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- FORUM -

THE USE OF DNA MARKERS FOR RAPID IMPROVEMENT OF CROPS IN AFRICA

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ABSTRACT

Genetic engineering and biotechnology are providing new tools for genetic improvement of food crops. Molecular DNA markers are some of these tools which can be used in various fields of plant breeding and germplasm management. For example, molecular markers have been used to confirm the identity of hybrids in breeding programmes. Another application of molecular markers is in determining phylogenetic relationships in related species. Information on phylogenetic relationships is useful in facilitating introgression of desirable traits from wild relatives to cultivated crop species. Molecular markers are also being used to construct genetic maps. A genetic map is a collection of genetic markers that have been grouped according to their linkage. Breeders can use DNA maps to carry out marker-assisted selection. This technique enables plants carrying desirable traits such as pest and disease resistance to be selected while still in the seedling stage. Ultimately, this enables the cloning of the genes to be used for crop improvement. The polymerase chain reaction (PCR) has become a popular technique for molecular genome mapping and the diagnosis of plant pathogens. The technique ensures amplification of specific DNA sequences by the use of primers and the enzyme Taq DNA polymerase. Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), microsatellites and Amplified Fragment Length Polymorphism (AFLP) are some of the most useful molecular markers for DNA fingerprinting. For viral, fungal and bacterial DNA fingerprinting and diagnosis as well as strain differentiation of rhizobia, PCR-RAPD and cDNA probes can be applied alongside with monoclonal antibodies.

Key Words: Crop improvement, DNA polymorphism, marker-assisted selection

RÉSUMÉ

Le génie génétique et la biotechnologie constituent de nouveaux outils pour l'amélioration génétique des cultures vivrières. Les marqueurs moléculaires figurent parmi ces outils qui peuvent être utilisés dans différents domaines de l'amélioration des plantes et de la gestion du matériel génétique. A titre d'exemple, les marqueurs moléculaires ont été utilisés pour confirmer l'identité des hybrides dans les programmes de sélection. Autre application des marqueurs moléculaires: la détermination des relations phylogénétiques entre les espèces apparentées. Les informations sur les relations phylogénétiques sont utiles, car elles facilitent l'introgression des caractères souhaitables des espèces sauvages dans les espèces cultivées. Les marqueurs moléculaires peuvent également servir pour tracer des cartes génétiques. Une carte génétique est une collection de marqueurs génétiques regroupés selon leur liaison génétique. Les sélectionneurs peuvent utiliser des cartographies de l'ADN pour procéder à la sélection à l'aide de marqueurs. Cette technique permet de sélectionner les plantes qui sont encore au stade de

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plantules et dotées de caractères souhaitables notammment la résistance aux ravageurs et aux maladies. Finalement, cela permet le clonage des gènes qu'on utilise en vue de l'amélioration des plantes. La réaction en chaîne de la polymérase (PCR) est devenue une méthode courante de cartographie du génome moléculaire et de diagnostic des phytopathogènes. Cette technique permet l'amplification de séquences d'ADN spécifiques suite à l'utilisation d'amorces et la polymérase Taq. Les polymorphismes de taille des fragments de restriction (RFLP), l'ADN polymorphique amplifié par séquence aléatoire (RAPD), les microsatellites, le polymorphisme de taille des fragments amplifiés (AFLP), constituent un groupe de marqueurs moléculaires utilisés pour la prise des empreintes d'ADN. Pour la prise d'empreintes d'ADN et le diagnostic des virus, des cryptogames et des bactéries, et pour différencier les rhizobiums, les sondes ADNc, les méthodes PCR- RAPD et les anticorps monoclonaux peuvent être utilisées.

Mots Clés: Crop improvement, DNA polymorphism, marker-assisted selection

INTRODUCTION

The use of molecular markers is based on naturally occurring polymorphism whose magnitude has only recently been appreciated. Biotechnology provides powerful new tools for crop improvement. Rapid advances in genome research and molecular biology have led to the use of DNA markers in plant breeding. Target genes in a segregating population can be identified with the assistance of DNA markers so as to accelerate traditional breeding programmes.

