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Utility of Molecular Markers in Molecular Breeding for Integrated Crop Improvement

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ABSTRACT

Molecular markers are the marvelous assets which are frequently used in the identification of particular genes/QTLs for the trait of interest as well as marker assisted back cross breeding. The utility of molecular markers in crop breeding for introgressions of traits are depends on the efficacy of the markers. Molecular marker based foreground selection allow the screening at seedling stage. The background selection is the third and last step of marker assisted back cross breeding which involves the selection of back cross progenies with maximum coverage of genomic region of recurrent parent using chromosome wise maximum molecular marker, for the batter recurrent parent genome recovery. There are several markers have identified and mapped gene/QTLs and associated DNA markers linked to the gene of interest in rice (Oryza sativa L.) for bacterial blight, blast, brow plant hopper, drought, submergence and salinity, in Maize (Zea mays L.) for drought tolerance, salinity tolerance, banded leaf sheath blight, polysora rust and leaf blight, in Wheat (Triticum aestivum L.

Key words: *Molecular markers, fore ground selection, Back ground selection, Recurrent parent genome recovery, Gene based, Gene linked markers*

INTRODUCTION

Molecular breeding is the techniques used for development of resilience for various biotic and abiotic stresses. The term molecular breeding is used for several breeding strategies like marker assisted selection, marker assisted recurrent parent selection, along with marker assisted back cross breeding and genomic selection[1]. The molecular breeding is the marvelous application of biotechnological strategies on the basis of genotypic assays used for the trait improvement or to alter plant traits [2]. Now days marker assisted back cross breeding is frequently used in various crops for the awareness of the presence of genes/quantitative locus and breeding populations.

Researchers have identified and mapped gene/QTLs and associated DNA markers linked to the gene of interest in rice (*Oryza sativa* L.) for bacterial blight, blast, brow plant hopper, drought, submergence and salinity, in Maize (*Zea mays* L.) for drought tolerance, salinity tolerance, banded leaf sheath blight, polysora rust and leaf blight, in Wheat (*Triticum aestivum* L.) for drought and heat tolerance, rust and pre-harvest sprouting.

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Marker assisted incorporation of two or more genes (called as gene pyramiding) provide the long durability of resistant power for the disease, insect and pest. However, Molecular breeding offers the opportunity for the plant breeders to develop stress tolerant high yielding cultivars. The molecular breeding would lay the foundation for the modern crop improvement in 12th century [3, 4].

Marker Assisted Selection

Term marker assisted selection (MAS) was first used by Beckmann and Soller [5]. Marker assisted selection is the section of the allele for trait of interest and marker assisted back cross breeding (MABCB) is the introgression of one or more than one allele from the genetic background of one cultivar to the other cultivar. Recurrent parent genome recovery is the recovery of the original genome of the recurrent parent that can be achieved by the several time back crossing with the recurrent parent[1] and genomic selection is the selection on the basis genome-wide coverage of molecular markers liked to the trait of interest[6,2]. The evolutions of next generation sequencing have also been used by the several workers for detection of genome-wide polymorphism, these tends would accelerate the genomic selection [7].

Mechanism of Marker Assisted Selection

The marker assisted back cross breeding accomplished in three steps *viz.* fore ground selection, recombinant selection and background selection followed by recurrent parent genome recovery[8]. The SSRs, other microsatellites and SNPs markers are frequently used in marker assisted back cross breeding for incorporation of genes or QTLs in most of the cereal crops. The details of the mechanism of marker assisted selection are summarized below:

(i) Foreground Selection

Foreground selection is used for the screening of incorporated allele that is less time consuming than conventional breeding [9]. Foreground selection allow the screening at seedling stage. In fore ground selection markers are used for the testing of targeted genes or QTLs [10].

