Genetic fingerprinting: Advancing the frontiers of crop biology research

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Genetic fingerprinting is one of the DNA-based techniques that have permeated a wide gamut of biological research, beginning with forensic biology and medicine and now extending to agriculture. The advent of polymerase chain reaction (PCR) ushered a revolutionary approach in producing genetic fingerprints, supplanting hybridization-based techniques. PCR-based methods can be accomplished using either arbitrary markers of unknown location in the genome or those markers that target specific genome sites. Among agricultural crops, rice and maize are the most intensively characterized for DNA markers. At present, genetic fingerprinting has also been applied in many aspects of crop biology, such as taxonomy and phylogeny, diversity analysis, hybridity testing, gene mapping, molecular breeding, and somaclonal variation. This paper describes genetic fingerprinting technology and discusses its applications in the major crops of the Philippines, highlighting the progress made by Filipino scientists.

Discovery and principle of genetic fingerprinting

The past century marked groundbreaking discoveries in the biological sciences particularly in the area of molecular biology. Since the discovery of the DNA structure in 1953, researchers have been increasingly empowered to analyze and manipulate genetics using the principles of nucleic acid biochemistry. The beginnings of DNA profiling or genetic fingerprinting can be traced in the work of geneticist Alec J. Jeffreys of the University of Leicester in Great Britain, on the gene for myoglobin, a protein that stores oxygen in muscle cells (Jeffreys et al., 1985). He found that the myoglobin gene contains many segments that vary in size and composition from individual to individual and that have no apparent function. Jeffreys called these segments minisatellites because they are small and are located near the gene that actually serves as a genetic blueprint. Minisatellites are tandemly arranged, repetitive DNA elements scattered throughout eukaryote and prokaryote genomes, the short basic units of which are mostly imperfectly reiterated at each locus (Epplen et al., 2005). Minisatellites comprise 10 – 100 nucleotide-long repeat units.

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DNA variation is the substrate of genetic fingerprinting. In simple organisms with small genomes, DNA variation can be detected simply by using restriction enzymes or restriction endonucleases that cut DNA strands at specific DNA sequences. Variation in the cutting sites on the DNA is manifested as a difference in the size of the digestion products. This difference is called Restriction Fragment Length Polymorphism (RFLP), which can be detected as follows: digesting DNA with restriction enzymes and separating the DNA fragments by gel

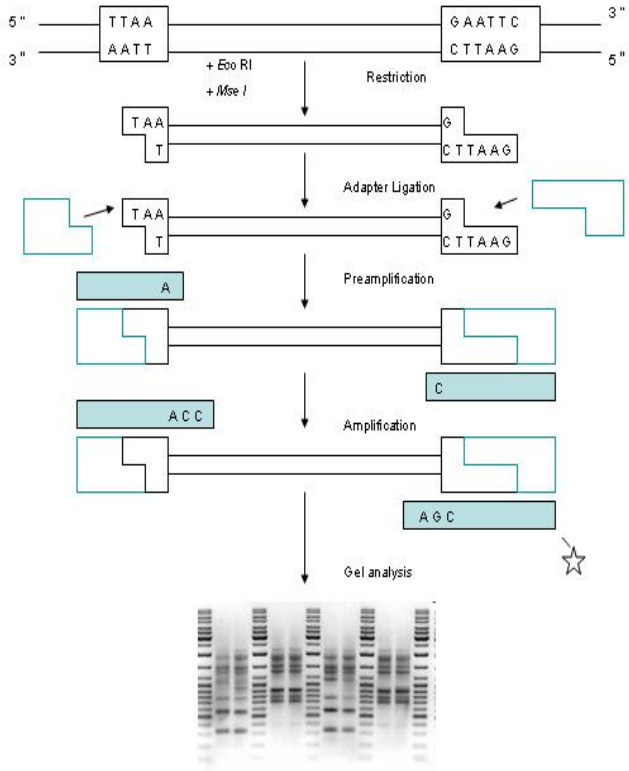

Figure 1. Principle of the AFLP strategy. Genomic DNA is cut with two restriction enzymes (here *Eco*RI and *Mse*I) and specific adapters are linked to both ends of all the resulting fragments. Two successive PCRs are then performed using specific primers complementary to the adapters and the restriction site with the 3'-ends extending by one or a few bases to effect selective amplification. Amplification products obtained by the second, selective PCR are separated on gels. Band detection can be through silver staining or labeling of one primer by a radioisotope or a fluorochrome (indicated by a star). (Weising et al., 2005).