Molecular markers include proteins and nucleic acids that are detectably different, i.e., polymorphic among individuals or populations. Molecular mapping techniques are becoming increasingly useful to scientists for various purposes. Examples are restriction fragment length polymorphism (RFLPs), polymerase chain reaction (PCR) based random amplified polymorphic DNAs (RAPDs), sequence characterised amplified regions (SCARs), sequence tagged sites (STS), single polymorphic length amplification test (SPLAT), and amplified fragment length polymorphism (AFLP). Others are fingerprinting of tandem repeats of 9 to 64 bp long sequence (minisatellites) also referred to as variable number tandem repeats (VNTRs), fingerprinting of tandem repeats of 2-6 bp long sequences (microsatellites) or simple sequence repeats (SSRs) or simple sequence length polymorphism (SSLP). The opportunities, pros and cons of these techniques are discussed below, and their current usage at the International Institute of Tropical Agriculture (IITA) described.

CHOICE OF DNA MARKERS FOR AFRICA

A number of techniques are known which can be used for the above applications – the choice of which depends a lot on the system one is working with, cost and time consideration, number of samples, how quickly you need the data, the technique that would best yield the maximum data, etc. For mapping and marker-assisted selection (MAS) one should be able to quickly analyse large number of plants. These consideration are evaluated and compared in Tables 1 and 2.

Restriction Fragment Length Polymorphism (RFLP). The detection and exploitation of naturally occurring DNA sequence polymorphisms represent one of the most significant recent developments in molecular biology. Useful polymorphisms have been obtained using Restriction Fragment Length Polymorphisms (RFLPs) with various restriction enzymes and nuclear genomic or cDNA probes.

Restriction endonuclease (RE) digestion of total genomic DNA followed by hybridisation with a labelled probe (radioactive or nonradioactive) reveals hybridising fragments of different sizes. Thus RFLPs has been used extensively for genetic studies (Tables 1 and 2).

Polymerase Chain Reaction (PCR). 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, amplification is achieved by a series of repeated cycles of heat denaturation of the DNA, annealing of the primers 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 synthesised in the previous cycle. The result is an exponential accumulation of the specific target fragment.

Random Amplified Polymorphic DNA (RAPD). A simple, sensitive and relatively cheap PCR-based technique in comparison to RFLP is RAPD. Amplification of DNA is based on the use of either arbitrary primer DNA sequences available commercially or representative DNA sequences. The amplification reaction depends on homology between the genomic DNA and these very short oligonucleotide primers (10 bp). The products are easily separated by standard electrophoretic techniques and visualised by ultraviolet (UV) illumination of ethidium-bromide stained gels. The amplification products will vary in size according to the distance between the sites of primer homology with the target DNA. If these distances vary between individuals, then a polymorphism will result. Generally, no prior sequence information is required to design the primers involved in the PCR reaction, hence the term "random" in the acronym. RAPD markers can be used in almost the same way as RFLP markers except that the former is a dominant marker while the RFLP is a co-dominant one.

RAPD markers have been used to generate molecular markers in a variety of organisms, including humans, fungi, and plants (Williams *et al.*, 1991), and more recently, insects (Black *et al.*, 1992).