(ii) Recombinant Selection

In recombinant selection the recombinant between flanking markers and loci of interest has to be selected. The size of incorporated chromosome, i.e., the donor chromosome having the target locus, is reduced by this selection. However, in conventional backcross breeding approach, the donor segment of the chromosome remain large even after many back cross generations >10.[11,12]. Recombinant selection gives better result in two back cross generations because double recombination events on both sides of target locus are usually rare[13].

(iii) Background Selection and recurrent parent genome recovery

Background selection is the third and last step of marker assisted back cross breeding which involves the selection of back cross progenies with maximum coverage of genomic region of recurrent parent using chromosome wise maximum molecular marker, for the batter recurrent parent genome recovery five or more than five markers per chromosome give robust result[13]. Hence, background selection is very useful for the recovery of recurrent parent genome.

Markers

Markers are those which mark the traits or features at external and internal level. Genetic markers are categorized in to three group namely morphological or phenotypic marker, biochemical markers and molecular marker.

(i) Phenotypic or Morphological Markers

In terms of crop research, morphological markers are defined as "indicator which indicates the survival of crops in open environment under the adverse climatic and ecological conditions". In other words morphological markers are those which mark the crops traits for the survival by avoiding particular stress related to traits. Plant height and structural orientation of the up ground and underground organs are the main morphological markers of the crop and tree plant. Morphological markers can easily be characterized phenotypic characters of the plants such as colour of flowers, shape of seeds, growth habits and pigmentation[14].

(ii) Biochemical Markers

Biochemical markers are differences in enzymes that are detected by electrophoresis and specific staining[15]. Most of the allozymes or isozymes are used as biochemical markers, they does not require DNA. These are easy, quick and cost efficient markers. Isoenzyme markers are the oldest technique as compared with molecular markers. Isozymes markers have been used in several crop improvement programmes[16,17,18]. They are codominant markers with the high level of reproducibility. The banding pattern of the Zymograms can easily be interpreted in terms of loci and allele and segregation analysis of the progeny. The lack of their abundance, low level of polymorphism and non differentiative mobility in electrophoresis are the major drawback of the allozymes [19]. (Table1)

However, allozymes have been used in studies like out crossing [20], population divergence[21], interspecific relationships[22], genetic inheritance[23], allelic frequency in germplasm[24], hybrid parents 580thidium580lites[25], diversity pattern and finger printing in crops[26, 27, 28, 29] (Table3)

Molecular Markers (Tools of molecular breeding)

Last two decades, DNA-based markers have been used in crop research for genetic diversity[30,31,32], sex identification[33] and mapping, tagging of genes[34]. According to Stansfield [35] the term MARKER is usually used for "LOCUS MARKERS" and each gene has a specific position along the chromosome called locus.

The utility of the molecular markers are the basis of naturally occurring DNA polymorphism**. "**Molecular markers are the DNA sequences that are linked with the particular genes/QTLs/traits and whose inheritance would be detected". Most of the molecular markers are used for the germplasm characterization and marker assisted indirect selection for the desirable traits[36]. An ideal molecular marker should have the following desirable traits- *Viz*.

(i) Frequently occurrence: Frequently distributed throughout the genome.

(ii) Molecular markers should must be polymorphic: The polymorphism is measured for the study of genetic diversity.

(iii) Codominant in nature: For the study of homozygous and heterozygous states of the organism.

(iv) Reproducibility: It should be highly reproducible.

(v) Easy asses: It should be easy fast and cheap to detect.

(vi) Selective neutral behavior and easy exchange of the molecular data between the laboratories.

A wide range of the molecular markers are available for the detection of polymorphism at DNA level[37]. Most of the molecular markers are need PCR and some of them have no need of PCR. However, on the basis of utility of PCR, molecular markers are categorized in to two groups i.e. Non-PCR based molecular marker and PCR based marker[36].

