International Journal of Current Agricultural Research Vol. 1, No. 1, PP.01-06 November 2012
http://www.wrpjournals.com/journals/V/IJCAR

Genomic Tools in Crop Improvement

Mushtaq Ahmad Dar, Gul Zaffar, S.M.Razvi, S. D. Mir, Z. A. Dar and M. R. Mir

Sher-E-Kashmir University of Agricultural Sciences &Technology of Kashmir Shalimar Campus, Srinagar -191 121

Accepted 25th October, 2012; Published 10th November, 2012

Genome is the entity of an organism's heredity information. It is encoded either in DNA or, for many types of viruses, in RNA. The genome includes both the genes and the non-coding sequences of the DNA. Genomics is a new field in biology that is concerned with the whole genome analysis, from sequence to function and derived information. The year 2010 marks the 10th anniversary of the first plant genome sequence (*Arabidopsis thaliana*). Triggered by advancement in sequencing technologies, many crop genome sequences have been produced, with eight published till date. To date, however, only the rice (*Oryza sativa*) genome sequence has been finished to a quality level similar to that of the *Arabidopsis* sequence. This trend to produce draft genomes could affect the ability of researchers to address biological questions of speciation's and recent evolution or to link sequence variation accurately to phenotypes. The increasing availability of DNA sequence information enables the discovery of genes and molecular markers associated with diverse agronomic traits creating new opportunities for crop improvement among different classes of molecular markers, SSR markers have proven to be the marker of choice for a variety of applications, particularly in breeding. In addition, DArT (Diversity array technology) markers represent another high-through put marker system, which can be used to prepare the whole genome map even without the availability of sequence data. In recent years, ESTs or gene sequences have been used to identify SSRs, SNPs and genetic molecular markers have been developed in several cereal crops species. Marker assisted selection (MAS) is a powerful tool for the indirect selection of difficult traits at an early stage before production of the next generation, thus speeding up the process of conventional plant breeding and facilitating the improvement of traits that cannot be improved easily by conventional method Association mapping might offer more power than linkage analysis for identifying genes responsible for the variation in a quantitative trait . Functional genomics involves the identification of the function of genes *per se* or those derived from a known allelic difference conferring an improved phenotype. Several techniques like SAGE, MPSS and micro and macro-arrays, are available, for the estimation of mRNA abundance for large number of genes simultaneously. Tilling has proven to be a practical, efficient, and an effective approach for functional genomic studies in numerous plants species. Eco-TILLING, which is a variant of TILLING, examines natural genetic variation in populations and has been successfully utilized in plants to discover SNPs including rare ones. Comparative genomics approach using bioinformatics tools might provide an opportunity for efficient transfer of information from model species and major crops to minor and orphan crops. Genomics research is generating new tools that could increase the efficiency and precision of crop improvement however high costs currently limit the implementation of genomic-assisted crop improvement particularly for minor crops. Nevertheless, marker-assisted breeding and selection will gradually evolve into genomic-assisted breeding for crop improvement .

Key words: Genome, Molecular Markers, Tilling, Eco-tilling, Functional Genomics, Comparative Genomics, QTL, SAGE, MPSS, Microarray, DArT.

INTRODUCTION

Genomics to describe the mapping, sequencing and characterization of genomes. It is the study of the global properties of genomes of related organisms which distinguishes it from Genetics which studies the properties of single gene or group of genes (Ridley, M. 2006). Plant genomes have been subjected to both structural and functional genomics research, which during the last two decades covered both basic and applied aspects. Fast evolution of novel technologies in the recent past has deepened our understanding from genome to gene level and has facilitated understanding about gene networks for plant development and agronomy in many model or major crop species (Feuillet *et al.,* 2010). These technologies included molecular markers, trait mapping, physical mapping, transcriptome/ genome sequencing and functional genomics. Further, comparative genomics studies especially in the species of the families Poaceae, Brassicaceae and Solanceae showed co-linearity in different genomes of corresponding species of a particular family . It has been suggested and demonstrated that the information gained from one plant species also benefits the improvement of syntenous species. More interestingly, genomics tools and approaches are revolutionizing the breeding methodology, a procedure referred as 'genomics-assisted breeding' through molecular breeding and directed mutagenesis that significantly enhances the efficiency of breeding for improvement of agronomical traits. In addition, genomics accelerates plant biotechnology by providing more native target genes (Rafalski, 2010). Many agronomical traits are under control of genes with unknown functions, which can be mapped and cloned based on their position on genetic maps (mapbased or positional cloning). The cloned genes, containing their own exons, introns and regulatory elements, are good resources for transformation into other varieties of the same crop or into other related crop species without additional modification.

