male}	7	1.86***	5	2.34***
SCA	20	1.06***	5	2.19***
Entry x Env	68	0.45	28	0.53***
GCA_{female} x Env	14	0.35	8	0.39
GCA_{male} x Env	14	0.59	10	0.78***
SCA x Env	40	0.41	10	0.38
Error	319	0.39	120	0.21

* $P \leq 0.05$, *** $P \leq 0.001$

Table 7.3 Combined analysis of variance across site and years for grain yield for the two sets of parental inbred lines used in F_1 hybrid formation

	Inbred line set 1		Inbred line set 2	
Source of variation	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Replication	1	0.071	1	0.11*
Entry	5	0.68***	8	0.51***
Environment	2	4.61***	2	9.69***
Year	1	0.72**	1	1.37***
Entry x Env	10	0.14	16	0.16***
Entry x Year	5	0.18	8	0.14***
Entry x Env x Year	12	0.19*	18	0.31***
Error	34	0.089	52	0.027

* $P \leq 0.05$, ** $P \leq 0.05$, *** $P \leq 0.001$

The reason why hybrid CML488TAS x CML488 was the poorest in yield performance is that both parents constituted the same line (CML488), the difference being that, one of the parental lines has the small tassel (*Fbr1*) gene, while the other parent is normal tasselled. Inbreeding depression may be the major cause of the serious yield reduction. Most hybrids that had high and positive SCA values for grain yield also had high mean grain yield and fall in complementary heterotic groups, for example hybrids 9x11, 1x6, 5x6 and 8x13 had high mean grain yield and positive SCA for grain yield. Lines that constituted these hybrids fall in complementary heterotic groups: A/B and B (Table 7.1). Thus, CIMMYT's predefined heterotic grouping of lines consistently predicts the performance of hybrids, suggesting that these heterotic groups were well defined.

7.4.2 Genetic diversity

7.4.2.1 Polymorphism of SNP markers

A total of 1051 out of the 1250 known SNPs that were called in the maize inbred lines that returned quality data, were polymorphic in all the lines, and had acceptable heterozygosity that made them fit for analysis of data. Average residual heterozygosity ranged from 0 to 32%, with an average of 8.65%, which is however, well above the expected ranges for residual heterozygosity found in maize inbred lines. Yan et al. (2009) found heterozygosity ranging from 0 to 9.9%, with an average of 2.5%, which they reported as within expected ranges. Xia et al. (2004) also found an average residual heterozygosity of 4.8% among CIMMYT maize inbred

lines investigated with SSR markers, which were in accordance with results reported by Heckenberger et al. (2002).

The PIC values for the 1051 SNP loci ranged from 0 to 0.38, with an average of 0.25. Thus, the SNP loci were informative and were able to detect differences among inbred lines based on their genetic relationships. The average PIC value was however lower than that reported previously for tropical and temperate maize lines (Dhliwayo et al., 2009; Betrán et al. 2003; Xia et al., 2004 ; Senior et al., 1998; Barata and Carena, 2006). There was therefore, relatively little genetic diversity among the germplasm used in this study, which is an indication that most of the inbred lines evaluated were close to fixation. The average inbreeding coefficient of 0.73 for the SNP loci further confirmed the fixation of the maize lines. Since the aim of this study was to identify normal and *Fbr1*-converted lines that are homozygous, and that can be used as parents in breeding programmes involving the *Fbr1* tassel mutation, these homozygous lines are useful in making crosses for test cross evaluations and in making mapping populations in planned marker assisted breeding.

7.4.2.2 Genetic distance among inbred lines and cluster analysis based on the SNP markers.
Most markers detected at least one allele for each of the inbred lines characterised; thus, all loci and individuals were included in the analysis. The mean genetic distance for all pair wise comparisons was 0.30, which is lower than that reported in previous studies for tropical germplasm (Xia et al., 2004; 2005), and that reported among elite CIMMYT and IITA tropical maize inbred lines (Dhliwayo et al., 2009). Reif et al. (2003), investigating the diversity among seven of CIMMYT's tropical maize populations with molecular markers, also identified low variance between populations. The lower average MRD suggests a high average degree of relatedness among the CIMMYT maize lines. Genetic distance ranged from MRD of 0.02 (between LaPosta SeqC7-F71 and LaPosta SeqC7-F18) to 0.39 (between La PostaSeqC7-F180 and CML312/CML445/TAS). UPGMA clustering showed two major clusters (Figure 7.1). One cluster (purple-coloured group) consisted of inbred lines in heterotic group B and A/B, while the other cluster (green-coloured group) consisted of inbred lines in heterotic group A and A/B. Hence, the maize lines were clustered according to heterotic grouping. This showed the efficiency of the SNP markers in characterising the inbred lines, thus placing them in their respective heterotic groups. The tight clustering of CML312/TAS and CML442/TAS was

surprising (4% dissimilar) since the lines are genetically different and do not have the same ancestry.

