

**GENETIC ANALYSIS OF TRADITIONAL ETHIOPIAN
HIGHLAND MAIZE (*Zea mays* L.) USING MOLECULAR
MARKERS AND MORPHOLOGICAL TRAITS: IMPLICATION
FOR BREEDING AND CONSERVATION**

By

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DECLARATION

I declare that the dissertation, which I here by submit for the degree of Doctor of Philosophy in Plant Breeding/Genetics at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at another University.

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PREFACE

This work was conducted at the Department of Genetics and Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. The project involved laboratory, glasshouse and field experiments. Various molecular marker techniques and quantitative genetics approaches were employed to accurately unravel the extent of genetic diversity and genetic relationships among traditional Ethiopian highland maize accessions with the view of establishing a strategic maize improvement program in the highlands of Ethiopia.

This dissertation is based on the following chapters, which were published, accepted or submitted for publication.

1. Phenotypic diversity for morphological and agronomic traits in traditional Ethiopian highland maize accessions (*South African Journal of Soil and Plant* 2005, 22: 100-105)
2. Genetic diversity in traditional Ethiopian highland maize accessions assessed by AFLP markers and morphological traits (*Journal of Biodiversity and Conservation, in press*).
3. Genetic diversity among traditional Ethiopian highland maize accessions assessed by simple sequence repeat (SSR) markers (*Journal of Genetic Resources and Crop Evolution, in press*).
4. A comparative study of molecular and morphological methods of describing genetic relationships in maize (*African Journal of Biotechnology* 2005,4:586-595)
5. Association of simple sequence repeats with quantitative traits in Ethiopian highland maize accessions and the effect of admixture.

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LIST OF ABBREVIATIONS

°C	Degree celsius
µl	Microliter
µg	Microgram
µM	Micromolar
AEI	Assay efficiency index
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
AOD	Analysis of distance
bp	Base pair
CA	Cluster analysis
CIMMYT	International Maize and Wheat Improvement Center
cm	Centimeter
cM	Centimorgan
CSA	Central statistical authority
DNA	Deoxyribonucleic acid
dNTPs	The mixtures of four deoxynucleotides triphosphate
DSK	Days to silking
DTS	Days to tasseling
DYM	Days to maturity
e.g.	Example
EARO	Ethiopian Agricultural Research Organization
ERD	Ear diameter
ERH	Ear height
ERL	Ear length
EST	Expressed sequence tag
FAPRI	Food and Agricultural Policy Research Institute
g	Gram
GA	Genetic advance
GCV	Genotypic coefficient of variability
GS _{NL}	Genetic similarity base on Nei and Li
GS	Genetic similarity
GS _J	Genetic similarity based on Jaccard
GS _{MR}	Genetic similarity based on Modified Roger's distance
GS _{SM}	Genetic similarity Simple matching
h	Hour
h ²	Broad sense heritability
ha	Hectare
HCl	Hydrochloric acid
IRDye	Infrared day
Kg ha ⁻¹	Kilogram per hectare
KCl	Potassium chloride
KLR	Kernels per row
LD	Linkage disequilibrium
LFL	Leaf length

LFW	Leaf width
MA	Millampere
MAS	Marker assisted selection
masl	Meters above sea level
Mb	Mega billion
MgCl ₂	Magnesium chloride
min	Minute
mM	Millimolar
mm	Millimeter
mnt	Million metric tons
NIL	Near isogenic line
NMSA	National metrological service agency
no.	Number
ns	Not significant
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
PIC	Polymorphism information content
PLH	Plant height
QTL	Quantitative trait loci
R/L	Restriction ligation
R ²	Multiple regression coefficient
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RWN	Rows per ear
s	Second
SDW	Seed weight
SNP	Single nucleotide polymorphism
Spp.	Species
SSR	Microsatellite or simple sequence repeat
St Dev	Standard deviation of the means
t	Ton
UPGMA	Unweighted paired group method using arithmetic averages
V	Volt
V _B	Between group variation
V _T	Total variation
V _W	Within group variation
W	Watt
w/v	Weight volume ratio
YD	Yield

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SUMMARY

Knowledge of the genetic variation of crop collections is essential for their efficient use in plant breeding programs. The Ethiopian Highland Maize Germplasm Collection Mission was launched throughout the highlands of Ethiopia in 1998 and 287 traditional maize accessions were collected from farmers' fields. To date, no information was available on the morphological and genetic diversity in this important collection. Various molecular marker techniques and quantitative genetics approaches were applied to accurately unravel the extent of phenotypic and genetic diversity, to study patterns of morphological and molecular variation and to determine association of molecular markers with quantitative trait variation, with the view of designing a sound breeding program and management strategy for maize in the highlands of Ethiopia.

The morphological study confirmed that traditional Ethiopian highland maize accessions contain large amounts of variation for agro-morphological traits. The broad

trait diversity observed among the accessions suggested ample opportunities for the genetic improvement of the crop through selection directly from the accessions and/or the development of inbred lines for a future hybrid program. Selection practices followed by local farmers are mostly consistent within agroecology and gave rise to morphologically distinct maize accessions in different agroecologies. This underscores the importance of considering farmers' knowledge of diversity in the collection and evaluation of local accessions.

The results of amplified fragment length polymorphism (AFLP) and microsatellite or simple sequence repeat (SSR) marker analyses showed that bulking leaf samples from 15 individual plants per out-bred accession is an effective means of producing representative profiles of individual plants, thereby reducing the cost of DNA extraction and subsequent marker analysis of open-pollinated varieties. Cluster analyses based on AFLP and SSR data showed that most of the accessions collected from the Northern agroecology were genetically distinct from the Western and Southern accessions suggesting that differentiation for adaptive traits for drought conditions may have occurred in the Northern accessions. However, there was very little genetic differentiation between the Western and Southern accessions suggesting gene flow between the two agroecologies and recent introduction of similar improved varieties in these agroecologies. In both marker systems, high mean genetic diversity was observed among the traditional Ethiopian highland maize accessions. This is possibly due to (i) the continuous introduction of maize from abroad by different organizations; (ii) genetic variation generated through farmers management practices; and (iii) the presence of different environmental conditions in the highlands of

Ethiopia to which local landraces may have been adapted.

The correlation between the morphological dissimilarity matrix and the matrices of genetic dissimilarity based on SSR and AFLP markers were 0.43 and 0.39, respectively ($p = 0.001$ in both cases). The correlation between SSR and AFLP dissimilarity matrices was 0.67 ($p = 0.001$). These significant correlations indicate that the three independent sets of data likely reflect the same pattern of genetic diversity, and validate the use of the data to calculate the different diversity statistics for Ethiopian highland maize accessions. From this study, three groups of maize accessions with distinctive genetic profiles and morphological traits were identified that will be useful for future collection, conservation and breeding programs of maize for the highlands of Ethiopia.

A pilot association study using SSR markers and quantitative trait variation indicated that molecular markers could be useful to identify genetic factors controlling earliness, tallness, grain yield and associated traits, which could be exploited by various breeding schemes. The analytical tools outlined in this dissertation can be a useful tool in managing genetic variation of open-pollinated crops and will aid in the conservation of unique genetic diversity. Production stability and global food security are linked to the conservation and exploitation of worldwide genetic resources and this research attempts to add to that body of knowledge.

Key words: AFLP markers, association mapping, bulked analysis, clustering, correlation, Ethiopia, genetic diversity, genetic resources, heritability, highland maize, quantitative traits, phenotypic diversity, regression analysis, SSR markers