
REVIEW ON: RELATIONSHIP OF HYBRID PERFORMANCE AND AFLP BASED GENETIC DISTANCE IN MAIZE (*ZEA MAYS L.*)***Zelalem Tafa and Abenezer Abebe**

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Abstract

Maize (*Zea mays L.*) is one of the cereal crops broadly adapted worldwide. Enhancement of maize production and productivity can be achieved through identification of potentially superior inbred line combinations in the form of hybrids. The objective of this paper is to understand the relationship of hybrids performance and AFLP based genetic distance and review the molecular basis for heterosis. Morphological markers have shortcomings to detect differences among closely related genotypes and influenced by prevailing environmental conditions. Molecular markers are not influenced by environmental factors and also fast, efficient and more sensitive than field testing to detect large numbers of distinct differences among genotypes at the DNA level. In maize, AFLP techniques have been applied to genome mapping, DNA fingerprinting, genetic diversity studies and hybrid performance prediction. Genetic markers represent genetic differences between individual organisms or species. There are three major types of genetic markers: (1) morphological markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA. Prediction of hybrid performance is one of the main goals in almost all maize hybrid breeding programmes. Information on germplasm diversity and relationships among elite materials is of great importance in maize hybrid development. Genetic distance has been used to predict hybrid performance and the efficiency of prediction was greater with cross between inbred lines from the same heterotic group than cross between inbred lines from different heterotic groups.

Keywords: Hybrid, Markers, Diversity, Heterosis.

INTRODUCTION

Maize (*Zea mays L.*) is one of the important cereals broadly adapted worldwide. In Ethiopia, it is grown in the lowlands, the mid-altitudes and the highland regions. It is an important field crop in terms of area coverage, production and utilization for food and feed purposes. However, maize varieties mostly grown in the highlands (altitude = 1,700 - 2,400 m.a.s.l.) of Ethiopia are local cultivars. They are low yielding, vulnerable to biotic and abiotic constraints and also exhibit undesirable agronomic performances such as late maturity and susceptibility to root and stalk lodging (EARO, 2000). Enhancement of maize production and productivity can be achieved through identification of potentially superior inbred line combinations in the form of hybrids (Bernardo 1999; Saleh *et al.*, 2002). Conventional breeding methods are expensive and time consuming. Furthermore, the large number of possible hybrid combinations to be produced from a relatively small number of inbred lines, render the evaluation of all possible combinations unfeasible (Bernardo 1992; Betran *et al.*, 2003). In addition, morphological markers have shortcomings to detect differences among closely related genotypes and are influenced by prevailing environmental conditions. Molecular markers are not influenced by environmental factors and are also fast, efficient and more sensitive than field testing to detect large numbers of distinct differences between genotypes at the DNA level (Melchinger, 1999). In maize, AFLP techniques have been applied to genome mapping (Marsan *et al.*, 2001), DNA fingerprinting (Oliveira *et al.*, 2004), genetic diversity studies (Garcia *et al.*, 2004) and hybrid performance prediction (Sheng and Rui 2000; Barbosa *et al.*, 2003).

Previous studies conducted to assess genetic diversity and to predict hybrid performance in maize were mostly focused on temperate germplasm (Melchinger, 1999). Using AFLP markers, some information on tropical maize germplasm is present but the genotypes studied were of lowland tropical origin (Sheng and Rui 2000; Barbosa *et al.*, 2003; Garcia *et al.*, 2004). Keeping this in mind, the objective of this review paper is: -To know the molecular concepts of plant breeding and application of AFLP for maize breeding for development of hybrids.

MOLECULAR MARKERS

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in elucidation of genetic relationships within and among species (Chakravarthi and Naravaneni, 2006). The theoretical advantages of using genetic markers and the potential value of genetic marker linkage maps and direct selection in plant breeding were first reported about thirteen years ago (Crouch and Ortiz 2004). DNA-based molecular markers have acted as versatile tools and have found their own position in various fields like taxonomy, plant breeding, genetic engineering (Joshi *et al.*, 2011).

Types of Markers

Genetic markers represent genetic differences between individual organisms or species (Collard *et al.*, 2006). Generally, they do not represent the target genes themselves but act as 'signs' or 'flags'. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the

phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes called 'loci' (singular loci). There are three major types of genetic markers: (1) morphological (also 'classical' or 'visible') markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA markers, which reveal sites of variation in DNA (Jones *et al.*, 1997; Winter and Kahl, 1995).

