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Review : The Importance of Molecular Markers in Plant Breeding Programmes

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Abstract - Since the advent of restriction fragment length polymorphism (RFLP) markers, a range of other markers such as Random Amplified Polymorphism DNA (RAPD), amplified fragment length (AFLP), Simple sequence repeats (SSRS), etc has been introduced during the last two decades of the 20th century to fulfill various demands of the last breeding programmes. Ever since their invention, they are being, constantly modified for enhanced utility as a means to solve problems and to bring about automation in the genome analysis, gene tagging, phylogenic analysis and selection of desirable genotypes. It is also evidence that molecular markers (non morphological markers) offer several advantages over the morphological markers (conventional phenotypic markers), as they provide data that can be analyzed objectively; giving new dimension to breeding especially with respect to the time required to developing new improved crop varieties. In terms of scientific progress, the old disciplines of quantitative genetics and plant taxonomy have been revived by the molecular marker approach, which have the immediate application in supportive research for advanced breeding programmes. Therefore, the success of DNA marker technology for bring genetic improvement in crop plants would depend on close interaction between plant breeders and biotechnologists, availability of skilled manpower and substantial financial investment on research.

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Review : The Importance of Molecular Markers in Plant Breeding Programmes

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Abstract - Since the advent of restriction fragment length polymorphism (RFLP) markers, a range of other markers such as Random Amplified Polymorphism DNA (RAPD), amplified fragment length (AFLP), Simple sequence repeats (SSR_s), etc has been introduced during the last two decades of the 20th century to fulfill various demands of the last breeding programmes. Ever since their invention, they are being, constantly modified for enhanced utility as a means to solve problems and to bring about automation in the genome analysis, gene tagging, phylogenic analysis and selection of desirable genotypes. It is also evidence that molecular markers (non morphological markers) offer several advantages over the morphological markers (conventional phenotypic markers), as they provide data that can be analyzed objectively; giving new dimension to breeding especially with respect to the time required to developing new improved crop varieties. In terms of scientific progress, the old disciplines of quantitative genetics and plant taxonomy have been revived by the molecular marker approach, which have the immediate application in supportive research for advanced breeding programmes. Therefore, the success of DNA marker technology for bring genetic improvement in crop plants would depend on close interaction between plant breeders and biotechnologists, availability of skilled manpower and substantial financial investment on research.

I. Introduction

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 eighty 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 e.t.c (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 and 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 umber 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).

Molecular markers for classification of genotype are abundant, but unlike morphological traits, markers are not affected by environment (Staub, *et al*, 1997. Collecting DNA marker data to determine whether

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phenotypically similar cultivars are genetically similar would therefore be of great interest in crop breeding programme (Duzyaman, 2005).

Molecular genetics or the use of molecular technique for detecting differences in the DNA of individual plants has many applications of value to crop improvement (Wammanda and Jonah, 2006). 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 use to select indirectly for the desirable allele and this represents the simplest form of marker- assisted selection (MAS). (Hoisington, *et al*, 2002).

Markers can also be used for dissecting polygenic traits into their Mendelian components or Quantitative Trait Loci (QTL) and this increasing understanding of the inheritance and gene action for such traits allows the use of markers – selection procedures (Anderson *et al*, 1993).

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). The discovery of polymerase chain reaction (PCR) was a land mark in this effort and proved to be a unique process that brought about a new class of DNA profiling marker, which has facilitated the development of marker-based gene tags, map-based cloning of agronomic important genes, variability studies, phylogenetic analysis, synteny mapping, market assisted selection of desirable genotypes e.t.c. DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analyzed objective. Therefore, several molecular marker types are available and they each have their advantages and disadvantages (Cadalen *et al*, 1998).