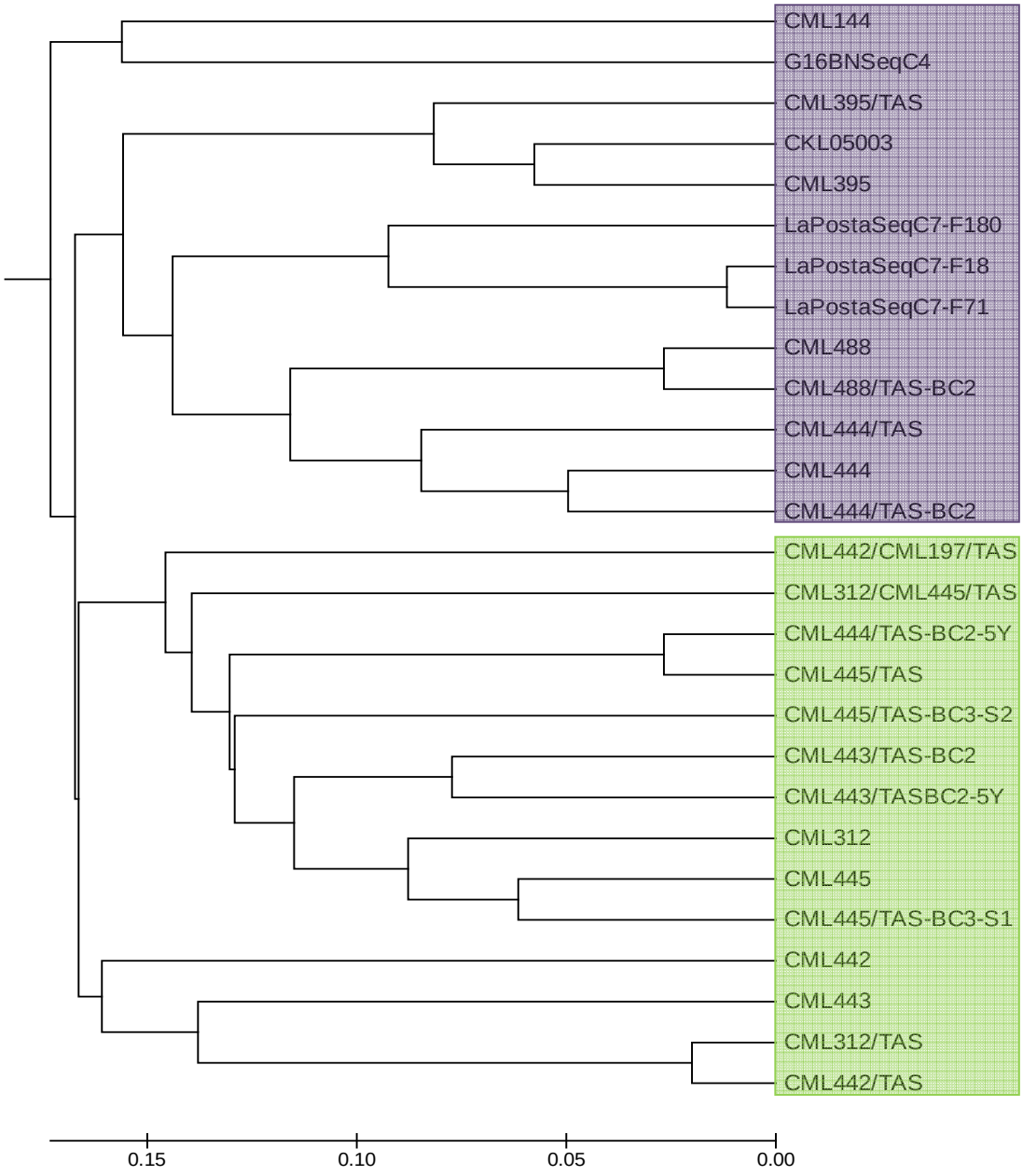

Figure 7.1 Dendrogram constructed using unweighted pair group method with arithmetic mean clustering of maize inbred lines from CIMMYT based on 1051 SNPs. The scale bar on the axis is expressed in Modified Roger's distance (MRD) (Wright, 1978; Goodman and Stuber, 1983)which shows percentage dissimilarity between or among genotypes.