The detection and exploitation of genetic variation have always been an integral part of plant breeding. DNA-based molecular markers are useful for detecting the genetic variation available in germplasm collections and/or breeding lines (Edwards and Batley,. 2010). During the past two decades, many different molecular markers have been developed for most major crop species. These markers have been used extensively for the development of saturated molecular genetic and physical maps and for the identification of genes or quantitative trait loci (QTLs) controlling traits of economic importance for marker-assisted selection (MAS) (Collard and Mackill, 2008). In addition to traditional trait or QTL mapping using biparental populations, new approaches such as association mapping , advanced back-cross QTL analysis, functional genomics, genetical genomics, allele mining, TILLING and EcoTILLING have become available in recent years. Genomics-assisted breeding is a holistic approach using different genomic strategies and tools. The prediction of phenotype from genotype using different genomic tools and strategies is the basis of genomics assisted breeding. By improving the precision and efficiency of predicting phenotypes from genotypes, the development of improved cultivars with enhanced resistance or tolerance to biotic and/or abiotic stresses and higher agronomic performance can be greatly accelerated. Indeed, successful examples of genomics-

***Corresponding author:** sahilmushtaqdar@rediffmail.com

assisted breeding have been demonstrated for several cereals. Genomics-assisted breeding approaches have greatly advanced with the increasing availability of genome and transcriptome sequence data for several model plant and crop species (Varsheny *et al*., 2007).

Classification of genomics

Genomics can be classified into

- Molecular Genomics
- Structural Genomics
- Functional Genomics
- Comparative Genomic

MOLECULAR GENOMICS

The concept of genetic markers is not a new one; Gregor Mendel used phenotype-based genetic markers in his experiment in the nineteenth century. Later, phenotype based genetic markers for Drosophila led to the establishment of the theory of genetic linkage. The limitations of phenotype based genetic markers led to the development of more general and useful direct DNA based markers that became known as molecular markers. A molecular marker is defined as a particular segment of DNA that is representative of the difference at the genome level. Molecular markers may or may not correlate with phenotypic expression of a trait. Molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell are not confounded by the environment, pleiotropic and epistatic effects.

Molecular marker techniques

The publication of Botstein *et al*., (1980) about the construction of genetic maps using restriction fragment length polymorphism (RFLP) was the first reported molecular marker technique in the detection of DNA polymorphism. Basic molecular marker techniques Basic marker techniques can be classified into two categories:

- non-PCR-based techniques or hybridization based techniques and,
- PCR-based techniques.

Non-PCR-based techniques

Restriction fragment length polymorphism (RFLP)

In RFLP, DNA polymorphism is detected by hybridizing a chemically labelled DNA probe to a Southern blot of DNA digested by restriction endonucleases, resulting in differential DNA fragment profile. This differential profile is generated due to nucleotide substitutions or DNA rearrangements like insertion or deletion or single nucleotide polymorphisms. The RFLP markers are relatively highly polymorphic, co-dominantly inherited and highly reproducible. Because of their presence throughout the plant genome, high heritability and locus specificity the RFLP markers are considered superior. The method also provides opportunity to simultaneously screen numerous samples. DNA blots can be analyzed repeatedly by stripping and reprobing (usually eight to ten times) with different RFLP probes. The technique is not very widely used because it is time consuming, involves expensive and radioactive/toxic reagents and requires large quantity of high quality genomic DNA. The requirement of prior sequence information for probe generation increases the complexity of the methodology. These limitations led to the conceptualization of a new set of less technically complex methods known as PCR-based techniques.

PCR-based techniques

After the invention of polymerase chain reaction (PCR) technology (Mullis and Faloona 1987), a large number of approaches for generation of molecular markers based on PCR were detailed, primarily due to its apparent simplicity and high probability of success. Usage of random primers overcame the limitation of prior sequence knowledge for PCR analysis and facilitated the development of genetic markers for a variety of purposes. PCR-based techniques can further be subdivided into two subcategories: (1) arbitrarily primed PCR-based techniques or sequence nonspecific techniques and (2) sequence targeted PCR-based techniques.