II. Molecular Markers

In the early part of the 20th century, scientist discovered that, Mendelian factor controlling inheritances (genes) are organized in linear order on cytogenetically defined structure called chromosomes. It was shown that, combination of genes can be inherited in a group (i.e. they are linked together because they are close to each other on the same chromosomes. The individual genes flanking within a defined close interval are known as molecular DNA markers. Molecular markers are identifiable DNA sequence, found at specific locations of the genome and associated with the inheritance of a trait or linked gene (FAO, 2004). Thottappilly *et al* (2000), refer to molecular markers as naturally occurring polymorphism which include proteins and nucleic acids that are detectably different. Rapid advances are genome research and molecular biology has led to the use of DNA markers in plant breeding. Target genes in a segregating population can be identified with the assistance of DNA makers so as to accelerate traditional breeding programs (Thottappilly *et al*, 2000). Markers must be polymorphic (i.e. they must exist in different forms so that the chromosome carrying the mutant gene can be distinguished from the chromosome with normal gene by form of the marker it carries. Polymorhism can be detected at three levels: morphological, biochemical or molecular. Recently, the term DNA fingerprinting /profiling is used to describe the combined use of several single locus detection systems and are being used as versatile tools for investigating various aspects of plant genomes. These include characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, plant breeding and diagnostics (Joshi *et al*, 2011) The development of DNA (or molecular markers) has irreversibly change the disciplines of plant genetics and breeding (Collard and Mackill, 2006), According to Joshi *et al* (2011),an ideal DNA makers should however poses the following properties.(i) Highly polymorphism, which is the simultaneous occurrence of a trait at the same population of two or more discontinues variants or genotypes.

(i) Co dominant inheritance- different form of marker should be detected in a diploid organism to allow discrimination of homozygote and heterozygote.
(ii) Frequent occurrence in genome
(iii) Selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices)
(iv) Easy access (availability)
(v) Easy and fast assay
(vi) Reproducible – highly reproducibility and
(vii) Easy exchange of data between laboratories.

It is extremely difficult for a single genetic marker to possess all properties above. Depending on the type of study to be undertaken a marker system can be identified that would fulfill at least a few of the above characteristics.

a) Types and description of DNA markers

i. *Non-PCR based genetic markers (Restriction fragment length polymorphism):*

The first and forest molecular markers system called the Restriction Fragment length Polymorphism (RFLP), was developed in early 1980 (Farooq and Azam, 2002). The RFLPs are simply inherited naturally occurring Mendelian characters. Genetic information is stored in the DNA sequence on a chromosome and variation in this sequence is the basis for the genetic diversity within species. Plants are able to replicate their DNA with high accuracy and rapidity, but many mechanisms causing changes (mutation) in the DNA

are operative (Joshi *et al*, 2011).This leads to simple or large-base pair changes as a result of inversion, translocation, transpositions or deletion which may occur, resulting in a loss or gain of a recognition sites and in turn lead to restriction fragment of different lengths. This marker was first reported by Botstein *et al*, (1980); in the detection of DNA polymorphism (Agarwal *et al,* 2008).

Genomic restriction fragment of different length between genotypes can be detected on southern blots and by a suitable probe. In this method, DNA is digested with restriction enzyme like EcoR1, which cut the DNA at specific sequences, electrophoresed, blotted on a membrane and probed with a labeled clone. RFLP marker provides a way to directly follow chromosome segments during recombination as they follow Mendelian rules and greatly aid in the construction of genetic maps. When an F_1 plants undergoes meiosis to produce gametes, its chromosomes will undergo recombination by crossing over and this recombination is the basis of conventional genetic mapping and when use, RFLP markers, require hybridization of probe DNA with sampled plant DNA.

III. Polymerase Chain Reaction Based Markers

A decade after the emergence of AFLP, there was another breakthrough which involves the use of PCR in 1990 (Farooq and Azam, 2002).

PCR is an in vitro method of nucleic acid synthesis by which a particular segment of DNA can be specifically replicated (Mullis and Faloona, 1987). The process involves two oligonucleotide primers that flank the DNA fragment of interest and amplification is achieved by a series of repeated cycles of heat denaturation of the DNA, annealing of the primer to their complementary sequences, and extension of the annealed primers with a thermophilic DNA polymerase. Since the extension products themselves are also complementary to primers, successive cycles of amplification essentially double the amount of the target DNA synthesized in the previous cycle and the result is an exponential accumulation of the specific target fragment.

Genomic DNA from two different individual often produces different amplification and a particular fragment generated from one individual but not for other represent DNA polymorphism and can be used as genetic markers. The pattern of amplified bands so obtained could be use for genomic fingerprint (Welsh and McClelland 1990).

a) Randomly - amplified polymorphic DNA marker

The randomly-amplified polymorphic DNA marker (RAPD), detects nucleotide sequence polymorphism in DNA by using a single primer of arbitrary nucleotide sequence (Oligonucleotide primer, mostly ten bases long) (William *et al*, 1991). In this reaction, a single species of primer anneals to the genomic DNA at two different sites on complementary strands of DNA template.