Genetic distances of the inbred lines that constituted the F_1 hybrids in this study are presented separately in Table 7.4. Genetic distance of the parental lines ranged from 0.04 in the combination CML442/TAS x CML312/TAS to 0.3657 in the combination CML445/TAS x CML442/TAS with an average value of 0.30, demonstrating a lower range of genetic variation in this set of inbred lines.

Table 7.4 Modified Roger's distance (MRD) based on the 1051 SNP loci, for the maize inbred lines constituting the F_1 hybrids

Inbred line	L12	L7	L14	L4	L1	L8	L5	L9	L13	L2	L10	L11	L6	L3
CML312/TAS (L12)	0.0000													
CML395/TAS (L7)	0.3398	0.0000												
CML442/TAS (L14)	0.0400	0.3521	0.0000											
CML443 (L4)	0.2806	0.3114	0.2706	0.0000										
CML443/TAS-BC2 (L1)	0.3390	0.3387	0.3322	0.2480	0.0000									
CML443/TASBC2-5Y(L8)	0.3574	0.3363	0.3526	0.2475	0.1545	0.0000								
CML444 (L5)	0.3053	0.2555	0.3111	0.2719	0.3000	0.3091	0.0000							
CML444/TAS(L9)	0.3525	0.2655	0.3612	0.3360	0.3500	0.3516	0.1378	0.0000						
CML444/TAS-BC2(L13)	0.3454	0.3192	0.3526	0.3386	0.3493	0.3503	0.0991	0.2005	0.0000					
CML444/TAS-BC2-5Y (L2)	0.3553	0.3414	0.3597	0.3261	0.2889	0.3061	0.2789	0.3407	0.3322	0.0000				
CML445/TAS(L10)	0.3571	0.3478	0.3657	0.3433	0.2985	0.3023	0.2959	0.3444	0.3351	0.0534	0.0000			
CML445/TAS-BC3-S1(L11)	0.3578	0.3465	0.3487	0.2637	0.1594	0.1886	0.2870	0.3443	0.3419	0.2282	0.2503	0.0000		
CML488(L6)	0.3453	0.3000	0.3509	0.3439	0.3325	0.3469	0.0993	0.2439	0.3306	0.3224	0.3351	0.3335	0.0000	
CML488/TAS-BC2(L3)	0.3468	0.3099	0.3509	0.3436	0.3317	0.3514	0.1369	0.2527	0.3271	0.3328	0.3489	0.3345	0.0534	0.0000

7.4.3 Correlation of genetic distance with hybrid performance and heterosis

Estimates of grain yield, mid-parent heterosis (MPH), high-parent heterosis (HPH) and specific combining ability (SCA), for the maize hybrids are presented in Table 7.5.

Table 7.5 Yield of the *Fbr1* maize hybrids in relation to mid-parent heterosis (MPH), high-parent heterosis (HPH) and specific combining ability (SCA)