Table 1. Characteristics of common fingerprinting techniques.

Molecular marker	PCR-based	Use of restriction enzymes	Polymorphism	Abundance	Gene action	Automation	Loci per assay	Specialized equipment
Restriction Fragment Length Polymorphisms (RFLP)	No	Yes	Moderate	Moderate	Co-dominant	No	1 to few	Radioactive isotope
Random Amplified Polymorphic DNA (RAPD)	Yes	No	Moderate	Moderate	Dominant	Yes	Many	Agarose gels
Amplified Length Polymorphism (AFLP)	Yes	No	Moderate	Moderate	Dominant	Yes	Many	Polyacrylamide gels or capillary
Simple Sequence Repeats or 'Microsatellites' (SSR)	Yes	No	Low	Moderate	Co-dominant	Yes	1 to about 20	Polyacrylamide gels or capillary
Cleaved Amplified Polymorphic DNA (CAP)	Yes	Yes	Variable	Moderate	Co-dominant	Yes	Single	Agarose gels
Simple Nucleotide Polymorphism (SNP)	Yes	No	Variable	Highest	Co-dominant	Yes	1 to thousands	Polyacrylamide gels, arrays, mass spectrometer, or plate readers

electrophoresis. In higher forms with large genomes, additional steps are necessary to display the variation, namely: blotting the digested fragments to a filter, and hybridizing a probe to the fragments (Southern, 1975). DNA fingerprinting has opened the possibility to identify genomic variation between individuals, and to follow DNA exchange in the progeny, making it a powerful tool for genotypic selection such as in animal and plant breeding.

The discovery of PCR afforded a very convenient way of assaying DNA variation without the blotting and probing steps in RFLP. With creative designs of primers and various thermal cycling strategies, PCR has brought about a new class of DNA profiling markers for fingerprinting and later has also become a major tool in biotechnology research and product development worldwide.

A wide array of molecular markers is now available for detecting DNA variation. They fall into three broad categories: (a) Hybridization-based approaches such as RFLP, (b) PCR arbitrary or multi-locus profiling techniques such as Random Amplified Polymorphic DNA (RAPD) and Amplified Length Polymorphism (AFLP), and (c) Site-Targeted-PCR techniques such as microsatellite or Simple Sequence Repeat (SSR), and Cleaved Amplified Polymorphic DNA (CAPs). RAPD makes use of short (10-mer) primers and low annealing temperature to amplify several regions of unknown map location in the genome (Welsh and McClelland, 1990; Williams et al., 1990). AFLP is based on the amplification of a subset of digested DNA using primers designed for selective extension at digestion sites fitted with adapters (Vos et al., 1995) (Figure 1).

SSRs are tandemly repeated simple sequences of about 2 - 6 nucleotides that vary in repeat number to as many as 100 times (Weber and May, 1989). CAPs are PCR products that contain sequence variation that can be detected by cleavage or digestion with restriction enzymes (Konieczny and Ausubel, 1993). Table 1 summarizes the properties of these commonly used markers in crop breeding and genetics (Edwards and McCouch, 2007). DNA markers are continually being modified to boost their utility, becoming more amenable to high-throughput, automated process of genome analysis.

The latest marker type is the Simple or Single Nucleotide Polymorphism (SNPs), the ultimate marker representing single base changes or short insertion/deletions in DNA. SNPs are revealed by sequencing, microarray-chip hybridization, or digestion of heteroduplex DNA from pairs of polymorphic lines. The very high densities of SNPs in a genome have made them a preferred molecular marker for fine-mapping studies (Rafalski, 2002).

Table 2. Abundance of DNA markers discovered and developed in major crops.