Morphological marker: Morphological markers are usually visually characterized phenotypic characters such as flower color, seed shape, growth habits or pigmentation. The major disadvantages of morphological markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant (Winter & Kahl, 1995). However, despite these limitations, morphological markers have been extremely useful to plant breeders (Eagles *et al.*, 2001).

Biochemical markers: A biochemical marker includes protein (Isozymes and allozymes) and Phytochemicals. Isozymes are allelic variants of the same enzyme, generally encoded by different loci, while allozymes are different proteins encoded by different genes performing the same enzyme function (Weeden and Wendel, 1989). Isozymes were the first molecular tool to be used for genetic characterization (Soltis and Soltis, 1990). The analysis can be carried out by preparing tissue extract and proteins will be separated according to their net charge and size by electrophoresis using a polyacrylamide or starch gel. The gel is stained for a particular enzyme by adding a substrate and a dye under appropriate reaction conditions, resulting in band(s) at position where the enzyme polypeptide has migrated showing relative enzyme activity. Depending upon the number of loci, their state of homo/heterozygosity in the individual, and the enzyme molecular configuration, one to several bands were visualized (Kumar *et al.*, 2018). Biochemical markers can be biased since these markers represent a small portion of the genome and generally they exhibit low polymorphism.

DNA markers: The theoretical advantages of using genetic markers and the potential value of genetic marker linkage maps and direct selection in plant breeding were first reported about thirteen years ago (Crouch and Ortiz 2004). However, it was not until the advent of DNA marker technology in the 1980s, that a large enough number of environmentally insensitive genetic markers generated to adequately follow the inheritance of important agronomic traits and since then DNA marker technology has dramatically enhanced the efficiency of plant breeding. DNA-based molecular markers have acted as versatile tools and have found their own position in various fields like taxonomy, plant breeding, genetic engineering (Joshi *et al.*, 2011). A number of breeding companies have in the past two decades to varying degrees started using markers to increase the effectiveness in breeding and to significantly shorten the development time of varieties and therefore plant geneticist consider molecular marker assisted selection a useful additional tool in plant breeding programs to make selection more efficient (Bueren *et al.*, 2010; Joshi *et al.*, 2011) over the last few decades plant genomics has been studied extensively bring about a revolution in this area, making molecular markers useful for plant genomic analysis, therefore becoming an important tool in this revolution (Joshi *et al.*, 2011). The

most significant breakthrough in agricultural biotechnology is coming from research into the structure of genomes and the genetic mechanisms behind economically important traits. The rapidly progressing discipline of genomics also known as molecular biology, is the provision of information on the identity, location, impact and function of genes affecting such traits which researchers have been identifying, cataloging and mapping single gene markers in many species of higher plants. Molecular markers include biochemical constituents (e.g. secondary metabolites in plants) and macro-molecules, viz proteins and deoxyribonucleic acid (DNA). Analysis of secondary metabolites is, however restricted to those plants that produce a suitable range of metabolites which can be easily analyzed and which can be distinguished by varieties (Joshi *et al.*, 2011). These metabolites which are being use as markers should be ideally neutral to environmental effects or management practices. Hence, amongst the marker molecular markers used, DNA markers are more suitable and ubiquitous to most of the living organisms.

Diversity based on phenotypic and morphological characters, usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification, but now the rapid development of biotechnology allows easy analysis of large number of loci distributed throughout the genome of the plants. Molecular makers have proven to be powerful tools in the assessment of genetic variation and in elucidation of genetic relationships within and among species (Chakravarthi and Naravaneni, 2006). Collecting DNA marker data to determine whether phenotypically similar cultivars are genetically similar would therefore be of great interest in crop breeding programme (Duzyaman, 2005). The differences are called molecular markers because they are often associated with specific gene and acts as a 'sign posts' to those genes and such markers when very tightly linked to genes of interest, can be used to select indirectly for the desirable allele and this represents the simplest form of marker- assisted selection (MAS) (Hoisington *et al.*, 2002).

The molecular markers are no longer looked upon as simple DNA fingerprinting markers in variability studies or as mere forensic tools, but they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis (Joshi *et al.*, 2011). Molecular markers work by highlighting differences (polymorphisms) within a nucleic sequence between different individuals. These differences include insertions, deletions, translocations, duplications and point mutations. They do not, however, encompass the activity of specific genes (Linda *et al.*, 2009; Wani, 2006). In addition to being relatively impervious to environmental factor, molecular markers have the advantage of: (i) being applicable to any part of the genome (introns, exons and regulation regions); (ii) not possessing pleiotrophic or epistatic effects; (iii) being able to distinguish polymorphisms which not produce phenotypic variation and finally, (iv) being some of them co-dominant. In addition, the different techniques can assess either multi-locus or single-locus markers. Multi-locus markers allow simultaneous analyses of several genomic loci, which are based on the amplification of casual chromosomal traits through oligonucleic primers with arbitrary sequences. These types of markers are also defined as dominant since it is possible to observe the presence or the absence of a band for any locus, but it is not possible to distinguish between heterozygote (a/-)