Hybrid[†]	Yield (kg/plot)	MPH (%)	HPH(%)	SCA	Hybrid	Yield (kg/plot)	MPH(%)	HPH(%)	SCA
1x2	1.54	47.30	20.50	0.037	7x10	1.41	73.09	10.26	-0.106
1x3	1.83	157.07	43.86	0.077	7x11	1.90	141.02	48.71	0.164
1x4	1.87	143.97	46.98	-0.044	7x12	2.16	167.87	69.38	0.095
1x5	1.49	63.74	16.65	0.073	7x13	1.96	131.19	53.69	0.193
1x6	1.95	147.58	53.02	0.348	7x14	1.80	153.93	40.93	0.088
2x3	1.78	88.31	39.64	-0.267	8x10	1.72	133.73	34.79	0.158
2x4	2.19	119.22	71.95	-0.007	8x11	1.21	70.69	-4.98	-0.568
2x5	2.46	115.44	92.67	-0.102	8x12	1.92	163.99	51.00	-0.188
2x6	1.95	91.40	53.11	0.033	8x13	1.99	158.63	56.32	0.320
3x4	1.79	166.52	40.18	0.263	8x14	1.70	169.70	33.41	-0.056
3x5	2.01	148.24	57.85	0.119	9x10	1.38	63.57	8.43	-0.005
3x6	0.49	-28.97	-61.53	-0.748	9x11	2.11	157.96	65.85	0.507
4x5	2.48	186.40	94.38	-0.139	9x12	2.26	169.00	77.06	0.265
4x6	1.83	145.13	43.25	-0.124	9x13	1.11	26.22	-12.82	-0.530
5x6	2.09	135.68	63.63	0.480	9x14	1.84	148.98	44.63	0.202
7x3	1.59	100.58	24.45	-0.220	10x11	1.67	69.60	30.80	0.025
7x8	1.87	247.85	46.97	-0.009	10x12	1.84	83.51	44.33	-0.135
8x3	1.84	157.68	44.33	-0.015	10x13	1.69	62.35	32.95	0.018
8x9	2.41	322.52	89.46	-0.058	10x14	1.69	87.47	32.95	0.009
9x3	1.47	78.58	15.43	-0.211	11x12	2.16	120.97	69.38	-0.062
10x3	1.69	70.70	32.23	-0.032	11x13	2.02	98.41	58.52	0.098
11x3	1.90	98.02	49.43	-0.059	11x14	1.72	95.57	34.79	-0.147
12x3	2.10	113.79	64.61	0.490	12x13	1.68	62.16	32.04	0.116
13x3	1.76	72.46	38.38	-0.027	12x14	1.00	11.77	-21.26	-0.506
3x14	1.80	103.95	41.26	0.086	13x14	2.06	119.46	61.73	0.097

[†] The pedigree information of the lines used to make the hybrids is shown in Table 7.1.

Grain yield ranged from 0.49 to 2.48 kg/plot for the hybrids: CML488 x CML488/TAS-BC2 and CML443 x CML444 respectively, with average grain yield of 1.80 kg/plot across all hybrid sets. The highest MPH (323%) was recorded for the hybrid 8x9, i.e. CML444/TAS1 x CML443/TAS1, while the lowest MPH (-28.97%) was detected in the combination CML488 x CML488/TAS, which are sister lines. It is worth noting that this particular hybrid also recorded the lowest mean grain yield, and had the smallest SCA for grain yield. HPH recorded an average of 41.51% in the maize hybrids, with hybrid CML488 x CML488/TAS similarly recording the

lowest HPH value of -61.53%, while CML443 x CML444 had the highest HPH of 94.38%. Betrán et al. (2003) also recorded the lowest SCA effects for grain yield for hybrids between sister lines LP4 and LP5. They found that SCA across environments was generally negative for hybrids involving inbred lines with the same germplasm origin or related by pedigree, and was greater for hybrids involving inbred lines of different source germplasm origin. Sister lines or lines related by pedigree lack the interaction of the genes, in favour of cumulative dominant alleles, which are useful in the expression of heterosis in F_1 hybrid combination (Qi et al., 2010).

Grain yield for hybrids across environments was positively correlated with MPH, HPH, SCA and genetic distance and the correlation coefficients were highly significant ($P \leq 0.001$, Table 7.6). The highest correlation was observed between grain yield and HPH. The correlation between grain yield and MPH was also relatively high (r = 0.73***), indicating that heterosis can predict hybrid performance better than SCA among parental lines or molecular marker-based genetic distance. On the contrary, Betrán et al. (2003) found that SCA among lines was highly correlated with grain yield across stress and non-stress environments and justified prediction of hybrid performance based on SCA. They argued that heterosis is highly dependent on the performance of inbred lines, and there is differential response of inbred lines to stresses and environmental conditions relative to hybrids, rendering predictions based on heterosis erratic and inconsistent across environments. SCA was positively correlated with MPH (r = 0.46***) and HPH (r = 0.63***) across environments.