TABLE 1. DNA marker technologies for crop improvement

Techniques	Application	Pros	Cons
RFLP	Alignment of linkage maps to chromosome numbers	Effectively infinite in number Single protocol for all markers Codominant Robust Transferable	Uses large quantities of DNA Variable-levels of polymorphism Difficult in large genome species Generally uses isotopes
RAPD	Construction of index markers Diversity studies Rare-allele-based selection	Quick Easty Primers available Polymorphism easily detected PCR-based Many markers can be screened using a single primer	Dominant markers Experimental conditions critical Poor transferability Poor reproducibility
SSLP	Constructing index maps Mapping in tetraploids Population genetics Examining gene flow Determining hybrids	Simple PCR-based Codominant Highly polymorphic Easily transferable Quick to perform Robust	Long development time Need specific primers Expensive to establish
AFLP	High resolution mapping DNA profiling Basis of allele specific diagnostics	Detects large number of loci Highly polymorphic Robust	Technically difficult Expensive to establish Uses radio isotopes Uses denaturing PAGE

TABLE 2. Properties of systems for generating genetic markers

Properties	RFLPs	RAPDs	SSLP	AFLP
Principle	Endonuclease restriction, Southern blotting Hybridisation	DNA amplification with random primers	DNA amplification of simple sequence repeats with specific primer pairs	Endonuclease restriction of PCR products
Type of polymorphism	Single base change insertions/deletions	Singe base changes insertions deletions	Changes in length of repeats insertions deletions	Single base changes insertions deletions
Genomic abundance	High	Very high	High	High
Level of polymorphism	Medium	Medium	High	Very high
Inheritance	Codominant	Dominant	Codominant	Dominant/ codominant
Quality of DNA required	Relatively pure	Crude	Relatively pure	Relatively pure
Quantity of DNA required	5-20 μg	10-25 μg	50-100 μg	0.2-2-5 μg
Genome sequence information required?	No	No	No	No
Primer sequence required?	No	Arbitrary primers	Yes	Yes
Use of detection system	Radioactive/ non-radioactive	Neither	Neither	radioactive/ non-radioactive
Start-up costs	Medium/High	Low	High	High
Reliability	High	Intermediate	High	Very high
Labour intensive	Yes	No	No	Yes
Automation	Difficult	Difficult	Difficult	Possible, but requires lot of skill
Recurring costs	High	High	High	Very high
Technically demanding	Intermediate	Low	Intermediate	High
Suitability in less advanced labs	Appropriate if equipment available	Appropriate	Appropriate if facilities exist	Difficult to run
Licensing required?	No	Probably yes	No	Probably yes
Application mapping	Genome	DNA finger-printing, genome mapping	DNA finger-printing, genome mapping	Genome mapping , DNA finger-printing
Isolation of genes by this method	Long process	Long process	Long process	Chances are high
DNA markers for traits	Possible	Chances are good	Chances are good to identify markers	Chances are high
Properties	RFLPs	RAPDs	SSLP	AFLP
Application in public sector labs	Yes	Yes	Yes	Yes
Application in private sector labs	Yes	Yes	Yes	Yes
IITA's stand until today	Yes	Yes	Already in use	Studying the feasibility and trying to get licensing

DNA Amplification Fingerprinting (DAF). DAF is quite similar to RAPD but DNA amplification is achieved using one or more arbitrary primers 5-6 nucleotides in length. DAF generates a complex and more detailed pattern when separated on a polyacrylamide gel which is visualised using the highly sensitive silver staining method (Caetano-Anolles *et al.*, 1991).

Repetitive DNA sequences. These are found at many locations throughout the genome of most eukaryotes (Tautz and Renz, 1984; Jeffreys *et al.*, 1985). A repetitive DNA sequence when used as a probe produces a very complex Southern hybridisation pattern that is called DNA fingerprint (Jeffreys *et al.*, 1985). DNA fingerprinting has been shown to be a powerful means of detecting variations among individuals.

a. Minisatellites, Variable Number of Tandem Repeats (VNTRs). Minisatellites or VNTRs are hypervariable regions of the genome which exhibit polymorphism due to variable number of tandem repeats of short sequences (9-64 bp).

b. Microsatellites. DNA sequences with short repeated motifs (2-6 bp) are called simple sequence repeats (SSRs), microsatellites (Hamada *et al.*, 1982; Litt and Luty, 1989; Epplen *et al.*, 1991; Todokoro *et al.*, 1995) or second generation markers (Davies, 1993). Because microsatellites are highly polymorphic, randomly distributed in the genome, and easily analysed by the PCR technique, they are regarded as a general and novel source of genetic markers. This polymorphism is now being used to construct high resolution genetic maps.