conditions and homozygote for the same allele (a/a). By contrast, single-locus markers employ probes or primers specific to genomic loci, and are able to hybridize or amplify chromosome traits with well-known sequences. They are defined as co-dominant since they allow discrimination between homozygote and heterozygote loci. The use of molecular markers allows for the tracking of the number and frequency of alleles, which determinate the population parameters that can increase the genetic gains in populations of recurrent selection (Ferreira *et al.*, 2000). Various scientists have been developed and see different marker techniques in different time. In recent years polymerase chain reaction (PCR) based molecular markers such as RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeat) or AFLP (Amplified Fragment Length Polymorphism) were explored to study genetic variability and diversity of many plant species (He *et al.*, 2009). Microsatellites or simple sequence repeats (SSR) stand out for their high information content, co dominance, large number of loci available for rice, wheat, barley and maize and ability to be amplified by polymerase chain reaction (PCR) (Semagn *et al.*, 2006). Yosef *et al.*, 2005 stated that, SSR markers had the highest expected heterozygosity, while AFLP markers had the highest effective multiplex ratio.

Table 1. Acronyms commonly used for different molecular markers

AFLP	Amplified Fragment Length Polymorphism
AP-PCR	Arbitrarily primed PCR
ARMS	Amplification Refractory Mutation System
ASAP	Arbitrary Signatures from Amplification
ASH	Allele-Specific Hybridization
ASLP	Amplified Sequence Length Polymorphism
ASO	Allele Specific Oligonucleotide
CAPS	Cleaved Amplification Polymorphic Sequence
CAS	Coupled Amplification and Sequencing
DAF	DNA Amplification Fingerprint
DGGE	Denaturing Gradient Gel Electrophoresis
GBA	Genetic Bit Analysis
IRAO	Inter-Retrotransposon Amplified Polymorphism
ISSR	Inter-Simple Sequence Repeats
ISTR	Inverse Sequence-Tagged Repeats
MP-PCR	Microsatellite-Primed PCR
OLA	Oligonucleotide Ligation Assay
RAHM	Randomly Amplified Hybridizing Microsatellites
RAMPs	Randomly Amplified Microsatellite Polymorphisms
RAPD	Randomly Amplified Polymorphic DNA
RBIP	Retrotransposon-Based Insertion Polymorphism
REF	Restriction Endonuclease Fingerprinting
REMAP	Retrotransposon-Microsatellite Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SAMPL	Selective Amplification of Polymorphic Loci
SCAR	Sequence Characterized Amplification Regions
SNP	Single Nucleotide Polymorphism
SPAR	Single Primer Amplification Reaction
SPLAT	Single Polymorphic Amplification Test
S-SAP	Sequence-Specific Amplification Polymorphisms
SSCP	Single Strand Conformation Polymorphism
SSLP	Single Sequence Length Polymorphism
SSR	Simple Sequence Repeats
STMS	Sequence-Tagged Microsatellite Site
STS	Sequence-Tagged-Site
TGGE	Thermal Gradient Gel Electrophoresis
VNTR	Variable Number Tandem Repeats
RAMS	Randomly Amplified Microsatellites

Source = Linda Mondini *et al.*, 2009

AFLP Concept

The AFLPs markers technique combines elements of RFLP and RAPD. They are PCR-based markers, simply RFLPs

visualized by selective PCR amplification of DNA restriction fragments. Technically, AFLP is based on the selective PCR amplification of restriction fragments from a total double-digest of genomic DNA under high stringency conditions, i.e., the combination of polymorphism at restriction sites and hybridization of arbitrary primers (Weising *et al.*, 2005). The usage of AFLP technologies results in the detection of higher levels of polymorphisms compared with RFLPs. AFLPs also have a much higher multiplex ratio (more markers per experiment) and better reproducibility than RAPDs. In the whole, AFLP markers allow the rapid generation of highly replicable markers, thus permitting high-resolution genotyping of fingerprinting quality. A drawback can be that most AFLP markers are dominant rather than co-dominant, due to the complex banding patterns (Meudt and Clarke, 2007).