Advantages associated with RAPD analysis include:

(i) Use of small amount of DNA which makes it possible to work with population that is not accessible with RFLP. It is fast and efficient in analysis having high-density genetic mapping as in many plant species such as alfafa (Kiss *et al*, 1993), fabean bean (Torress *et al*, 1993) and apple (Hammat *et al*, 1994)

(ii) Non involvement with radioactive assays (Kiss *et al*, 1993)

(iii) Non – requirement of species specific probe librararies

(iv) Non – involvement in blotting or hybridization.

Limitations of RAPD markers are:

(i) Its polymorphisms are inherited as dominant or recessive characters causing a loss of information relative to markers which show co-dominance.

(ii) Primers are relatively short, a mismatch of even a single nucleotide can often prevent the primer from annealing, hence leads to a loss of band.

(iii) Suffers from problems of repeatability in many systems, especially when transferring between populations or laboratories as is frequently necessary with marker assisted selection programs (Liu *et al*, 1994).

b) Amplified Fragment Length Polymorphism (AFLP)

$AFLP_S$ are fragments of DNA that have been amplified using directed primers from restriction of genomic DNA (Metthes *et al*, 1998). In this approach the sample DNA is enzymatically cut up into small fragments (as with RFLP analysis), but only a fraction of fragments are studied following selective PCR amplification (Liu *et al*, 1994). It is a combination of RFLP and RADP methods.

AFLP technique shares some characteristic with both RFLP and RAPD analysis (Farooq and Azam, 2002) and combines the specifically of restriction analyses with PCR amplification.

AFLP is extremely sensitive technique and the added use of fluorescent primers for automated fragment analysis system and software packages to analyze the biallelic data makes it well suitable for high thorough put analysis.

The major advantages of AFLP techniques (Farooq and Azam, 2002) are: (i) generation of a large number of polymorphism.

(i) No sequence information is required

(ii) The PCR technique is fast with high multiplex ratio which makes the AFLP very attractive choice.

The problems associated with AFLPs are of three types and all are related with practical handling, data generation and analysis. These problems are not unique to AFLP technology but also associated with other markers systems.

An ideal marker should have sufficient variation for the problem under study, be reliable and simple to generate and interpret. Unfortunately, neither AFLP nor other DNA markers exhibit these qualities. Thus a specific technique or techniques selected on the basis of objectives be utilized collectively to achieve the best results (Kharp *et al*, 1997; Harris, 1999).

c) Simple sequence repeat or short tandem repeats (SSRs) or micro satellites

These are ideal genetic markers for detecting differences between and within species of genes of all eukaryotes (Farooq and Azam, 2002).

It consist of tandemly repeated 2-7 base pair units arranged in repeats of mono-, di-, tri-, tetra and penta-nucleotides (A,T, AT, GA, AGG, AAAG etc) with different lengths of repeat motifs. These repeats are widely distributed throughout the plants and animal genomes that display high level of genetic variation based on differences in the number of tandemly repeating units of a locus. The variation in the number of tandemly repeated units results in highly polymorphic banding pattern (Farooq and Azam, 2002) which are detected by PCR, using locus specific flanking region primers where they are known.

Some of the prominent features of these markers are that they are dominant fingerprinting markers and codominant sequence tagged microsatellites (STMS) markers (Joshi *et al*, 2011).

The reproducibility of microsatellites is such that they can be used efficiently by different research laboratories to produce consistent data (Saghai Maroof *et al*, 1994). Locus-specific micro-satellite-based markers have been reported from many plant species such as Lettuce (*Lactuca sativa* L.) (Van de Wiel *et a,l* 1999), barley (*Hordeum vulgare* L.) (Saghai Maroof *et al*, 1994) and rice (*Oryza Sativa* L) (Wu and Tanksley, 1993).