Amplified Fragment Length Polymorphism (AFLP). AFLP is a PCR-based technology for marker-assisted breeding and genotyping. AFLP represents a significant breakthrough compared to the currently available methods in terms of facility, precision, flexibility, speed and cost. Essentially, AFLP enables the generation of thousands of DNA markers from a genome of any complexity and without prior knowledge of the genome's structure or sequence. The method of data collection and processing is a proprietary software package developed by Keygene. This technology is expected to revolutionise the process of plant breeding in the years to come and also, has significant proven utility in fields beyond the sphere of crop improvement, such as microbial typing.

AFLP involves the amplification of small restriction fragments, obtained by cleaving genomic DNA with restriction enzymes, to produce high resolution DNA "fingerprinting" patterns on denaturing polyacrylamide gels. The rationale of the AFLP technique is based on the use of specifically designed PCR primers which selectively amplify a small subset of restriction fragments, or "markers", out of a complex mixture comprising as many as several million different fragments. The products of the reaction can be visualised by conventional DNA staining or DNA labelling procedures using either radioactive or non-radioactive methods.

AFLP is an extremely flexible technology which offers multiple applications in the field of crop breeding and plant genome analysis, especially in the fields of genotying, marker-assisted breeding and plant genome analysis.

i) Genotyping:
- Genetic distance analysis
- Identification of parental lines and cultivars
- Seed quality control/hybrid purity

ii) Marker-assisted breeding:
- Development of DNA markers linked to agronomically important traits
- Identification and selection of QTLs
- Indirect selection using DNA markers
- Introgression of new traits:
  - reduction in backcross generations
  - selection against linkage drag

iii) Plant genome analysis:
- Construction of high resolution genetic maps
- Positioning of traits on a genetic map
- Construction of physical maps
- Map-based gene identification and cloning

DNA MARKER APPLICATIONS AT IITA

Crop and germplasm enhancement. In order to determine the feasibility of using RAPD marker

technology to detect genetic diversity in cowpea, a survey of 95 selected accessions of cowpea from diverse geographical areas has been undertaken. After screening over 120 random primers, nine were selected to genotype the cowpea germplasm. RAPD fingerprint patterns obtained showed a high degree of genetic polymorphism among the cowpea germplasm. The usefulness of this marker system for the study of genetic structure of cowpea germplasm has been demonstrated (Mignouna *et al.*, 1998b).

Similar to the genetic diversity studies, phylogenetic relationships between the cultivated Guinea yams (*Dioscorea rotundata* and *D. cayenensis*) and some wild *Dioscorea* species of Nigeria were established based on RAPD markers. This study revealed that *D. rotundata* and *D. cayenensis* are two separate species and that *D. rotundata* is genetically close to the wild species, *D. abyssinica* and *D. praehensislis*, while *D. cayenensis* was genetically related to *D. burkilliana* (Mignouna *et al.*, 1998a).

Genetic analysis of African cassava varieties resistant to CMV. Along with five improved varieties and one cassava mosaic virus (CMV) resistant genetic stock (58308) of cassava, 35 landraces from West Africa with varying degrees of resistance to cassava mosaic virus (CMV) were analysed using RAPD. The objective was to assess the utilisation of RAPD markers to differentiate the different cassava varieties and to determine the genetic diversity among the 41 accessions. This study showed that most of the cassava landraces which are resistant to CMV are genetically distant from the breeding line 30572 and the genetic stock 58308. These results have some implication on the CMV resistance breeding at IITA (Mignouna and Dixon, 1997).