Crop	Genome size (MB)	RFLP	RAPD	SSR	AFLP	SNP[a]
Rice (*Oryza sativa* L.)	415-460[b]	3,553[b]	133[b]	12,992[b]	1,062[b]	5,418,373
Maize (*Zea mays* spp. *mays*)	2,300-2,700[b]	9,355[b]	-	2,243[b]	501[b]	2,018,337
Wheat (*Triticum aestivum*)	16,500[b]	874[b]	16[b]	1,103[b]	731[b]	1,051,736
Oats (*Avena sativa*)	11,300[b]	507[b]	10[b]	3[b]	27[b]	485
Sorghum (*Sorghum bicolor*)	750-770[b]	1,082[b]	-	229[b]	5,344[b]	209,814
Barley (*Hordeum vulgare*)	4,900[b]	1,001[b]	15[b]	196[b]	336[b]	501,336
Sugar cane (*Saccharum* spp.)	10,000[c]	57[d]	67[e]	221[f]	614[g]	1,632[h]
Pineapple (*Ananas* spp.)	440-520[i]	18[j]	93[k]	379[k]	19[k]	-
Banana (*Musa* spp.)	500-600[l]	47[m]	50[n]	12[n]	119[p]	-
Coconut (*Cocos nucifera*)	234[q]	-	86[r]	92[r]	>1,000[q]	-

[a]www.ncbi.nlm.nih.gov, [b]Gramene Web Browser (www.gramene.org), [c]Arruda and Silva (2007), [d]Garcia et al. (2006), [e]Liang et al. (2004), [f]Pan (2006), [g]Aitken et al. (2006), [h]Bundock et al. (2009), [i]Carlier et al. (2007), [j]Duval et al. (2001), [k]Carlier et al. (2006), [l]Lysák et al. (1999), [m]Venkatachalam et al. (2008), [p]Xiao et al. (2007). [q]Sniady et al. (2003), [r]Manimekalai et al. (2007).

Table 3. Recommended websites for crop markers, genomes and genetic maps.

GRAMENE Web Browser http://www.gramene.org/	Gramene is a data resource for comparative genome analysis in the grasses, in particular the cereals: rice, maize, oats, etc. It provides comprehensive and in-depth information regarding markers used for mapping plant species such as RAPD, SSR, AFLP and RFLP.
TropGENE DB http://tropgenedb.cirad.fr/	TropGENE DB is a database that manages genetic and genomic information about tropical crops - banana, cocoa, coconut, coffee, cotton, oil palm, rice, pineapple, rubber tree, and sugarcane - studied by Centre de coopération internationale en recherche agronomique pourle développement (CIRAD).
MaizeGDB http://www.maizegdb.org/	MaizeGDB is the community database for biological information about the crop plant *Zea mays* ssp. *mays*. Genetic, genomic, sequence, gene product, functional characterization, literature reference, and person/organization contact information are among the datatypes accessible through this site.
MSU Rice Genome Annotation Project http://rice.plantbiology.msu.edu/	This website provides genome sequence from the Nipponbare subspecies of rice and annotation of the 12 rice chromosomes.
Wheat Genome Database http://www.tigr.org/tdb/e2k1/tae1/	This website is dedicated in developing a bioinformatics resource for annotating the wheat genome.
GrainGenes: **A Database for *Triticeae* and *Avena*** http://wheat.pw.usda.gov	GrainGenes is a suite of services for the *Triticeae* and oat communities, including databases, documents, tools, data files, web sites, announcements, curation, and community assistance.
SorghumDB - **A *Sorghum bicolor* Genome Database** http://algodon.tamu.edu/sorghumdb.html	SorghumDB is a database that contains extensive genome information on *Sorghum bicolor*. Additional references, gene maps, disease information, and other extensive sorghum information can be found within the database.

Marker abundance in crop genomes

Table 2 provides the present abundance of markers discovered and developed in the major crop species. Model plant species and major crops are the most intensively characterized for DNA markers. Rice, being both a model species and the staple of most people, is the most advanced in terms of genome characterization. Its small genome and huge economic importance stimulated vigorous international efforts by the International Rice Genome Sequencing Project/ IRGSP, which was established in 1997, leading to the completion of rice genome sequencing in 2005 (Matsumoto et al., 2005). The genome sequence information has served as an excellent platform to uncover SNPs of rice that is now more than 5 million. Rice SNP resources are publicly accessible in www.oryzasnp.org to aid gene localization and expression studies (McNally, 2006). SSR is the next most abundant marker in rice numbering to 13,000.