Some other microsatellites based on the same principle include the following:

(i) ***Randomly Amplified Microsatellite Polymorphism (RAMP):*** This is a micro satellite – based marker which show a high degree of allellic polymorphism, but they are labor-intensive (Agarwal and Shrivastava, 2008). On the other hand RAPD markers are inexpensive but exhibit a low degree of polymorphism. To compensate for the weaknesses of these approaches, a technique termed as RAMP was developed (Wu *et al*, 1994). The technique involves a radiolabeled primer consisting of a 5^1 anchor and 3^1 repeats which is used to amplify genomic DNA in the presence or absence of RAPD primers. (Agarwal and Shrivastava, 2008).

(ii) **The Sequence Characterized Amplified Region (SCAR):** The SCARS are PCR-based markers that represent genomic DNA fragments at genetically defined loci that are identified by PCR amplification using sequence specific oligonucleotide primer (McDermoth *et al*, 1994).

(iii) **Simple Primer Amplification Reaction (SPAR):** SPAR uses the single SSR oligonucleotide principles.

(iv) **Sequence – Related Amplified Polymorphism (SRAP):**The aim of SRAP technique (Li and Quiros, 2001) is the amplification of open reading frames (ORFs). It is base on two-primer amplification using the AT- or GC- rich cores to amplify intragenic fragment for polymorphism detection (Agarwal and Shirvastava, 2008).

(v) **Target region amplification polymorphism (TRAP):** The TRAP technique (Hu and Vick, 2003) is a rapid and efficient PCR-based technique, which utilizes bioinformatics tools and expressed sequence tag (EST) database information to generate polymorphic markers, around targeted candidate gene sequences.

IV. Marker Assisted Selection (MAS)

MAS which is sometimes referred to as genomics is a form of biotechnology which uses genetic finger printing techniques to assist plant breeders in matching molecular profile to the physical properties of the variety. It is the identification of DNA sequences located near genes that can be tracked to breed for traits that are difficult to observe (Barloo and Stam, 1999). MAS refer to the use of DNA markers that are tight-linked to target loci as a substitute for or to assist phenotype screening. By determining the allele of a DNA marker, plants that possess particular genes or quantitative trait loci (QTL) may be identified based on their genotype rather than their phenotype.

Collard and Mackill (2006), reported the fundamental advantages of MAS compared to conventional phenotypic selection which are:-

(i) Simpler compared to phenotypic breeding
(ii) Selection may be carried out at breeding stage and single plants may be selected with high reliability.

In this technique, linkages are sought between DNA markers and agronomic important traits such as resistance to pathogens, insects and nematodes, tolerance to biotic stresses, quality parameters and quantitative traits.

MAS is in contrast to genetic engineering which involves the artificial insertion of such individuals genes from one organism into the genetic material of another (typically, but not exclusively from other unrelated species (Wamanda and Jonah, 2006).

V. Breeding of Polygenic Traits

The utilization of markers can obviously prevent loss of quantitative trait loci (QTL) common with some crops DNA markers and this allow us to unravel the genetic basis of traits expressing continuous phenotypic variations as they are abundant and scattered throughout the genome. By using dense genetic marker maps, the contributions of separate regions of the genome on the trait values can be estimated once the mapping population is sufficiently large. In addition, agronomic important traits like nutritional quality, yield, flower time and durable resistance which appear to follow complex, polygenic inheritance patterns with multiple genes having small effects on the trait value can easily by analyzed using markers. Evidences obtained from various crops indicate that even such complex traits appear to be determined by only a few major factors/genes. (Frary *et al*, 2000 and Thornsberry *et al*, 2001).

VI. Application of Molecular Markers in Plant Genome Analysis and Breeding

Molecular markers have been look upon as a tools 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).

a) Application of MAS in vegetative propagated crops

The first generation of DNA markers analysis of vegetative propagated crops at IITA was focused on germplasm characterization, construction of preliminary linkage maps and development of disease diagnostics in plantain/banana, cassava and yam (Ortiz, 2004).

i. *Plantain / Banana*

At IITA plantain improvement was nominated as the model system for developing molecular breeding systems within this Institute (Crouch and Tenkoune, 1999). Parthenocarpy (ability to develop fruit in the absence of seed development) was chosen as an ideal character for the initiation of a marker assisted selection program.