Genetic diversity in cassava germplasm from Benin Republic. Using RAPD markers, the genetic variation among 28 cassava accessions collected in farmers' fields in the Republic of Benin was also investigated. A set of 10 random sequence 10-mer primers out of 100 screened generated 78 amplified polymorphic DNA. Based on cluster analysis, the accessions were categorised into 6 groups, none of the accessions was clustered according to the geographic origin. This study has shown the efficiency of molecular markers system for cassava germplasm characteristaion, utilisation and management (Tonukari *et al.*, 1997). A similar study on a more wider base of farmer's cassava germplasm collected all over African countries is being analysed using RAPD, AFLP and microsatellite markers.

RFLP. Wild *Vigna* germplasm has potentially useful genes for pest and disease resistance that can be transferred to cultivated cowpea. Classical breeding approaches are being applied by crossing improved cowpea varieties to their wild relatives and subsequent selection of the progenies by visual or morphological means. Now molecular markers offer more reliable and accurate tools to detect interspecific hybrids.

Interspecific hybrids in *Vigna* have been confirmed by the use of restriction enzymes and nuclear genomic probes, especially a nuclear probe encoding for the 35S sub-unit of ribosomal DNA (SR Schnapp, unpublished data). In addition, RFLP markers are currently being used as anchor markers to establish genetic linkage maps of yams.

AFLP. AFLP marker-system has been adapted and used for DNA fingerprinting of cowpea and yams germplasm. This marker-system is efficient in detecting the genetic diversity among the cultivars and has been used to identify duplicates in yam germplasm. A preliminary genetic linkage map consisting of four linkage groups is being generated in *D. rotundata* using AFLP markers. A similar map is being developed for *D. alata*. Effort is on-going to produce a genetic linkage map with molecular markers that cover the ten chromosomes of yam genome.

Banana/plantain. Molecular genetic analysis based RFLP appears to have limited application in *Musa*. This is due to the low number of polymorphic loci which can be detected using this technique. Hence the PCR-based analysis systems are now the technique of choice at IITA, Onne.

PCR primers are being generated from *Musa* microsatellite sequences in collaboration with the Birmingham University, U.K. Initial studies

focussed on two main areas: (i) germplasm characterisation and definition of heterotic groups to maximise hybrid vigour, and (ii) marker assisted selection of those characters which are difficult to score or expressed late in the growth season. Through molecular genotyping it should be possible to estimate the level of useful variation which could be directly or indirectly available to the *Musa* breeder.

Molecular genetic analysis may also locate duplicate accessions and this will result in improved conservation and management of *Musa* genetic resources. These techniques will also be used to test putative theories of *Musa* taxonomy, phylogeny and genetic behaviour. Such information will be of crucial importance to the development of efficient breeding strategies.

Onne scientists have initiated studies to define the scope with which marker assisted selection (MAS) can be effectively applied to specific traits in *Musa* breeding. This would then be the first programme to apply marker-assisted-selection at IITA. Recently, microsatellite markers for cowpea and yams have been developed in collaboration with University of Saskatchewan, Canada. These microsatellites are being used for genetic studies and generation of genetic linkage map in these crops.

Detection and fingerprinting of plant pathogens. PCR is an extremely sensitive and specific technique for the detection and identification of plant pathogens, and it can be used to investigate the genetic diversity of pathogen populations. Because PCR amplifies nucleic acids, the technique could be useful in overcoming many of the present difficulties associated with serological detection methods, e.g. low titer of antigen, cross-reaction of antibodies with heterologous antigens, and developmental or environmental regulation of antigen production.

Geminiviruses. Geminiviruses are well suited to detection and identification by PCR because they replicate via a double-stranded, circular DNA intermediate- the replicative form which can serve as a template for amplification by PCR. The complete nucleotide sequences of several bipartite, whitefly-transmitted geminiviruses have been published. These sequences were aligned so that highly conserved regions could be identified for use in designing degenerate PCR primers.

a. Cowpea Golden Mosaic Disease (CGMD). In 1977 cowpea golden mosaic disease was first discovered at Onne, Nigeria. Infected cowpea plants have large bright yellow patches on their leaves, and, under severe infection, the entire leaf surface turns bright yellow. The virus is transmitted by the whitefly (*Bemissia tabaci*) in a persistent manner but is not seed– or sap-transmitted.