Table 7.6 Correlation coefficients between molecular-based genetic distance (MRD), grain yield, mid-parent heterosis (MPH), high-parent heterosis (HPH), and specific combining ability (SCA)

Crosses	Grain Yield (kg/plot)	MPH (%)	HPH (%)	SCA
MPH	0.73***			
HPH	0.99***	0.73***		
SCA	0.63***	0.46***	0.63***	
MRD	0.50***	0.42*	0.50***	0.45**

* $P \leq 0.05$, ** $P \leq 0.05$, *** $P \leq 0.001$

Parental genetic distance was positively correlated with grain yield, heterosis (MPH and HPH), and SCA. Mladenovic-Drinic et al. (2002) also found positive correlation between genetic

distance and these parameters. Ajmone-Marsan et al. (1998) also obtained highly significant but modest estimates of correlation coefficients between genetic distance and yield within a set of 78 maize hybrids studied, for the two classes of molecular markers, RFLP and AFLP. The correlation coefficient of genetic distance and SCA was moderate and significant (r = 0.45***). Previous experiments with diallel crosses indicated correlation between genetic distance and SCA for grain yield ranging from very low (Dudley et al., 1991), medium (Melchinger et al., 1990, 1992), to rarely, very high (Lee et al., 1989).

The correlation between MPH, and HPH for grain yield with genetic distance were positive and moderate (r = 0.42* and r = 0.50*** respectively). Thus, genetic distance of parental lines, to some extent, determines hybrid vigour expected in hybrid progeny. Boppenmaier et al. (1992), Dhillon et al. (1993) and Ajmone-Marsan et al. (1998) found relatively low values of correlation coefficients between genetic distance and heterosis. Betrán et al. (1997) studied germplasm of tropical white maize using RFLP markers and obtained low values of correlation coefficients between genetic distance and SCA, and between genetic distance with grain yield and heterosis. On the other hand, Smith et al. (1990) obtained very high correlation (r = 0.87) between RFLP-based genetic distance and heterosis in crosses of inbred lines from the same and different heterotic groups.

Dhliwayo et al. (2009), however, found no significant association of genetic distance with grain yield, MPH and SCA. Regarding RAPD markers, Rinaldi et al. (2007) also did not infer a significant correlation between heterosis and productivity in Brazilian popcorn populations. Bernardo (1992) and Melchinger (1999) summarised some theoretical considerations that often lead to poor predictive value of genetic distance for hybrid performance; these include the small role of dominance gene action, low heritability of the trait (as is the case for grain yield), and few trait-relevant QTL linked to the molecular markers. In this study, SCA effects for grain yield were mostly significant (Table 7.2), thus dominance gene action played a major role in determining yield of hybrids and broad sense heritability for grain yield as determined by the formula ($1- 1/F_{value}$) was relatively high (an average broad sense heritability for the two hybrid sets was H = 0.79). Thus, according to Bernardo (1992) and Melchinger (1999), high predictive value of genetic distance for hybrid performance was expected.

Lack or low correlation of genetic distance with hybrid performance, heterosis, and SCA was also suggested to be a result of lack of linkage between genes controlling the traits under analysis, unequal or insufficient genome coverage, random marker distribution and diversified effect of dominance (Melchinger, 1990; Charcosset et al., 1991; Kwon et al., 2002). Prediction of heterosis based on marker loci would therefore, be more efficient if the markers are selected *a priori*, for their relationship to the alleles implicated in the heterotic traits.

According to Mladenovic-Drinic et al. (2002), the absence of linkage between molecular markers used to estimate divergence and the genes controlling heterosis for the studied traits could explain low correlation observed between heterosis and genetic distance. Therefore, markers must be in linkage disequilibrium with QTLs to have a predictive value. Charcosset and Essioux (1994) suggested that necessary conditions for prediction efficiency should be fulfilled at the within-group level and at a general level. Linkage disequilibria between markers and QTLs generally differ randomly from one heterotic group to another, thus genetic distance based on neutral marker loci will not be predictive for the performance of between-groups hybrids.