Arbitrarily primed PCR-based markers

Random amplified polymorphic DNA (RAPD)

The basis of RAPD technique is differential PCR amplification of genomic DNA. It deduces DNA polymorphisms produced by "rearrangements or deletions at or between oligonucleotide primer binding sites in the genome'' using short random oligonucleotide sequences (mostly ten bases long). As the approach requires no prior knowledge of the genome that is being analyzed, it can be employed across species using universal primers. The major drawback of the method is that the profiling is dependent on the reaction conditions so may vary within two different laboratories and as several discrete loci in the genome are amplified by each primer, profiles are not able to distinguish heterozygous from homozygous individuals (Bardakci 2001). Due to the speed and efficiency of RAPD analysis, high-density genetic mapping in many plant species such as alfalfa , faba bean and apple (Hemmat *et al*. 1994) was developed in a relatively short time. The RAPD analysis of NILs (non-isogenic lines) has been successful in identifying markers linked to disease resistance genes in tomato (Lycopersicon sp.) and common bean (*Phaseolus vulgaris*) (Adam-Blondon *et al.* 1994). Arbitrarily primed polymerase chain reaction (AP-PCR) and DNA amplification fingerprinting (DAF) techniques are independently developed methodologies, which are variants of RAPD. For AP-PCR a single primer (about 10–15 nucleotides long) is used. The technique involves amplification for initial two PCR cycles at low stringency. Thereafter the remaining cycles are carried out at higher stringency by increasing the annealing temperature). This variant of RAPD was not very popular as it involved autoradiography but it has been simplified as fragments can now be fractionated using agarose gel electrophoresis. The DAF technique involves usage of single arbitrary primers shorter than ten nucleotides for amplification and the amplicons are analysed using polyacrylamide gel along with silver staining.

Amplified fragment length polymorphism (AFLP)

To overcome the limitation of reproducibility associated with RAPD, AFLP technology (Vos *et al*., 1995) was developed. It combines the power of RFLP with the flexibility of PCR-based technology by ligating primer recognition sequences (adaptors) to the restricted DNA and selective PCR amplification of restriction fragments using a limited set of primers. The primer pairs used for AFLP usually produce 50–100 bands per assay. Number of amplicons per AFLP assay is a function of the number selective nucleotides in the AFLP primer combination, the selective nucleotide motif, GC content, and physical genome size and complexity. The AFLP technique generates fingerprints of any DNA regardless of its source, and without any prior knowledge of DNA sequence. Most AFLP fragments correspond to unique positions on the genome and hence can be exploited as landmarks in genetic and physical mapping. The technique can be used to distinguish closely related individuals at the

sub-species level (Althoff *et al*. 2007) and can also map genes. Applications for AFLP in plant mapping include establishing linkage groups in crosses, saturating regions with markers for gene landing efforts (Yin *et al.* 1999) and assessing the degree of relatedness or variability among cultivars (Mian *et al*. 2002). For high-throughput screening approach, 123 fluorescence tagged primers are also used for AFLP analysis. The amplified fragments are detected on denaturing polyacrylamide gels using an automated ALF DNA sequence with the fragment option.

Sequence specific PCR based markers

With the advent of high-throughput sequencing technology, abundant information on DNA sequences for the genomes of many plant species has been generated . The Arabidopsis Genome Initiative 2000; Yu *et al*., 2002). ESTs of many crop species have been generated and thousands of sequences have been annotated as putative functional genes using powerful bioinformatics tools. In order to correlate DNA sequence information with particular phenotypes, sequence-specific molecular marker techniques have been designed.