Parthenocarpy seems to be controlled by just a few genes yet a high proportion of current breeding populations are non-parthenocarpic but can not be identified as such until close to harvest. As such MAS for parthenocarpy at seedling stage would have a dramatic influence on breeding efficiency. Plantain and banana current priorities for the molecular breeding of Musa crops focus on the development of appropriate MAS schemes for parthenocarpy,apical dominance/regulated suckering and short cropping cycle. Thereafter, the focus will turn to markers for post-harvest characters and for favorable alleles contributing to heterosis in yield components.

ii. *Cassava*

The development of markers for post-harvest characters and virus resistances appears to warrant the greatest emphasis for cassava breeders. Based on the urgent need, William, (1999) proposed that attention should be focus on the development of DNA markers for tolerance to abiotic stress and for storage characteristics.

iii. *Legumes*

a. Bambara groundnut

Amplified fragment length polymorphism (AFLP) was used to assess genetic diversity among 100 selected bambara groundnut (*Vigna subterranea* L Verdc).

The results showed that bambara groundnut landraces from Tanzania form a genetically diverse population. Therefore, AFLP markers can be effectively employed to assess genetic diversity and to measure genetic relationship among accessions (Ntundu *et al*, 2004)

b. Cowpea

Cowpea, a legume crop grown in the semi-arid tropics is attached by insect pests. Thus, in cowpea, the development of markers for resistance to thrips, bruchids, maruca and pod borer is considered of great priority. In the long term, markers for resistance to parasitic weed (striga) and markers for genes contributing to drought resistance are considered a high priority intervention (Morales *et al*, 2000).

c. Soybean

Tremendous advances in all aspects of the molecular breeding of soybean are being made in advanced laboratories particularly in the USA. These may provide substantial background understanding many of the constraints to soybean cropping in sub-Saharan Africa which are very different. Therefore, a high priority for example, could be the use of marker-assisted breeding for selecting lines with the ability to cause suicidal germination of *Striga hermonthica*, a parasitic weed affecting maize but not soyabean (trap crop). In the longer term, increased nodulation and resistance to pod shattering would be highly important candidates for MAS systems (Ortiz, 2004).

d. Chickpea

Ascochyta rabiei (pass) Labri is the most severe fungal disease limiting chickpea production and studies in Syria revealed the occurrence of three pathotypes for *A. rabiei*. A set of micro satellite and RAPD markers were also used which lead to identification of suitable RAPD markers, allowing a more precise determination of the pathotypes. Furthermore, the availability of markers for

pathotype I and II allow the monitoring of the pathotype distribution, which gives the recommendation for the planting of suitable chickpea cultivars (Baum, 2003)

IV. *Cereal*

a. Maize

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 thousand 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-dominate 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/QLTs for diverse traits of agronomic and scientific interest (Prasanna and Pixley, 2010).

QLTs 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 world wide in optimizing MAS for improvement of both qualitative and quantitative inherited traits using maize as a model system. 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 02 gene and phenotypic selection for endosperm modifiers in the parental lines (Buba *et al,* 2005).Using MAS Scientist at IARI have pyramided major genes /QTL_S 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.

b. Sorghum

The development of DNA markers for resistance to pests and diseases in sorghum is receiving great priority e.g. in breeding new populations for striga prone environment (Crouch and Ortiz, 2004). Five genomic regions (QTL) associated with stable striga resistance from resistant line N13 have been identified across a range of 10 field trials in Mali and Kenya and two independent samples of a mapping population involving this resistance source, indicating that the QLT are biological realities.

v. *Vegetable - Okra*

Okra is an important vegetable in India, West Africa, south-east, Asia, USA, Brazil, Australia and Turkey, which provides and important input of vitamin and mineral salts including calcium (IBPGR, 1990).

Omahinmin and Osawaru (2005) reported that high degree of wide morphological variation exist among accession of okra which requires further evidence using molecular markers to clarify. Among wide relatives of okra, *Abelmoschus angulosus* showed complete resistance to yellow vein mosaic virus (YVMV) and powdery mildew disease. *A. ficulneus* and *A. moschatus* accompany a high degree of resistance only to powdery mildew and these germplasm can be potential genetic resources in breeding okra for YVMV and powdery mildew resistance (Samarajeewa and Rathnayaha, 2004). Furthermore, Aladele *et al, (*2008), reported that 93 accessions of okra were assessed for genetic distinctiveness and relationships using RAPD (i.e 75 primers used). 59 showed strong and clear amplification, 7 showed weak amplification, while 9 primers did not show any application.

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