Corn is the second most important crop worldwide as well as in the Philippines. Its genome, which is 6 times the size that of rice, is being sequenced by an international consortium led by the US Department of Agriculture and National Science Foundation Plant Genome Research Program (http://www.nsf.gov/bio/dbi/dbi_pgr.htm). The SNPs in corn are now more than 2,000,000, followed by 9,000 RFLPs. Except for oats, the rest of the major cereals have more than 200,000 SNPs. The recent completion of sorghum genome sequencing (Paterson et al., 2009) will certainly lead to the discovery of many more SNPs. In the non-cereal crops, SSR is the most abundant in pineapple, while it is AFLP in banana and coconut. Table 3 lists the websites hosting genomics information for the major crops.

Applications of genetic fingerprinting

Genetic fingerprinting has given new impetus to the biological sciences. Because of its versatility, it was rapidly adopted as a research tool in medicine (Nakamura et al., 1987), forensic science (Lewin, 1989), and animal behavior (Burke, 1989). At present, genetic fingerprinting has also been applied in many aspects of crop biology, from analysis of genetic diversity within breeding populations in plants, to differentiation between cultivars, as well as to identification of plants containing a gene of interest, to name a few. Table 4 shows the current crop research activities involving DNA markers, which are being lead by Filipino scientists.

Taxa identification and phylogeny

The use of molecular markers is a major advance in evaluating genetic variation and in elucidating the genetic relationships within and among species. The first application of microsatellites in agriculture has been in cultivar identification, wherein breeders/scientists are able to determine cultivated varieties from traditional varieties and vice versa thru the use of microsatellites. This paved the way for diverse materials like rice, wheat, grapevine (*Vitis unifera*), and soybean to be genotyped, using unique genetic information instead of phenotypic expression or physical appearance to discern between closely similar or identical materials or varieties.

In addition, the microsatellite markers are useful in sex identification of dioeceous plants. Normally, sex-specific morphological differences of these types of plants only show during the flowering stage, however, a microsatellite probe (GATA)4, used as a diagnostic marker, reveals sex-specific DNA variation at any stage of plant development, which allows sex-identification possible beyond the limitation of morphologically expressed differences of the plant. Likewise, a RAPD marker detected pseudo-autosomal plant sex chromosome in *Silene dioica* (L.) (Joshi et al., 1999).

Umali of Del Monte Fresh Produce Division in Davao developed CAP markers for banana identification during his graduate work in Japan. The markers, which were based on a maternally inherited chloroplast DNA mutation, helped establish the lineage of banana cultivars as well as the contribution of wild progenitors to cultivated types (Umali and Nakamura, 2003).

Diversity analysis

Diversity analysis measures the level of genetic similarity or differences among materials, which is vital information in crop conservation and varietal development. DNA markers are extensively used in assessing the genetic diversity in most crop species due to its high efficiency, easy to use, co-dominance nature, reproducibility, and high degree of polymorphism. This information can serve as basis for rational use and conservation of genetic resources, since the collection, storage and maintenance of germplasm generally requires expensive equip-

Table 4. Current crop research lead by Filipino scientists involving DNA markers.

Organization	Lead Investigator	Markers	Research
Philippine Rice Research Institute	Antonio A. Alfonso	SSR	Marker-aided introgression of Golden Rice in the Philippines
	Nenita V. Desamero	Sub1 transgene	Introgression of Sub1 into Philippine locally adapted and high-yielding rice cultivars
	Loida M. Perez	SSR	Molecular fingerprinting of commercial hybrid rice varieties and parental inbreds
		RFLP	Efficiency of two-gene marker-assisted selection for restorer line identification in PhilRice Hybrid Breeding Program
	Gabriel O. Romero	AFLP, SSR, CAP	QTL analysis of rice tungro resistance
		SNP	Association mapping of drought resistance
		AFLP, SSR	DNA fingerprinting of rice germplasm
	Dindo A. Tabanao	SSR and Rep and Xa21 transgenes	Marker-assisted pyramiding of transgenic resistance to bacterial blight and tungro in popular rice varieties
International Rice Research Institute (IRRI)	Glen B. Gregorio	SSR	Genetic diversity analysis of Sub-Saharan Africa (*O. glaberrima*) rice germplasm using microsatellite markers
Mindanao State University and University of the Philippines, Mindanao	Florence L. Zapico and Severo. T. Bastian Jr.	RAPD	Molecular characterization of selected rice varieties in Lake Sebu, Philippines using RAPD markers
Institute of Plant Breeding University of the Philippines, Los Baños	Josefina O. Narciso and Desiree M. Hautea	Bt transgene	Marker-aided selection in the development of transgenic fruit and shoot borer resistant Philippine eggplant

ment, highly controlled environments and infrastructure. In conjunction with pedigree records, DNA fingerprinting provides an effective method of assessing the diversity of varieties as well as breeding lines.