Microsatellite-based marker technique

Microsatellite or short tandem repeats or simple sequences repeats are monotonous repetitions of very short (one to five) nucleotide motifs, which occur as interspersed repetitive elements in all eukaryotic genomes (Tautz and Renz 1984). Variation in the number of tandemly repeated units is mainly due to strand slippage during DNA replication where the repeats allow matching via excision or addition of repeats. As slippage in replication is more likely than point mutations, microsatellite loci tend to be hyper variable. Microsatellite assays show extensive inter-individual length polymorphisms during PCR analysis of unique loci using discriminatory primers sets. The PCR amplification protocols used for microsatellites employ loci-specific either unlabelled primer pairs or primer pairs with one radio labelled or fluoro-labelled primer. Analysis of unlabelled PCR products is carried out using polyacrylamide or agarose gels. The employment of fluorescent labelled microsatellite primers and laser detection (e.g., automated sequencer) in genotyping procedures has significantly improved the throughput and automatisation . However, due to the high price of the fluorescent label, which must be carried by one of the primers in the primer pair, the assay becomes costly. Schuelke (2000) introduced a novel procedure in which three primers are used for the amplification of a defined microsatellite locus: a sequence-specific forward primer with M13(–21) tail at its 50 end, a sequence-specific reverse primer and the universal fluorescent-labelled M13(–21) primer which proved simple and less expensive. Microsatellites are highly popular genetic markers because of their co-dominant inheritance, high abundance, enormous extent of allelic diversity, and the ease of assessing SSR size variation by PCR with pairs of flanking primers. The reproducibility of microsatellites is such that, they can be used efficiently by different research laboratories to produce consistent data. Locus-specific microsatellite-based markers have been reported from many plant species such as lettuce (*Lactuca sativa* L.) (van de Wiel *et al*. 1999), barley and rice (Oryza *sativa* L.) (Wu and Tanksley 1993).

Single nucleotide polymorphism (SNPs)

Single nucleotide variations in genome sequence of individuals of a population are known as SNPs. They constitute the most abundant molecular markers in the genome and are widely distributed throughout genomes although their occurrence and distribution varies among species. Maize has 1 SNP per 60–120 bp (Ching *et al*., 2002), while humans have an estimated 1 SNP per 1,000 bp (Sachidanandam *et al*. 2001). The SNPs are usually more prevalent in the non-coding regions of the genome. Within the coding regions, an SNP is either non-synonymous and results in an amino acid sequence change, or it is synonymous and does not alter the amino acid sequence. Synonymous changes can modify mRNA splicing, resulting in phenotypic differences (Richard and Beckman 1995). Improvements in sequencing technology and availability of an increasing number of EST sequences have made direct analysis of genetic variation at the DNA sequence level possible (Buetow *et al*., 1999; Soleimani *et al*., (2003). Majority of SNP genotyping assays are based on one or two of the following molecular mechanisms: allele specific hybridization, primer extension, oligonucleotide ligation and invasive cleavage (Sobrino *et al*., 2005). High throughput genotyping methods, including DNA chips, allele-specific PCR and primer extension approaches make single nucleotide polymorphisms (SNPs) especially attractive as genetic markers. They are suitable for automation and are used for a range of purposes, including rapid identification of crop cultivars and construction of ultra high-density genetic maps.

Advances in molecular marker techniques

The technical advancements and genome based discoveries has lead to the enhancement of molecular marker techniques. These advanced molecular marker techniques are an amalgamation of the advantageous characteristics of several basic techniques as well as incorporation of modifications in the methodology to increase the sensitivity and resolution to detect genetic discontinuity and distinctiveness. Organelle microsatellites Plant organelle genomes such as chloroplast DNA and mitochondrial DNA have been increasingly applied to study population genetic structure and phylogenetic relationships in plants. Due to their uniparental mode of transmission, chloroplast and mitochondrial genomes exhibit different patterns of genetic differentiation compared to nuclear alleles (Provan *et al*., 1999a, b). Thus, for evolution, three interrelated genomes must be considered, so in addition to nuclear microsatellites, marker techniques based on the chloroplast and mitochondrial microsatellites have also been developed**.**