(A) Non-PCR based molecular marker

Restriction Fragment Length Polymorphism (RFLP) and Minisatellites/Variable Number of Tandem Repeats (VNTR), are comes under the category of non-PCR based markers. In RFLP, the DNA sequence variation is detected by the digestion of genomic DNA with restriction endonuclease, which cut the DNA at specific sequence, electrophoresed, blotted on the membrane and probed with the labeled clone. These markers are codominant in nature[38,39]. RFLP markers were used for the first time in the construction of genetic maps[40]. RFLPs can be used in diversity analysis, phylogenic studies, gene mapping [41], relationship between closely related texta[39,42], finger printing[43], 580thidium580lite of the genes[44] and construction of genetic map [40].

Minisatellites, Variable Number of Tandem Repeats (VNTRs) can also be used as molecular markers. The 580thidium580lites was introduced[45]. These loci contain repeat units between genotypes and are referred to as variable number of tandem repeats (VNTRs). Minisatellites are particularly useful in genetic identity and structure analysis, identification of varieties and cultivars[45,46], and population studies[47]. (Table3)

(B) PCR based molecular marker

Amplified fragment length polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple sequence Repeat (SSR), Inter Simple Sequence Repeats (ISSR), Single-Strand Conformation Polymorphism (SSCP), Cleaved Amplified Polymorphic Sequence (CAPS), Sequence Characterized Amplified Region (SCAR), and Single Nucleotide Polymorphism (SNP) are PCR based molecular markers[48].

(i) Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) is an intermediate between RFLPs and PCR. AFLP needs two restriction enzymes. In AFLPs the DNA is digested with restriction enzyme, ligated with oligonucleotide adapters, pre-amplification of the ligated products directed by the primers complementary to the adopters and restriction site sequences, amplification and labelling of the amplified products and finally labelled products are amplified polyacrylamide gel electrophoresis (PAGE)[49,50]. AFLPs are codominant in nature, highly reproducible and sensitive method of polymorphism at DNA level[51]. It can be used in genetic diversity, identification of pedigree and fingerprinting of cultivars[52]. AFLPS have been used in genetic diversity by several workers in crop plants[53]. used AFLPs in the genetic study of Peanut cultivars, Soybean[54], and Maize[55]. (Table 1 & 2)

(ii) Random Amplified Polymorphic DNA

Random Amplified Polymorphic DNA (RAPD) is very quick and easy molecular marker widely distributed in genome of the most of the cereal crops. In RAPD, the polymorphism of DNA is detected by single primer of arbitrary nucleotide sequence which anneals the genomic DNA at two different sites on complementary strands of DNA template. RAPDs are dominant in nature. This is frequently used in the polymorphism studies between the individuals[56]. In RAPDs the primers are short synthetic (10bp) of random sequence and their amplified products are amplified by Agarose gel in the presence of carcinogenic agent 581thidium bromide[50]. RAPDs have been used for genetic identity and diversity in several crops[57,58]. used RAPD for the distinguish *mugo* and *uncinata* their subspecies. (Table1&2).

(iii) Simple sequence Repeat

Simple sequence Repeat (SSR) is known as microsatellite. They are present in all eukaryotic genome. The term microsatellites were coined by Litt & Lutty[59]. In SSRs, ranges of the alleles of different loci do not overlap[60]. SSRs are the tandemly repeats of mono, di, tri, tetra and penta nucleotides with different length of the repeating motif. They are widely distributed throughout the genome and display high level of genetic variations based on the differences in the tandemly repeating units at a locus. They are amplified by the PCR using flanking region of the primers where sequences are known. However, SSRs are the top class of molecular markers associated for the target trait in many crops[61] identified two EST-SSR markers linked to the photoperiod response gene (ppd) in wheat. A large number of SSRs found to be associated with the wheat genome[62,63,64]. In general, SSR gives high level of polymorphism and can be used in genetic studied, molecular breeding,[65,66,67], germplasm collections[68], phenotypic variations[69] and functional diversity in relation to adaptive variation[70]. Simple sequence repeat (SSR) was very useful to identify date palm cultivars, and a high polymorphism has been detected in date palm cultivars[71]. (Table 1& 2).