Rivera et al. (1999) of the Philippine Coconut Authority in Zamboanga City used microsatellites in assessing the diversity of their coconut collection. The microsatellites revealed high polymorphism in the coconut germplasm, indicating broad genetic diversity, and thus attesting to the agency's effective collecting and gene-banking strategies. A highly polymorphic set of coconut microsatellites has been assembled for routine evaluation of both *in situ* and *ex situ* genetic resources of coconut.

Hybridity testing

In hybrid technology, hybridity or the 50:50 parental gene contributions in the progeny should be ascertained to draw maximum performance of the hybrid variety in farmers' fields. Identification and selection is necessary: a) for proper identification and varietal protection, b) for genetic identity stability, c) for complete characterization and measurement of crop genetic diversity, and d) for uniformity of appearance and agronomic performance of produced variety that will meet the demand of the farmers and consumers (Smith and Register, 1998). Detection of hybridity is, however, hampered by the paucity of markers. Seed characteristics are unreliable as they are largely controlled by the maternal parent. Biochemical markers are of limited use as they can be affected by developmental stage and environment, and can only be assayed with considerable tissue material. Genetic fingerprinting is therefore an ideal approach to hybridity testing.

At the Philippine Sugar Research Institute (PhilSURIN), Manigbas and Villegas screened fifty SSR markers and found one SSR that amplified highly polymorphic bands (Manigbas and Villegas, 2004). The SSR correctly identified the true hybrids among 918 progenies derived from four sugarcane crosses. With this molecular hybridity testing system, the conventional 7-year breeding cycle for sugarcane can be reduced by 1-2 years.

Gene mapping

With the growing density of genetic markers in crop genomes and the increasing power of associated statistical methods, it is now possible to determine specific genomic regions that are responsible for the expression of important physiological and agronomic traits, many of which are quantitative traits. DNA markers have been efficiently employed in tagging numerous individual traits that are extremely vital for a breeding program like yield, disease resistance, stress tolerance, seed quality, etc. Moreover, a causal link between coding structural genes and quantitative variation can be established by analyzing quantitative trait loci (QTL). QTL can lead to the identification of candidate genes for the trait of interest.

Molecular marker technology has been useful in detecting QTL in rice by Filipino graduate students and researchers. Redoña, while in University of California, Davis, identified the QTL for rice seedling vigor through RAPD markers (Redoña and Mackill, 1996). Tabien used RFLP markers to determine the QTL for blast resistance as a student in Texas A&M (2000). At Cornell, Sebastian localized the QTL for tungro resistance using both RFLP and RAPD markers (Sebastian et al., 1996). At PhilRice, Romero et al., (2008) used SSR and CAPS to move closer to the tungro resistance QTL. Gregorio's team at the International Rice Research Institute (IRRI) determined rice genotypes with salt tolerance using microsatellite markers associated with the saltol QTL (Mohammadi-Nejad et al., 2008).

At the Institute of Plant Breeding (IPB) at the University of the Philippines in Los Banos (UPLB), QTL analysis with DNA markers was used in investigating disease resistance. Balatero and Hautea (2002) discovered that seven AFLP markers and one Resistance Gene Analogs (RGA) marker were putatively associated with resistance to bacterial wilt strains Tm-22 and Tm-151.

Marker-aided introgression

Introgression or transfer of desirable traits into target varieties or organisms can be facilitated or accomplished thru the aid of DNA mark-

ers. For traits that are recessive, expressed late in development and/or require progeny testing, the use of markers can significantly hasten and improve selection efficiency. DNA markers allow plant breeders to monitor genetic variation and identify genotype in the absence of a phenotype; thus, accelerating breeding work to produce and select crops with desirable traits.