Chloroplast microsatellite

The analysis of the chloroplast organelle provides information on the population dynamics of plants that is complementary to those obtained from the nuclear genome. Numerous studies have shown that chloroplast microsatellites consisting of relatively short and several mononucleotide stretches such as (dA)nx(dT)n are ubiquitous and polymorphic components of chloroplast DNA. Chloroplast genome based markers uncover genetic discontinuities and distinctiveness among or between taxa with slight morphological differentiation, which sometimes cannot be revealed by nuclear DNA markers as interbreeding and genetic exchange has obscured the evidence of past demographic patterns (Wolfe *et al.,* 1987). The conservation and homology of sequence in chloroplast genome makes it possible to compare genes across the plant kingdom and examine phylogenetic relationships in taxa that have diverged for hundreds of thousands to millions of years. Chloroplast microsatellites bare now becoming firmly established as a high-resolution tool for examining patterns of cytoplasmic variation in a wide range of plant species (Provan *et al*., 2001). Chloroplast microsatellites are particularly effective markers for studying mating systems, gene flow via both pollen and seeds, and uniparental lineage. Chloroplast microsatellite based markers have been used for the detection of hybridization and introgression and the analysis of the genetic diversity (Clark *et al*., 2000) and phylogeography of plant populations. One limitation of the approach is the need of sequence data for primer construction. Primer sequences flanking chloroplast microsatellites are usually inferred from fully or partially sequenced chloroplast genomes. In general, these primer pairs produce

polymorphic PCR fragments from the species of origin and their close relatives, but transportability to more distant taxa is limited. Attempts to design universal primers to amplify chloroplast microsatellites have resulted in a set of consensus chloroplast microsatellite primers (ccmp1– ccmp10) that aims at amplifying cpSSR regions in the chloroplast genome of dicotyledonous angiosperms. Most of the primer pairs derived from A or T mononucleotide repeats (n = 10) identified in the tobacco chloroplast genome, were functional as genetic markers in the Actinidiaceae, Brassicaceae and Solanaceae (Chung and Staub 2003). Universal primers for the amplification of chloroplast microsatellites in grasses (Poaceae) have also been developed (Provan *et al.,* 2004).

Mitochondrial microsatellites

In contrast to animal mtDNA, which typically has a size of 10 MDa (MDa) per mitochondrial genome, plant mtDNA is far more complex, e.g., the maize mitochondrial genome has been estimated to be 320 MDa (Sederoff *et al*., 1981). In addition to larger size, plant mtDNA is characterized by molecular heterogeneity observed as classes of circular chromosomes that vary in size and relative abundance. In plants, mitochondrial genomes are not usually used for phylogenetic analysis due to a high rate of sequence reorganization. However, mitochondrial haplotype diversity related to sequence rearrangement proved useful in population differentiation of pine and fir taxa (Sperisen *et al*., 2001). Mitochondrial repeats have been used for trait-based segregation of population (Rajendrakumar *et al.,* 2007).

Sequence characterized amplified regions (SCAR)

In order to utilize markers identified by arbitrary marker analysis (RAPD, AFLP, etc.) for map-based cloning, a single locus must be identified unequivocally. In addition, both arbitrary marker techniques are sensitive to changes in the reaction conditions. In order to bridge the gap between the ability to obtain linked markers to a gene of interest in a short time and the use of these markers for map-based cloning approaches and for routine screening procedures, SCAR marker technique was developed and applied. 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 primers (McDermott *et al.,* 1994). Derivation of SCARs involves cloning the amplified products of arbitrary marker techniques and then sequencing the two ends of the cloned products. The sequence is thereafter used to design specific primer pairs of 15–30 bp which amplify single major bands of the size similar to that of cloned fragment. Polymorphism is either retained as the presence or absence of amplification of the band or can appear as length polymorphisms convert dominant arbitrary primed marker loci into co-dominant SCAR markers. As SCARs are primarily defined genetically, they can be used both as physical landmarks in the genome and as genetic markers. Co-dominant SCARs are more informative for genetic mapping than dominant arbitrary-primed molecular markers, as they can be used to screen pooled genomic libraries by PCR and for physical mapping (Chelkowski and Stephen 2001), defining locus specificity as well as comparative mapping (Guo *et al*., 2003) and homology studies among related plant species.

Cleaved amplified polymorphic sequences (CAPS)