(iv) Inter Simple Sequence Repeats

Inter Simple Sequence Repeats (ISSRs) are DNA fragments of about 100–3000 bp located between adjacent, oppositely oriented microsatellite regions. This technique reported by Zietkiewicz [72]. The ISSRs have been involved in the several genetic studies[72,73]. ISSRs have been used in the genetic studies of the tree plants[71,73]. Monocotyledon species such as the genus *Poa*[74] and durum wheat[75] have to informative of ISSRs. (Table 1).

(v) Single Strand Conformation Polymorphism

Single Strand Conformation Polymorphism (SSCP), Cleaved Amplified Polymorphic Sequence (CAPS) and Sequence Characterized

Amplified Region (SCAR) have also been used several studies like mutation detection[76], gene mapping[77,78] and marker assisted selection[79]. (Table1).

(vi) Single Nucleotide Polymorphism

Another novel type of PCR based molecular marker (Single Nucleotide Polymorphism) has been recently preferred in many genetic studies. SNPs are single base position in genomic DNA where two or more different nucleotide occurs in the different individuals. This type of polymorphism is due to substitution, deletion or insertion. The Single Nucleotide Polymorphism (SNPs) have wide range of linkage map score[80], heterogenetic study, positional cloning of mutant locus and linked gene inheritance in molecular breeding. (Table1&2).

Potential Application and Future prospects of the marker Assisted Selection:

Molecular markers have been used as tools in the various genetic studies in crop plants. Now a day's molecular marker are used in genomic studies for the development of tolerant verities for biotic and abiotic stresses. A lot genes or QTLs, associated traits have been identified with the involvement of molecular markers for salinity resilient in the major crops like rice, wheat, maize, chickpea, brassica and sorghum. Fruit crops, vegetables and oil yielding crops have also been remarkable using molecular marker. However, in future crop improvement programme molecular markers will prove an asset or marvellous gift for crop the researchers and may play crucial role in national food security as well as worldwide.

Table 1: A brief account of the molecular markers and their classification

S.No.	Name of the	Technique Discoverer
1.	Biochemical markers, Allozymes	(81, 82)
2	Molecular markers	
(a)	Non-PCR based techniques	
	Restriction Fragment Length Polymorphisms (RFLP)	(40, 41)
	Minisatellites or Variable Number of Tandem Repeats (VNTRs)	(45)
(b)	PCR-based techniques	
(i)	DNA sequencing, Multi-copy DNA, Internal Transcribed Spacer regions of nuclear ribosomal genes (ITS)	(83)
	Single-copy DNA, including both introns and exons	(84)
(ii)	Sequence-Tagged Sites (STS)	
(iii)	Microsatellites, Simple Sequence Repeat (SSR), Short Tandem Repeat (STR), Sequence Tagged Microsatellite (STMS) or Simple Sequence Length Polymorphism (SSLP)	(59, 65, 66, 67)
(iv)	Amplified Sequence Length Polymorphism (ASLP)	(85)
(v)	Sequence Characterized Amplified Region (SCAR)	(79,86, 87)
(vi)	Cleaved Amplified Polymorphic Sequence (CAPS)	(77, 78)
(vii)	Single-Strand Conformation Polymorphism (SSCP)	(76)
(viii)	Denaturing Gradient Gel Electrophoresis (DGGE)	(88)
(xix)	Thermal Gradient Gel Electrophoresis (TGGE)	(89)
(x)	Heteroduplex Analysis (HAD)	(90)
(xi)	Denaturing High Performance Liquid Chromatography (DHPLC)	(91, 92, 93)
(xii)	Multiple Arbitrary Amplicon Profiling(MAAP)	(94, 95)
(xiii)	Random Amplified Polymorphic DNA (RAPD)	(56, 57)
(xiv)	DNA Amplification Fingerprinting (DAF)	(94)
(xv)	Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)	(56, 96)
(xvi)	Inter-Simple Sequence Repeat (ISSR)	(72, 73)
(xvii)	Single Primer Amplification Reaction (SPAR)	(97)
(xviii)	Directed Amplification of Minisatellites DNA (DAMD)	(98, 99)
(xix)	Amplified Fragment Length Polymorphism (AFLP)	(52)
(xx)	Selectively Amplified Microsatellite Polymorphic Loci (SAMPL)	(51)