7.5 Conclusions and recommendations

The 1051 SNP marker loci used to characterise the maize parental lines indicated that the mean genetic distance for all pairwise comparisons of lines was low (0.30) suggesting a high level of relatedness among lines. Inbred lines can therefore be isolated from this germplasm for future breeding work involving the *Fbr1* tassel mutation. Grain yield for the hybrids ranged from 0.49-2.48 kg/plot, with an average of 1.80 kg/plot and hybrids constituted of closely related parental lines (according to SNP-based genetic distances) had the lowest mean grain yield, lowest SCA effects for grain yield, and low heterosis. Although determination of the genetic basis of hybrid performance and measuring the relationship between marker-based genetic distance and complex agronomic traits like yield are reported to be quite complex, significant and positive correlations of genetic distance with grain yield, heterosis and SCA were found in this study. Thus, SNP-based genetic distances could be used as efficient predictors of hybrid performance in this maize germplasm. Results of this study suggest that SNP-based genetic distance information would aid in the selection of genetically wide lines to include in breeding programmes where inclusion of diverse lines as parents is critical, for example, in synthetic variety formation. Although few breeding programmes rely less on recurrent selection schemes, DNA-based genetic distance could be useful in guiding the introgresion of exotic germplasm into existing local heterotic

germplasm, or in initial grouping of uncharacterised germplasm. Our results showed that SNP-based genetic distances were effective in grouping CIMMYT maize lines into predefined heterotic groups. However, it would also be important to test the utility of these SNP-based genetic distances for selecting lines for use in formation of synthetic varieties or in defining a new pair of complementary heterotic populations for subsequent exploitation.

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Chapter 8

General conclusions and recommendations

This study was conducted to investigate the value of the *Fbr1* tassel mutation in maize breeding programmes targeting stress environments. The tassel mutation is a novel trait that was introduced into CIMMYT maize germplasm and information had to been gathered to develop recommendations for breeders on whether to incorporate the trait into their maize breeding germplasm or not. Relatedness and homozygosity levels of the *Fbr1* maize lines were assessed using SNP markers. The information would be important for future use in CIMMYT breeding programmes and in assessing the efficiency of the backcross procedures done to convert the elite normal tasselled lines to *Fbr1*. Yield performance and stability of the *Fbr1* lines and hybrids under abiotic stress environments were evaluated since, during the breeding process, yield and stability in performance are handed as one complex and accumulation of environmentally stable yield genes equates with better performance under stress. The evaluation of GxE interaction showed significant variation in stability of *Fbr1* lines and hybrids as measured by mean yield and ASV. The AMMI and the GGE biplots ranked the best and poorest genotypes similarly in terms of yield performance and stability.Thus, the *Fbr1* hybrids that were identified as high yielding and stable could be used for additional exploitation in CIMMYT breeding programmes aimed at increasing maize grain yield.

The *Fbr1* tassel mutation did not have a positive effect on grain yield under abiotic stress conditions and *Fbr1* x *Fbr1* hybrids had lower grain and pollen yield, and were less adapted to abiotic stress conditions. This could raise questions on the validity of incorporating such a trait in breeding programmes targeting stress tolerance.The reason why the *Fbr1* mutation did not have any positive effect on grain yield could be that grain yield is a complex trait conditioned by the interaction of various growth and physiological processes within the plant. The effects of many factors additively contribute to increased grain yield and considering only one trait (tassel size) may not cause significant yield improvement. The appropriate knowledge of interrelationships between grain yield and its contributing components can significantly improve the efficiency of breeding programmes through the use of appropriate selection indices. The nature of association between grain yield and its components determine the appropriate traits to be used in indirect selection for improvement in grain yield.

This means small tassels may not necessarily confer improved biomass per ear/grain yield, because there can be variability for biomass partitioning to the ear among genotypes. In maize, past genetic gains in yield potential were achieved by a reduction in the ASI which was evidence of a decline in apical dominance obtained through the selection for small tassels and the concomitant enhanced biomass partitioning to the ear. The reduction in tassel size together with improved agronomic practices like selecting the best sowing date for an optimum setting of the critical period in relation to the environmental condition seems less useful on their own, for further increasing potential and actual grain yields.

Therefore, incorporation of the *Fbr1* tassel trait should accompany selection for other traits associated with stress tolerance under low N and drought conditions, e.g. the "stay green" trait, factors associated with premature senescence, synchrony between male and female flowering and decreased barrenness. Furthermore, future gains in grain yield should depend upon a more detailed knowledge of the responses of different genotypes to varying stress conditions (and also on fine-tuning the phenotyping of the *Fbr1* genotypes, for example, to assess response of genotypes with varying number of tassel branches to different stress levels).