Application of molecular markers in maize genome analysis and breeding

Molecular markers have been look upon as a tool for a large number of applications ranging from localization of a gene to improvement of plant varieties by marker-assisted selection, called genome analysis which has generated a vast amount of information and a number of databases are being generated to preserve and popularize it (Joshi *et al.*, 2011). Prasanna and Pixley (2010) stress the importance of efforts in meeting the growing demand for maize and provide examples of the recent use of molecular markers with respect to (i) DNA finger printing and genetic diversity analysis of maize germplasm (inbreds and landraces/OPVs), (ii) QTL analysis of important biotic and abiotic stresses and (iii) MAS for maize improvement. Advances in genome analysis led to the identification of numerous DNA markers in maize includes thousands of mapped micro-satellite markers and more recently, single nucleotide polymorphisms (SNPs) and insertion-deletion (INDEL) markers. With the SSRs and SNPs, a large number of genes controlling various aspects of plant development, biotic and abiotic stress resistance, quality characters etc, have been cloned and characterized in maize, which are excellent assets for molecular-assisted breeding (Prassana and Pixley, 2011). At present SSRs are the most widely used markers by maize researchers due to their availability in large numbers in the public domain including their simplicity and effectiveness (Maize CrDB; <http://www.maizegdb.org>). These PCR-based, genetically co-dominant marker are robust, reproducible, hyper variable, abundant, and uniformly dispersed in plant genomes (Powell *et al.*, 1996). Also, both SSRs and SNPs can be reliably applied on a large scale and therefore offer significant advantages for genetic and breeding purposes. SSR markers have been successfully used for DNA finger printing and analysis of genetic diversity in china, India, Indonesia and Thailand (Prassana and Pixley, 2010). Following the first report on QTLs for yield related traits in maize (Stuber *et al.*, 1987), maize researchers worldwide have generated numerous reports of molecular markers tagging genes/QTLs for diverse traits of agronomic and scientific interest (Prasanna and Pixley, 2010). QTLs for several important traits affecting maize such as plant height, downy mildew resistance, Maize dwarf Mosaic Virus resistance, head smut resistance, drought stress tolerance, water logging, nutrient components under low nitrogen and high-oil content. Further, significant progress has been made worldwide in optimizing MAS for improvement of both qualitative and quantitative inherited traits using maize as a model system.

Table 2. Advantages and disadvantages of AFLP marker for QTL analysis

Molecular marker	Co-dominant or Dominant	Advantages	Disadvantages	Reference
AFLP	Dominant due to the complex banding patterns	Reliable and stable Moderate cost Diversity analysis parentage detection DNA fingerprinting Prediction of hybrid performance	Large amounts of DNA required Complicated methodology	Vos <i>et al.</i> , (1995)

Table 3. Comparison of most widely used DNA marker systems in plants

Feature and description	RFLP	RAPD	AFLP	SSR	SNP
Genomic abundance	High	High	High	Moderate to high	Very high
Expression/inheritance	Co-dominance	Dominant	Dominant/ co-dominant	Co-dominant	Co-dominant
Level of polymorphism	Moderate	High	High	High	High
PCR-based	Usually no	Yes	yes	yes	yes
Reproducibility/ reliability	High	Low	High	High	High
Technically demanding	Moderate	Moderate	low	Low	High
Ease of use	Not easy	Easy	Moderate	Easy	Easy
Development/start-up cost	Moderate to high	Low	Moderate	Moderate to high	High
Cost per analysis	High	Low	Moderate	Low	Low
Number of polymorphic loci per analysis	1.0 – 3.0	1.5 – 5.0	20 – 100	1.0 – 3.0	1.0
Primary application	Genetics	Diversity	Genetics and diversity	All purposes	All purposes

Source: Collard *et al.*, (2005), Semagn *et al.*, (2006a), Xu (2010)

One successful example of MAS for maize development and of particular use is the utilization of opaque 2-specific SSR markers in conversion of maize lines in quality protein maize (QPM) lines with enhanced nutritional quality (Buba *et al.*, 2005). A MAS-derived QPM hybrid is the “Vivek QPM hybrid 9,” recently released in Almora, India, which was developed through marker-assisted transfer of the O2 gene and phenotypic selection for endosperm modifiers in the parental lines (Buba *et al.*, 2005). Using MAS Scientist at IARI have pyramided major genes /QTLs for resistance to turicum leaf blight and Polysora rust in five elite Indian lines (Prassana *et al.*, 2009b) and these are CM 137, CM138, CM139, CM150 and CM151 which are parents of three single-cross hybrids.