Table 2: Features of the commonly used molecular markers in crop study

S.N.	Feature	RFLP	RAPD	AFLP	SSRs	SNPs
1	Nature	Codominant	Dominant	Codominant	Codominant	Codominant
2	DNA Require(µg)	10	.02	.5-1.0	.05	.05
3	DNA quality	High	High	Moderate	Moderate	High
4	PCR based	No	Yes	Yes	Yes	Yes
5	Polymorph loci analysed (No.)	1-3	1.5-50	20-100	1-3	1
6	Ease of use	Not Easy	Easy	Easy	Easy	Easy
7	Amenable to automation	Low	Moderate	Moderate	High	High
8	Reproducibility	High	Unreliable	High	High	High
9	Development Cost	Low	Low	Moderate	High	High
10	Cost per analysis	High	Low	Moderate	Low	Low

Table 3: Advantages and disadvantages of Isozyme and molecular markers

Type of markers	Advantages	Disadvantages
ISOZYMES	Evolutionary studies, Isolation easier than DNA, used across species, No radioactive labelling, No need for sequence information	Laborious, less polymorphism, Expensive, Not easily automated
Restriction Fragment Length Polymorphism (RFLP)	High genomic abundance, Co-dominant markers, Highly reproducible, Good genome coverage, map based cloning	Need large amount DNA, Laborious, Need radioactive labelling
Randomly Amplified Polymorphic DNA (RAPD)	High genomic abundance, Good genome coverage, Less amount of DNA, No radioactive labelling, Relatively faster	Dominant markers, Not reproducible, not used across species, Not very well-tested
Simple Sequence Repeat (SSR)	High genomic abundance, highly reproducible, good genome coverage, high polymorphism, Easy to automate, Multiple alleles	Cannot be used across species, Need sequence information, Not well-tested
Amplified Fragment Length Polymorphism (AFLP)	High genomic abundance, polymorphism, No need for sequence information, Can be used across species, Useful in mapping	Very tricky due to changes in patterns with respect to materials used, Not reproducible
Sequence-Tagged Site (STS)	Useful mapping, good genome coverage, highly reproducible	Laborious, Unable to detect mutations, Need sequence information

REFERENCES

1. Ribaut, J.M., Vicente, M.C., Delannay, X., Molecular breeding in developing countries: challenges and perspectives. *Curr. Opin. Plant Biol*. **13:** 1-6 (2010).
2. Jiang, G.L., Molecular markers and marker assisted breeding in plants. In: S.B. Anderson eds., Plant Breeding from Laboratories to Fields. *InTech, Croatia, pp*. 45-83 (2013).
3. Whitford, R., Gilbert, M., Langridge, P., Biotechnology in agriculture. In: M.P. Reynolds, ed., Climate change and crop production, *CABI Series in Climate Change* **1:** 219-244 (2010).
4. Moose, S.P., Mumm, R.H., Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiol.* **147(3):** 969-977 (2008).
5. Beckmann, J.S., Soller, M., Restriction fragment length polymorphisms and genetic improvement of agricultural species. *Euphytica* **35:** 111-124 (1986).
6. Bernardo, R., Yu, J., Prospects for genome wide selection for quantitative traits in maize. *Crop Sci.* **47:** 1082-1090 (2007).
7. Mir, R.R., Varshney, R.K., Future prospects of molecular markers in plants. *In:* Molecular markers in plants, R.J.

Henry, eds., Blackwell Publishing Ltd., Oxford, UK, pp: 169-190 (2013).