The CAPS marker technique provides a way to utilize the DNA sequences of mapped RFLP markers to develop PCR based markers thereby eliminating the tedious DNA blotting (Konori and Nitta 2005). Therefore CAPS are also known as PCR-RFLP markers. The CAPS deciphers the restriction fragment length polymorphisms caused by single base changes like SNPs, insertions/deletions, which modify restriction endonuclease recognition sites in PCR amplicons (Chelkowski and Stephen 2001). The CAPS assays are performed, by digesting locus-specific PCR amplicons with one or more restriction enzyme, followed by separation of the digested DNA on agarose or polyacrylamide gels. The primers are synthesized based on the sequence information available in databank of genomic or cDNA sequences or cloned RAPD bands. The CAPS analysis is versatile and can be combined with single strand conformational polymorphism (SSCP), SCAR, AFLP or RAPD analysis to increase the possibility of finding DNA polymorphisms. The CAPS markers are co-dominant and locus specific and have been used to distinguish between plants that are homozygous or heterozygous for alleles (Konieczny and Ausubel 1993). Thus, CAPS proves useful for genotyping, positional or map based cloning and molecular identification studies (Spaniolas *et al*., 2006; Weiland and Yu 2003) where sequence-based identification is not feasible. The technique is limited by mutations, which create or disrupt a restriction enzyme recognition site. In dCAPS analysis, a restriction enzyme recognition site, which includes the SNP is introduced into the PCR product by a primer containing one or more mismatches to template DNA. The modified PCR product is then subjected to restriction enzyme digestion and the presence or absence of the SNP is determined by the resulting restriction pattern. The method is simple, relatively inexpensive, and utilizes the ubiquitous technologies of PCR, restriction digestion and agrose gel analysis. This technique proved useful for following known mutations in segregating populations and positional based cloning of new genes in plants (Haliassos *et al*., 1989).

Randomly amplified microsatellite polymorphisms (RAMP)

Microsatellite-based markers show a high degree of allelic polymorphism but they are labor-intensive. On the other hand RAPD markers are inexpensive but exhibit a low degree of polymorphism. To compensate for the weaknesses of these two approaches, a technique termed as random amplified microsatellite polymorphisms (RAMP) was developed (Wu *et al.,* 1994). The technique involves a radio labeled primer consisting of a 50 anchor and 30 repeats which is used to amplify genomic DNA in the presence or absence of RAPD primers. The resulting products are resolved using denaturing polyacrylamide gels and as the repeat primer is labeled, the amplification products derived from the anchored primer are only detected. The melting temperatures of the anchored primers are usually 10–15^{0}C higher than those of the RAPD primers thus at higher annealing temperature only the anchored primer would anneal efficiently, whereas in PCR cycles at low annealing temperature both anchored microsatellite and RAPD primers would anneal. So the PCR program was modified such that there is switching between high and low annealing temperatures during the reaction. Most fragments obtained with RAMP primers alone disappear when RAPD primers are included, and different patterns are obtained with the same RAMP primer and different RAPDs, indicating that RAPD primers compete with RAMP primer during the low annealing temperature cycle. RAMP has been employed in genetic diversity studies of the cultivars of barley (Sanchez de la Hoz *et al*., 1996) and peach (Cheng *et al*., 2001).

Sequence-related amplified polymorphism (SRAP)

The aim of SRAP technique (Li and Quiros 2001) is the amplification of open reading frames (ORFs). It is based on two-primer amplification. The technique uses primers of arbitrary sequence, which are 17–21 nucleotides in length. It uses pairs of primers with AT- or GC- rich cores to amplify intragenic fragments for polymorphism detection. The primers consist of the following elements:

- Core sequences, which are 13–14 bases long, where the first 10 or 11 bases starting at the 50-end, are sequences of no specific constitution (''filler'' sequences), followed by the sequence CCGG in the forward primer and AATT in the reverse primer.
- The core is followed by three selective nucleotides at the 30-end. The filler sequences of the forward and reverse primers must be different from each other and can be 10 or 11 bases long.

For the first five cycles the annealing temperature is set at 35C. The following 35 cycles are run at 35C.The amplified DNA fragments are fractionated by denaturing acrylamide gels and detected by autoradiography. Sequence-related amplified polymorphism (SRAP) combines simplicity, reliability, moderate throughput ratio and facile sequencing of selected bands (Li and Quiros 2001). SRAP targets coding sequences in the genome and results in a moderate number of co-dominant markers. Sequencing demonstrated that SRAP polymorphism results from two events, fragment size changes due to insertions and deletions, which could lead to co-dominant markers, and nucleotide changes leading to dominant markers. The SRAP marker system has been adapted for a variety of purposes in different crops, including map construction, gene tagging and genetic diversity studies (Gulsen *et al.,* 2006).