There are similar descriptions of diseases in cowpea that are not sap-transmissible but only whitefly-transmitted. A similar disease occurs in Kenya, Tanzania, and Niger. Also, a whitefly-transmitted cowpea disease is reported from Brazil. These diseases may all be caused by a similar or identical virus. So far, no information is available on virus particles of any of these diseases. Various attempts to purify this virus by IITA and Vancouver Research Station have so far failed.

Because of the increasing importance of cowpea golden mosaic disease, rapid and accurate methods are needed for virus detection and subsequent identification. Such methods would greatly facilitate studies of the epidemiology and the genetic diversity of these viruses. This information would have important implications in designing strategies for breeding for disease resistance and disease control.

Two primer combinations, PARLv722 and PAL1c1960, gave the best PCR-amplification.

b. Cassava Mosaic Geminivirus. It was possible to amplify CMV in infected tissues and whiteflies. This work is being continued to study the recovery phenomenon in resistant cassava lines after CMV infection.

Banana streak badnavirus (BSV). PCR is used both at Onne and Ibadan for the detection of BSV An immunocapture polymerase chain reaction (IC-PCR) method for a highly sensitive analysis of BSV in infected plants has been developed in the Biotechnology Research Unit (BRU) at IITA. In this method, DNA extraction from the plant

material is not necessary. Viruses are enriched during the assay by antibody trapping in the PCR microtubes. The procedure is sensitive enough to allow a simple detection of BSV in *in vitro* cultured plants in which the detection of BSV by conventional immunological methods is difficult or even impossible.

Potyviruses. Several primer combinations are available for the detection of potyviruses in yam and cowpea. Also, the immunocapture-RT-PCR developed by Natural Resources Institute (NRI), UK is applied routinely at IITA for the detection of yam mosaic virus.

Cucumber mosaic virus. BRU has developed the detection of cucumber mosaic virus in cowpea and banana by RT-PCR as well as by immunocapture RT-PCR. Several primer combinations are available. Right now we are studying the sensitivity of immunocapture-RT-PCR in comparison to ELISA.

Fungal pathogens. One of the major obstacles for the development of cultivars resistant to pathogens is the great variability and occurrences of pathogen races. At IITA, we have just started the application of RAPD technique as a fast and reliable alternative method to distinguish fungal races. Initial results have been encouraging.

Bacterial pathogens. Studies have been initiated to apply RAPD technique as a fast and reliable alternative to distinguish bacterial blight of cowpea. Likewise, the results so far are very encouraging.

Genetic studies of *Striga*. Genetic variability in *Striga aspera* and *S. hermontica* and their hybrids were assessed using morphological characters and RAPD markers (Aigbokhan, unpublished). This study showed that these species were genetically distinct and the true hybrids were confirmed by RAPD marker.

Characterisation of biotypes of whiteflies and beetles using RAPD-PCR. The whitefly *B. tabaci* has become an increasingly important pest of crops in the tropics. Besides being a pest, whiteflies also transmit many plant viruses. Although host-induced characteristics were noted for the whiteflies, there is presently no evidence of consistent morphological differences that distinguish these adapted biotypes. Further, the taxonomic relationship between these biotypes is not clear. The ability to differentiate biotypes of *B. tabaci* using DNA-based markers would facilitate genetic and physiological research involving this insect. The preliminary results demonstrate the use of RAPD to differentiate the host-adapted biotypes of *B. tabaci*.

Cowpea bruchid (*Callosobruchus maculatus*) is a serious pest of cowpea. In order to determine differences in biotypes, if any, beetles collected from different locations in Nigeria were tested by RAPD. It was initially difficult to get good DNA preparations from beetles. After modifying several methods, we were able to get good quality DNA which can now be used for RAPD. Work will be continued to study whether any polymorphism exists between beetles from different locations.