A number of elite CIMMYT lines have been successfully converted to *Fbr1*, and were homozygous for the 1074 SNP loci used, thus could be used in breeding programmes involving these new tassel mutants. The UPGMA cluster analysis unravelled two discrete clusters of the inbred lines according to predefined CIMMYT heterotic groups. Additionally, the 1074 SNP markers clearly separated maize lines according to tassel size (*Fbr1 versus* normal), hence the SNP loci were effective in characterising the maize inbred lines. SNP-based genetic distances were positively correlated with hybrid performance, SCA, and heterosis indicating that genetic distance could accurately predict hybrid performance in this set of germplasm. Hybrids constituted of closely related parental lines had the lowest mean grain yield; lowest SCA effects for grain yield, and had the lowest heterosis values. SNP-based genetic distance information would be useful for effective selection by avoiding genetically similar lines when selecting parents for breeding programmes that require genetically diverse parental lines. Thus, SNP markers are newer genomic-related tools that can be used to facilitate efficient characterisation and selection of target genotypes in breeding programmes. The SNP markers are the marker of choice when handling maize germplasm with the *Fbr1* mutation because of their wide coverage

of the maize genome. Additionally, considering that the tassel mutation is a point mutation (G/C transitions), SNP markers would give the highest polymorphism among genotypes for the trait.

The *Fbr1* trait cannot be classified as a less useful trait in breeding programmes, until more work is done on this trait, especially with a larger population. The lack of association between the trait and yield potential could be a result of the small sample size used. Although the small tassel morphology poses a challenge in pollen production when maize lines with the trait are used as males in seed and in hybrid production, lines with the *Fbr1* trait can be used as female parents to make hybrids ideally suited for stress environments.

Summary

Maize is among the most commonly bred crops in the world and maize breeding programmes are increasingly using molecular tools to enhance the efficiency and speed of developing productive cultivars. Breeding efforts of CIMMYT have focused on incorporating drought tolerance into elite germplasm. The incorporation of the *Fbr1* gene into its elite germplasm was one such effort as the improvement of drought tolerance relies on manipulation of adaptive traits that limit yield under the target stress. The aim of this study was to find the genetic basis and effect of the *Fbr1* tassel mutation on maize grain yield under stress and non-stress environments.

A number of elite CIMMYT lines have been successfully converted to the *Fbr1* mutation, and were homozygous for the 1074 SNP loci used, thus could be used in breeding programmes involving these new tassel mutants. The UPGMA cluster analysis revealed two discrete clusters of the inbred lines according to predefined CIMMYT heterotic groups. In the principal component analysis, the SNP loci were effective in characterising the maize inbred lines since they separated maize lines according to tassel size. Positive relationships between grain yield and pollen yield and its components were found, under drought conditions. However, the *Fbr1* tassel trait did not have a positive effect on yield under stress and *Fbr1* x *Fbr1* hybrids had lower grain and pollen yield, and were less adapted to abiotic stress conditions. This raised questions on the value of incorporating such trait in breeding programmes targeting stress tolerance. Therefore, incorporation of the *Fbr1* tassel trait should accompany selection for other traits associated with stress tolerance under low N and drought conditions, such as the “stay green” trait, factors associated with premature senescence, synchrony between male and female flowering and decreased barrenness if yield gain is to be realized. Estimates of genetic components of variance revealed importance of both additive and dominance components in the determination of grain yield, pollen yield and their components. Additive gene action was predominant in determining tassel size and pollen yield, thus progress can be made by selecting within segregating progenies when improving maize populations for the *Fbr1* trait.

The investigation of GxE interaction showed significant variation in stability of *Fbr1* lines and hybrids as measured by mean yield and ASV. The AMMI and the GGE biplots ranked the best and poorest genotypes similarly in terms of yield performance and stability. The two classification models could be used simultaneously to make selection of genotypes more precise.

Genetic distances were positively correlated with hybrid performance, SCA, and heterosis indicating that genetic distance could accurately predict hybrid performance in this set of germplasm.

This study showed that yield is a complex trait and its improvement under stress should involve simultaneous selection of other traits associated with stress tolerance. The SNP markers are the marker of choice in genetic characterisation and determination of marker-based genetic distances because of their wide coverage of the maize genome. A number of lines homozygous for the *Fbr1* tassel trait, has been identified in this study. These lines could be used in future research such as the developing of mapping populations aimed at tagging the *Fbr1* trait, since the position of the *Fbr1* gene in the maize genome is still unknown.