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 bn candidate gene sequences. The technique uses two primers (18 nucleotides in length) to generate markers. One bp of the primers, the fixed primer, is designed from the targeted EST sequence in the database; the second primer is an arbitrary primer with either an AT- or GC-rich core to anneal with an intron or exon. As the TRAP technique can be used to generate markers for specific gene sequences, it is useful for genotyping germplasm and generating markers associated with desirable agronomic traits in crop plants for marker-assisted breeding (Hu *et al*., 2005). The technique has been effectively used in fingerprinting lettuce (*Lactuca sativa* L.) cultivars , In estimating genetic diversity (Alwala 2006) and mapping QTL in a wheat (*Triticum aestivum* L.) intervarietal recombinant inbred population (Liu *et al*., 2005).

Single strand conformation polymorphism (SSCP)

Single strand conformation polymorphism is the mobility shift analysis of single-stranded DNA sequences on neutral polyacrylamide gel electrophoresis, to detect polymorphisms produced by differential folding of single-stranded DNA due to subtle differences in sequence (often a single base pair) (Orita *et al.,* 1989). In the absence of a complementary strand, the single strand experiences intra strand base pairing, resulting in loops and folds, that gives it a unique 3D structure which can be considerably altered due to single base change resulting in differential mobility. The SSCP analysis proves to be a powerful tool for assessing the complexity of PCR products as the two DNA strands from the same PCR product (Hayashi 1992) often run separately on SSCP gels, thereby providing two opportunities to score a polymorphism and secondly, resolving internal sequence polymorphisms in some PCR products from identical places in the two parental genomes. The PCR-based SSCP analysis is a rapid, simple and sensitive technique for detection of various mutations, including single nucleotide substitutions, insertions and deletions, in PCR-amplified DNA fragments (Hayashi 1993). Thus, it is a powerful technique for gene analysis particularly for detection of point mutations (Fukuoka *et al*., 1994). The technique shares similarity to RFLPs as it can also decipher the allelic variants of inherited and genetic traits. However, unlike RFLP analysis, SSCP analysis can detect DNA polymorphisms and mutations at multiple places in DNA fragments. The SSCP gels have been used to increase throughput and reliability of scoring during mapping by PCR fingerprinting in plants (Li *et al*., 2005). Fluorescence-based PCR-SSCP (F-SSCP) is an adapted version of SSCP analysis involving amplification of the target sequence using fluorescent primers (Makino *et al*., 1992). The major disadvantage of the technique is that the development of SSCP markers is labor intensive and costly and cannot be automated.

CONCLUSION

Integration of the above-mentioned genetic and genomic approaches, together with transcriptomics, proteomics, metabolics and tools of bioinformatics, is essential for the effective use of genomics in breeding: we term this holistic approach as 'genomics-assisted breeding' (GAB). However, there are several challenges for successful exploitation of GAB that need to considered or tackled. Some of these challenges include precise phenotyping, low heritability of traits, epistasis, epigenetics, regulatory variation, technical difficulties and cost-investment issues. Particularly when using functional genomics and expression genetics approaches for identification of genes and their application in breeding programmes, it is important to consider the phenomenon of gene networks, such as epistatic interactions, and the relationships between gene silencing, DNA methylation, RNA interference (RNAi) and heterochromatic DNA, demonstrating the complexity of RNA regulation operating through small non-coding RNAs. Indeed, in the post-genomic era, owing to the availability of high-throughput approaches combined with automation, the rapid increase in sequence data in the public domain and good expertise and tools in the area of bioinformatics. Genomics holds great potential to facilitate the prediction of a phenotype more precisely, thereby, increasing the efficiency of breeding. Large scale application of genomics to breeding will result from new technologies that reduce the costs and increase the throughput of the assays. Although the newly developed genetic and genomics tools will certainly enhance the prediction of phenotype, they will not entirely replace the conventional breeding process. Superior varieties can result from the discovery of novel genetic variation, improved selection techniques, and/or the identification of genotypes with improved attributes due to superior combinations of alleles at multiple loci assembled through marker-assisted selection.