DNA fingerprinting of rhizobia. One of the major constraints in the successful application of rhizobial inocula in the field situation is identifying the fate of strains introduced in the field. It is not possible to distinguish individual strains of rhizobia based on visual observation. Also, it is not possible to readily determine which legume nodule was formed by which strain except in rare cases where one of the rhizobial strains produces a pigment such as melanin. Classical approaches, such as serology and antibiotic resistance have been used for strain identification. However, antibiotic resistance pattern has limitations because it is labourious and time-consuming. Enzyme-linked Immunosorbent Assay (ELISA) has been successfully applied for studies on microbial ecology (Asanuma *et al.*, 1985; Rao *et al.*, 1985).

In order to study inoculum establishment and survival, it is necessary to have a sensitive and reliable means of specifically detecting the inoculated strain in the field. Furthermore, it is important to determine the right strains for inoculation when several isolates are available. Some isolates may be duplicates. This can be eliminated through the use of molecular markers.

Our aim was to determine whether RAPDs are useful for strain discrimination and identification of tropical rhizobia. We have explored the

possibility of using RAPD to analyse 33 isolates of rhizobia collected from four legume hosts in Nigeria and our results are encouraging.

LOOKING AHEAD

Physical mapping and *in* situ hybridisation. *In situ* hybridisation is an important tool for the physical mapping of numbers and locations of sequences and is particularly valuable for identifying the sites of highly repetitive genes that are difficult to map by other methods. The *in situ* hybridisation technique is being applied to both cowpea and *Musa*.

Chromosome walking and gene isolation. High density genetic maps based upon tightly linked DNA markers can provide starting points for chromosome walking experiments to mark along the chromosome to the gene of interest. Examples of genes isolated using map-based cloning are the genes for *Pseudomonas (Ralstonia)* resistance in tomato using RAPD markers (Martin *et al.*, 1991) and bacterial blight (*Xanthomonas*) resistance genes in rice using RFLP markers (Song *et al.*, 1995). Using this 'map based cloning' technique, the gene is then excised with restriction enzymes, cloned, characterised and ultimately used in transformation, but need finer mapping to actually locate the gene for cloning.

Information from DNA markers serves many diverse purposes in crop breeding such as in wide crosses to identify genes, tag valuable traits from exotic germplasm and infer evolutionary relationships.

Marker-assisted selection (MAS). One can use DNA markers that are tightly linked to the gene of interest in diagnosing agronomically specific characters. One of the practical applications of DNA markers in plant breeding is marker-assisted selection (MAS) of those characters which are difficult to score or expressed late in the season. The significance of MAS is the diagnosis of the presence of the gene, without having to wait for the gene's effects is still to be seen. However, the practical applications of DNA markers are now increasingly adopted by breeders.

CONCLUSION

DNA marker techniques are still tedious, labourious, and, to some extent, expensive to generate and to implement in a MAS where large populations are generated in breeding programmes. Nevertheless, effort is being made to overcome these constraints, particularly by developing efficient methods to screen large population at low cost. They are not going to replace classical plant breeding. However, DNA markers can complement and significantly accelerate many breeding efforts, which is more efficient, and allow manipulations not possible through traditional means. In recent years, SSLP has gained more popularity in crop improvement than AFLP because AFLP is more difficult to run. Furthermore, DNA markers may provide new approaches to attain objectives which have proven difficult to achieve with classical techniques, such as the introgression of valuable traits e.g., disease or pest resistance from exotic germplasm, into agronomically desirable varieties. Using the saturated map constructed from DNA markers, map-based cloning can be achieved for direct benefit to crop improvement.

Plant breeders are using these genetic maps to undertake efficient breeding programmes so that agronomic traits can be more clearly understood providing alternative screening strategies for easy assessment of the characters.

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