PhilRice has developed several products of marker-aided breeding. Tabien et al. (2003) introduced bacterial leaf blight resistance genes into three popular varieties namely IR64, PSB Rc14, and BPI RI-10, producing elite breeding lines for anther culture and hybridization. Recently, two irrigated lowland rice varieties namely NSIC Rc142 (Tubigan 7) and NSIC Rc154 (Tubigan 11) were released with resistance genes Xa4 and Xa21 against BLB (Babb et al., 2007; Padolina et al., 2008). These are the first two rice varieties produced under molecular marker technology in the Philippines.

Somaclonal variation

Somaclonal variation refers to variation seen in plants being produced via plant tissue culture. It requires multiple genetic and/or epigenetic events which affect patterns of expression, or result in mutational alteration of genes. Several molecular mechanisms such as DNA damage and mutation, alteration of cell ability to repair damaged and mutated DNA, alteration of genes for cell-cycle control mechanisms, and DNA methylation (Merlo et al., 1995) are responsible for somaclonal variation. The physical bases include deletions, amplifications, translocations, insertions, recombination, and chemical alteration (Stoler et al., 1999).

Their widespread distribution in the genome makes microsatellites an effective detector of random plant genomic instability. Its variation, Inter-Simple Sequence Repeats (ISSR), can reveal genetic instability at early stages in in vitro culture. This method has been applied in *in vitro* cultures of cauliflower leaves and calli (Leroy et al., 2000).

Plant patenting

DNA fingerprinting occupies a unique place in the area of plant variety protection. Conventionally, differences in morphology, cultural characteristics, and pedigree serve as the initial basis to differentiate the proposed variety from existing ones (Anderson and Wu, 2007). When these are deemed inadequate, DNA fingerprinting becomes the final solution to document the distinctness of the new variety in the patenting process. Although not yet acceptable as proof of unique identity by The International Union for Protection of New Varieties of Plants (UPOV), the DNA fingerprint may strengthen claim for protection as supplementary information. In India, three chilli varieties sold under the brand name of an elite variety were found by fingerprinting to be spurious (Bhat, 2005). DNA analysis confirmed the adulteration in retail Basmati rice in the UK (Food Standards Agency, 2004) and established the origin of the different Basmati types in the US (Woolfe et al., 2001). Efforts are now underway at the Rice Technical Working group of the Philippine National Seed Industry Council to establish DNA fingerprinting using SSR to screen applications for registration and accreditation of new rice hybrids.

Conclusion

DNA fingerprinting is advancing the frontiers of crop biology research in broad strides, ushering a new age for genomic research and biotechnology. What began as a human profiling tool is now a prominent fixture in almost all areas of basic and applied biology including agriculture. DNA fingerprinting techniques which were tedious and time-consuming have now evolved into simpler, rapid and straightforward endeavors owing largely to the flexibility of PCR. DNA fingerprinting has greatly expanded the arsenal of scientists in the study and improvement of crop genetics. Many of its potential applications in agriculture are beginning to be realized and steady progress is also being made in the Philippines.

References

Aitken KS, Li JC, Jackson P, Piperidis G, McIntyre CL. AFLP analysis of genetic diversity within *Saccharum officinarum* and comparison with sugarcane cultivars. Aust J Agric Res 2006; 57 (11): 1167-1184.

Anderson MP, Wu YQ. Fingerprinting of bermuda grass DNA. USGA Turfgrass Environ Res Online 2007; 6 (14): 1-8.

Arruda P, Silva TR. Transcriptome analysis of the sugarcane genome for crop improvement. In: Varshney RK, Tuberosa R, eds. Genomics-Assisted Crop Improvement Volume 2: Genomics Applications in Crops. Netherlands: Springer, 2007: 483-494.

Babb GM, Padolina TF, Braceros RC, Duque MJC, Castres JG, dela Cruz AA et al. Use of classical and DNA marker-aided backcrossing to transfer bacterial blight resistance and other important traits to IR64, BPI RI-10 and PSB Rc14. Phil Rice R&D Highlights 2006; 14-16.

Balatero CH, Hautea DM. Major QTL associated with a co-dominant AFLP marker controls bacterial wilt resistance in Hawaii. Phil J Crop Sci 2002; 27 (1): 34.

Bhat KV. DNA fingerprinting and cultivar identification. In: Parsad R, Gupta VK, Bhar LM, Bhatia VK, eds. Advances in Data Analytical Techniques. New Delhi: IASRI, 2008: 101-109.