REFERENCE

Adam-Blondon AF, Sevignac M, Bannerot H, Dron M (1994) SCAR, RAPD and RFLP markers linked to a dominant gene (Are) conferring resistance to anthracnose in common bean. Theor Appl Genet 88:865–870

Alwala S, Suman A, Arro JA, Veremis JC, Kimbeng CA (2006) Target region amplification polymorphism (TRAP) for assessing genetic diversity in sugarcane germplasm collection. Crop Sci 46:448–455

Anderson, J.R. and Lubberstedt, T. 2003. Functional markers in plant. *Trends Plant Sci* 8 : 554-560.

Azhaguvel, P. 2006. Methodological advancement in molecular markers to delimit the gene(s) for crop improvement. In : Floriculture, Ornamental and Plant Biotechnology : Advances and Topical Issues. [Ed. J.A. Silva], pp. 460-469. Global Science Books.

Azhaguvel, P. 2006. Methodological advancement in molecular markers to delinit the gene(s) for crop improvement. In :

Floriculture, Ornamental and Plant Biotechnology : Advances and Topical Issues (Vol. 1) (Ed. J.A. Teieira de Silva), Global Science Books, pp. 460-469.

Bachem CWB, Oomen RJFJ, Visser GF (1998) Transcript imaging with cDNA-AFLP: a step-by-step protocol. Plant Mol Biol Rep 16:157 Bardakci F (2001) Random amplified polymorphic DNA (RAPD) markers. Turk J Biol 25:185–196

Bachem CWB, van der Hoeve RS, de Bruijn SM, Vreugdenhil D, Zabeau M, Visser RGF (1996) Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development. Plant J 9:745–753

Bachmann K, Hombergen E-J. 1997. From phenotype via QTL to virtual phenotype in *Microseris* (Asteraceae): predictions from multilocus marker genotypes. *New Phytologist* 137: 9-18.

Barkley, N.K. and Wang, M.K. 2008. Application of tilling and ecotilling as reverse genetic approaches to elucidate the function of genes in plants and animals. *Current Genomics* 9 : 212-226.

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 32:314–333

Branco CJS, Vieira EA, Malone G, Kopp MM, Malone E, Bernardes A, Mistura CC, Carvalho FIF, Oliveira CA (2007) IRAP and REMAP assessments of genetic similarity in rice. *J. Appl Genet* 48:107–113

Buetow KH, Edmonson MN, Cassidy AB (1999) Reliable identification of large numbers of candidate SNPs from public EST data. *Nat Genet* 21:323–332

Casa AM, Brouwer C, Nagel A, Wang L, Zhang Q, Kresovich S, Wessler SR (2000) The MITE family Heartbreaker (Hbr): molecular markers in maize. *Proc Natl Acad Sci* USA 97:10083–10089

Chang RY, O'Donoughue LS, Bureau TE (2001) Inter-MITE polymorphisms (IMP): a high throughput transposon-based genome mapping and fingerprinting approach. *Theor Appl Genet* 102:773–781

Chelkowski J, Steˆpien˜ L (2001) Molecular markers for leaf rust resistance genes in wheat. *J Appl Genet* 42:117–126

Cheng H-Y, Yang W-C, Hsiao J-Y (2001) Genetic diversity and relationship among peach cultivars based on random amplified microsatellite polymorphism (RAMP). *Bot Bull Acad Sin* 42:201–206

Ching ADA, Caldwell KS, Jung M, Dolan M, Smith OS, Tingey S, Morgante M, Rafalski A (2002) SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. *BMC Genet* 3:19

Chung S-M, Staub JE (2003) The development and evaluation of consensus chloroplast primer pairs that possess highly variable sequence regions in a diverse array of plant taxan. *Theor Appl Genet* 107:757–776

Clark CM, Wentworth TM, Malley DMO (2000) Genetic discontinuity revealed by chloroplast microsatellites in eastern North American Abies (Pinaceae). *Am J Bot* 87:774–778

Close, T. et al. (2004) A new resource for cereal genomics: 22k barley genechip comes of age. *Plant Physiol*. 134, 960–968

Collard, B.C.Y. and Mackill, D.J. 2008. Marker-assisted selection : an approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc.* 363 : 557-572.

Comai, L. 2004. Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. *Plant Jour*. 37 : 778-786.

Cronn RC, Adams KL (2003) Quantitative analysis of transcript accumulation from genes duplicated by polyploidy using cDNA SSCP. Biotechniques 34:726–734

Devos, K.M. (2005) Updating the 'crop circle'. Curr. Opin. Plant Biol. 8, 155–162

****