ESTIMATION OF GENETIC VARIABILITY

Genetic variability analysis is an essential process for clear and sound identification of the genetic relatedness of the available genetic resources. It is also required for effective choice of parents for subsequent crossing and selection of the progenies (Ahsan Iqbal *et al.*, 2013). Genetic variability within a population can be estimate through: (1) The number (and percentage) of polymorphic genes in the population (2) The number of alleles for each polymorphic gene (3) The proportion of heterozygous loci per individual (Primack, 2009). Understanding the molecular basis of the essential biological phenomena in plants is crucial for the effective conservation, management, and efficient utilization of plant genetic resources (PGR) (Linda Mondini *et al.*, 2009). Mendelian methods of making crosses and scoring the phenotypes of the offspring in one or more generations are insufficient for a detailed estimate of genetic variability. The process is restricted to phenotypic characters primarily, which are limited in number; the process is too time consuming to wait for future generations in many species; the process does not always yield precise information on genotype (homozygous dominant vs heterozygote); There are too many gene loci in most organisms for this process to yield reliable estimates of the genetic variability (Bader, 1998, Swanepoel, 1999). These limitations can be overcome by using the techniques of molecular genetics. DNA markers do not have such limitations.

They can be used to detect variation at the DNA level and have proven to be effective tools for distinguishing between closely related genotypes and for precise estimation of genetic variation in crop species (Beyene *et al.*, 2005; Iqbal *et al.*, 2013). Even if different types of molecular markers have been used to assess the genetic diversity in crop species, no single technique is universally ideal (Mohammadi and Prasanna, 2003; Beyene *et al.*, 2005; Semagn *et al.*, 2006). Therefore, the choice of the technique depends on the objective of the study, financial constraints, skills and facilities available.

Hybrid Performance and AFLP based Genetic Distance

Prediction of hybrid performance is one of the main goals in almost all maize hybrid breeding programmes. Information on germplasm diversity and relationships among elite materials is of great importance in maize hybrid development (Hallauer and Miranda, 1988; Choukan and Warburton, 2005; Ristić *et al.*, 2013, Vančetočić *et al.*, 2015.). Maize breeders extensively exploited phenomenon of heterosis although its genetic basis is still not completely understood. It is well known that the best hybrid combinations are obtained by crossing parental lines of distant genetic background (Buhiniček *et al.*, 2009). However, Moll *et al.*, (1965) reported that increase of genetic distance between parental lines, values of heterosis increase up to the certain level, after which decline. Better understanding of genetic diversity is useful in planning crosses for hybrid and line development, in assigning lines to heterotic groups and in plant variety protection (Pejic *et al.*, 1998). Genetic distance has been used to predict hybrid performance and the efficiency of prediction was greater with cross between inbred line from the same heterotic group than cross between inbred lines from different heterotic groups (Melchinger, 1999). Linkage disequilibrium between DNA markers and genes involved in the expression of target traits is required for GD and hybrid performance to be correlated (Betran *et al.*, 2003). Genetically diverse parents are, to a certain extent more likely to give heterotic hybrids than those genetically related. From a plant breeder's viewpoint, increase over better parent (heterobeltiosis) and standard variety (standard heterosis) is more relevant. It is reported that a positive correlation exists between genetic distance and heterosis. In maize, AFLP

markers have been employed for genetic distance analysis, variety identification, characterization of accessions, assigning of lines and populations into heterotic groups, as well as for hybrid prediction (Sheng & Rui, 2000; Barbosa *et al.*, 2003; Oliviera *et al.*, 2004). The relationship between DNA marker based genetic distance and single cross maize hybrid yields were studied by many researchers (Betran *et al.*, 2003; Reif *et al.*, 2003; Xiu *et al.*, 2004; Phuminchai *et al.*, 2008; Drinić *et al.*, 2012).

Conclusion

Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies. A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines and populations. These methods have relied on pedigree data, morphological data, agronomic performance data, biochemical data, and more recently molecular (DNA-based) data. The major disadvantages of morphological markers are that they may be limited in number and are influenced by environmental factors while molecular markers are not influenced by environmental factors and are also fast, efficient and more sensitive than field testing to detect large numbers of distinct differences between genotypes at the DNA level. In maize, AFLP techniques have been applied to genome mapping, DNA fingerprinting, genetic diversity studies and hybrid performance prediction. Previous studies conducted to assess genetic diversity and to predict hybrid performance in maize were mostly focused on temperate germplasm. Using AFLP markers, some information on tropical maize germplasm is present but the genotypes studied were of lowland tropical origin. Advances in genome analysis led to the identification of numerous DNA markers in maize includes thousands of mapped micro-satellite markers and more recently, single nucleotide polymorphisms (SNPs) and insertion-deletion (INDel) markers.

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