Opsomming

Mielies is van die mees algemene gewasse waarvoor teling gedoen word in die wêreld. Mielie teelprogramme gebruik toenemend molekulêre tegnieke om die effektiwiteit en die spoed van vrystelling van nuwe, produktiewe cultivars te verseker. Teling by CIMMYTfokus op die verbetering van droogtetoleransie in elite kiemplasma. Die insluiting van die *Fbr1* geenin die elite kiemplasma was een so ‘n aksie, omdat die verbetering van droogtetoleransie berus op die manipulasie van aanpassingseienskappe wat opbrengs beperk onder spesifieke stremmingstoestande. Die doel van hierdie studie was om die genetiese basis en die effek van die *Fbr1* pluimmutasie op mieliegraanopbrengs te bepaal in beide stremmings en optimale omgewings.

‘n Aantal elite CIMMYT lyne is suksesvol omgeskakel na die *Fbr1* mutasie, en is homosigoot vir die 1074 SNP loci wat gebruik is. Hulle kan dus aangewend word in teelprogramme wat hierdie nuwe pluimmutasie gebruik. Die UPGMAgroeperingsanalise het twee diskrete groepe ingeteelde lyne getoon volgens die voorafbepaalde CIMMYT heterotiese groepe. Die SNP loci was effektief in die karakterisering van die mielie ingeteelde lyne in die hoofkomponent analise omdat dit die mielielyne duidelik geskei het volgens die pluimgrootte.Daar was ‘n positiewe verwantskap tussen graanopbrengs en stuifmeelopbrengs en verwante eienskappe daarvan onder droogtestremming. Die *Fbr1*pluimmutasie het egter nie ‘n positiewe effek op opbrengs onder stremming gehad nie, en *Fbr1* x *Fbr1* basters het laer graan en stuimeelopbrengs as die ander basters gehad en was swakker aangepas onder abiotiese stremming. Dit het vrae laat ontstaan oor die waarde van insluiting van hierdie eienskap in teelprogramme vir stremmings toleransie. Dit is belangrik dat die insluiting van die *Fbr1*pluimmutasie gepaardgaan met die seleksie vir ander eienskappe wat geassosieer word met stremmingstoleransie onder beide droogte en lae N toestande, soos die “stay green” eienskap, faktore wat geassossieer word met vroeë verdroging, sinkronisasie tussen manlike en vroulike antese en ‘n afname in steriliteit, as verbeterde graanopbrengs gerealiseer moet word. Bepaling van genetiese komponente van variansie het die belangrikheid van beide additiewe en dominansie komponente in die bepaling van graanopbrengs en stuifmeeloprengs en verwante eienskappe getoon. Additiewe geenaksie was die belangrikste met die bepaling van pluimgrootte en stuifmeelopbrengs, dus kan genetiese vooruitgang gemaak word deur seleksie binne segregerende nageslagte as mieliepopulasies vir die *Fbr1*eienskap verbeter word.

Die GxE interaksie studie het betekenisvolle variasie in die stabiliteit van die *Fbr1* lyne en basters aangetoon soos gemeet met die gemiddelde opbrengs en die ASV. Die AMMI en die GGE grafieke het die beste en die swakste genotipes in ooreenstemmende rangordes geplaas in terme van graanopbrengs en stabiliteit. Die twee klassifikasie modelle kan gelyktydig gebruik word om die seleksie van genotipes meer effektief te maak. Genetiese afstande was positief gekorreleer met baster opbrengs, SCA en heterose wat wys dat genetieseafstand baster prestasie effektief kan voorspel in hierdie genotipes.

Hierdie studie het getoon dat opbrengs ‘n komplekse eienskap is en dat die verbetering daarvan onder stremming ook die seleksie van ander eienskappe moet insluit wat geassosieër word met stremmings toleransie. SNP is die merker van keuse in genetiese karakterisering en bepaling van merker-gebasseerde afstande omdat dit ‘n goeie dekking van die hele mieliegenoom gee. ‘n Aantal lyne homosigoot vir die *Fbr1*pluimmutasie is geïdentifiseer in hierdie studie. Hierdie lyne kan in toekomstige studies gebruik word, soos die posisionering van die *Fbr1* eienskap, omdat die posisie van die *Fbr1* geen in die mieliegenoom nog onbekend is.