Bundock PC, Eliott FG, Ablett G, Benson AD, Casu RE, Aitken KS et al. Discovering SNPs for gene mapping in sugarcane using deep sequencing. Plant Anim Genome XVII Conference, 2009: W241.

Burke T. DNA Fingerprinting and other methods for the study of mating success. Trends Ecol Evol 1989; 4(5): 139-144.

Carlier JD, Nancheva D, Leitão JM, D'Eeckenbrugge GC. Genetic mapping of DNA markers in pineapple. Proc Fifth Int Pineapple Symp, 2005: 79-86.

Carlier JD, d'Eeckenbrugge GC, Leitão G. Pineapple. In: Kole C, ed. Genome Mapping and Molecular Breeding in Plants 4: Fruits and nuts. Verlag Belin Heidelberg: Springer, 2007: 331-342.

Duval MF, Noyer JL, Perrier X, d'Eeckenbrugge C, Hamon P. Molecular diversity in pineapple assessed by RFLP markers. Theor Appl Genet 2001; 102 (1): 83-2001 90.

Edwards JD, McCouch SR. Molecular markers for use in plant molecular breeding and germplasm evaluation. In: Guimaraes EP, Ruane J, Scherf BD, Sonnino A, Dargie JD, eds. Marker-Assisted Selection: Current Status and Future Perspectives in Crops, Livestock, Forestry and Fish. Rome: FAO, 2007: 29-49.

Epplen JT, Kunstmann EM. Minisatellites. In: O'Daly A, ed. Encyclopedia of Life Sciences. Bochum, Germany: Wiley-Blackwell, 2005: DOI 10-1038.

Garcia AAF, Kido EA, Meza AN, Souza HMB, Pinto LR, Pastina MM et al. Development of an integrated genetic map of a sugarcane (*Saccharum* spp.) commercial cross, based on a maximum-likelihood approach for estimation of linkage and linkage phases. Theor Appl Genet 2006; 112 (2): 298-314.

Jeffreys AJ, Wilson V, Thein SL. Hypervariable 'minisatellite' regions in human DNA. Nature 1985; 314: 67-73.

Joshi SP, Ranjekar PK, Gupta VS. Molecular markers in plant genome analysis. Curr Sci 1999; 77 (2): 230 -240.

Konieczny A, Ausubel FM. A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 1993; 4 (2): 403-10.

Leroy XJ, Leon K, Branchard M. Plant genomic instability detected by microsatellite-primers. Electron J Biotechnol 2000; 3 (2): 140-148.

Lewin R. Limits to the DNA fingerprinting. Science 1989; 243: 1549-1551.

Liang C, Nan L, Zhen H, Pin W, Chuanbei L, Mingshan S. Molecular assay of novel pasturage, sugarcane-grass 94-42 using RAPD markers. Genet Resour Crop Evol 2004; 351-354.

Lysák MA, Doleelová M, Horry JP, Swennen R, Doleel J. Flow cytometric analysis of nuclear DNA content in *Musa*. Theor Appl Genet 1999; 98: 1344-1350.

Manigbas NL, Villegas LC. Microsatellite markers in hybridity tests to identify true hybrids of sugarcane. Phil J Crop Sci 2004; 29: 23-32.

McNally KL, Bruskiewich R, Mackill D, Buell CR, Leach JE, Leung H. Sequencing multiple and diverse rice varieties connecting whole-genome variation with phenotypes. Plant Physiol 2006; 141: 26-31.

Manimekalai R, Nagarajan P, Kumaran PM. Comparison of effectiveness of RAPD, ISSR and SSR markers for analysis of coconut (*Cocos nucifera* L.) germplasm accessions. J Trop Agric 2007; 18: 217-226.

Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC et al. 5' CpG island methylation is associated with transcriptional silencing of the human suppresser p16/CDKN2/MTS1 in human cancers. Nature Med 1995; 1: 686-692.

Mohammadi-Nejad G, Arzani A, Rezai AM, Singh RK, Gregorio GB. Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the saltol QTL. Afr J Biotechnol 2008; 7 (6): 730-736.

Nakamura Y, Mark L, O'Connell P, Wolff R, Holm T, Culver M et al. Variable number tandem repeat (VNTR) markers for human gene mapping. Science 1987; 235: 1616-1622.

Pan YB. Highly polymorphic microsatellite DNA markers for sugarcane germplasm evaluation and variety identity testing. Sugar Tech 2006; 8 (4): 246-256.

Padolina TF, Arocena EC, Dimaano YA, Orcino JA, Osoteo GM, Babb GM et al. Development of rice varieties with high yield potential, good grain quality and resistance to major insect pests and diseases. Phil Rice R&D Highlights 2007: 3-5.

Rafalski JA. Novel genetic mapping tools in plants: SNPs and LD-based approaches. Plant Sci 2002; 162: 329-333.

Redoña ED, Mackill DJ. Mapping quantitative trait loci for seedling vigor in rice using RFLPs. Theor Appl Genet 1996; 92: 395-402.

Rivera R, Edwards KJ, Barker JHA, Arnold GM, Ayad G, Hodgkin T, Karp A. Isolation and characterization of polymorphic microsatellites in *Cocos nucifera* L. Genome 1999; 42: 668-675.

Romero GO, Panes VA, Messing J. Molecular genetics of tungro resistance. In: Tiongco ER, Sebastian LS, eds. The Rice Tungro Virus Disease: A Paradigm in Disease Management in the Philippines. Nueva Ecija: PhilRice and Honda Research Inc., 2008: 153-162.

Sebastian LS, Ikeda R, Huang N, Imbe T, Coffman WR, McCouch SR. Molecular mapping of resistance to rice tungro spherical virus and green leafhopper. Phytopathology 1996; 86: 25-30.

Shu PN, Xu LB, Yan L, Huang BZ, Ge XJ. Genome composition and genetic diversity of *Musa* germplasm from China revealed by PCR-RFLP and SSR markers. Sci Hort 2007; 114 (4): 281-288.

Smith JSC, Register JC. Genetic purity and testing technologies for seed quality: A company perspective. Seed Sci Res 1998; 8: 285-293.

Sniady V, Becker D, Herran A, Ritter E, Rohde W. A rapid way of physical mapping in coconut and oil palm. Deutscher Tropentag Conf Proc 2003; 282.

Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975; 98: 503-510.

Stoler DL, Cheng N, Basik M, Kahlenberg MS, Rodriguez-Bigas MA, Petrelli NJ et al. The onset and extent of genomic instability in sporadic colorectal tumour progression. Proc Natl Acad Sci U S A 1999; 96:15121-15126.

Tabien RE, Li Z, Paterson AH, Marchetti MA, Park WD, Stansel JW et al. Mapping four blast resistance genes from 'Lemont' and 'Teqing' and evaluation of their combinatorial effect at the blast nursery. Theor Appl Genet 2000; 101: 1215-1225.

Tabien RE, Abalos MC, Fernando MP, Corpuz ER, Dimaano YA, Sebastian LS et al. DNA marker-aided selection & evaluation of IR64, PSBRc14, and BPI Ri-10 for resistance to bacterial leaf blight. Phil J Crop Sci 2003; 28: 3-19.

Umali R, Nakamura I. Identification of dCAPS markers that discriminate A and B cytoplasms in banana (*Musa* spp.). Plant Biotech J 2003; 20: 159-164.

Venkatachalam LR, Sreedhar V, Bhagyalakshmi N. The use of genetic markers for detecting DNA polymorphism, genotype identification and phylogenetic relationships among banana cultivars. Mol Phylogenet Evol 2008; 47 (3): 974-85.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M et al. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 1995; 23: 4407-4414.

Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989; 44: 388-396.

Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 1990; 18: 7213-7218.

Williams JG, Kubelik AR, Livak KJ, Rifalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Res 1990; 18: 6531-6535.

Weising K, Nybom H, Wolff K, Kahl G. DNA Fingerprinting in Plants: Principles, Methods and Applications 2nd Edition. CRC Press, 2005; 444 pp.

Woolfe M, Kelly S, Johnston M, Blackhall N. Varietal and geographical origin of five commercial USA rice samples labeled as Basmati rice. Working Party on Food Authenticity Methodology Report 2001; 5-11.

Xiao W, Xiao L, Chiang TY, Roux N, Hao G, Ge XJ. Genetic diversity of wild banana (*Musa balbisiana* Colla) in China as revealed by AFLP markers. Genet Resour Crop Evol 2007; 54 (5): 1125-1132.