8. Holland, J.B., Implementation of molecular markers for quantitative traits in breeding programs-challenges and opportunities. *Proc. 4th Int. Crop Sci. Congress*., Brisbane, Australia, 26 September-1 October, (2004).
9. Hospital, F., Charcosset, A., Marker-assisted introgression of quantitative trait loci. Genetics 147: 1469–1485 (1997).
10. Collard, B.C.Y., Mackill, D.J., Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363:** 557-572 (2008).
11. Ribaut, J.M., and Betran, J., Single large-scale marker-assisted selection (SLSMAS). *Mol. Breeding* **5:** 531-541 (1999).
12. Salina, E., Dobrovolskaya, O., Efremova, T., Leonova, I., Roder, M.S., Microsatellite monitoring of recombination around the Vrn-B1 locus of wheat during early backcross breeding. *Plant Breed* **122:** 116–119 (2003).
13. Frisch, M., Bohn, M. and Melchinger, A.E., Comparison of selection strategies for marker-assisted backcrossing of a gene. *Crop Sci*. **39:** 1295-1301 (1999).
14. Sumarani, G.O., Pillai, S.V., Harisankar, P. & Sundaresan, S., Isozyme analysis of indigenous cassava germplasm for identification of duplicates, *Gene. Resor. and Crop Evol.* **51:** 205-209 (2004).
15. Pillai, S.V., Sundaresan, P., Harisankar & Sumarani, G.O., Molecular characterization of germplasm in tropical tuber crops, *DAE-BRNS Symposium, Mumbai*. (2000).
16. Vallejos, C.E., Enzyme activitysatining. In Isozymes in Plant Genetics and Breeding (eds Tankley, S.D. and Orton, T.S, Elsvier, Amsterdam, pp. 469-516 (1983).
17. Glaszmann, J.C., Fautret, A., Noyer, J.L., Feldmann, P., Lanaud, C., Biochemical Genetic-Markers in Sugarcane. *Theor Appl Genet* **78:** 537-543 (1989).
18. Baes, P., Custsem, V., Electrophoretic analysis of eleven isozyme system and their possible use as biochemical markers in breeding chicory (Chychorium intybus L.). *Plant Breed* **110:** 16-23 (1993).
19. Kreiger, M., Ross, K.G., Identification of a major gene regulating complex social behaviour. *Science,* **295:** 328–332 (2002).
20. Erskine, W., Muehlbauer, F.J., Allozyme and morphological variability, Out crossing rate and core collection formation in lentil germplasm. *Theor. and Appl. Genet.* **83:** 119–125 (1991).
21. Freville, H., Justy, F., Olivieri, I., Comparative allozyme and microsatellite population structure in a narrow endemic plant species, *Centaurea corymbosa* Pourret (Asteraceae). *Molec Ecol* **10:** 879–889 (2001).
22. Garvin, D.F., Weeden, N.F., Isozyme evidence supporting a single geographic origin for domesticated tepary bean. *Crop Science* **34:** 1390–1395 (1994).
23. Warnke, S.E., Douches, D.S., Branham, B.E., Isozyme analysis supports allotetrapoloid inheritance in tetraploid creeping bluegrass (*Agrostis palustris* Huds.). *Crop Science*, **38:** 801– 805 (1998).
24. Reedy, M.E., Knapp, A.D., Lamkey, K.R., Isozyme allelic frequency changes following maize (*Zea mays* L.) germplasm regeneration. *Maydica* **40:** 269– 273 (1995)
25. Parani, M., Singh, K.N., Rangasamy, S., Ramalingam, R.S., Identification of *Sesamum alatum* × *Sesamum indicum* hybrid using protein, isozyme and RAPD markers. *Ind J Genet Plant Breed* **57:** 381–388 (1997).
26. Hamrick, J.L., Godt, M.J.W., Allozyme diversity in cultivated crops. *Crop Science* 37:26–30 (1997).
27. Tao, R., Sugiura, A., Cultivar identification of Japanese persimmon by leaf isozymes. *Hort Science* 22:932–935 (1987).
28. Lamboy, W.F., McFerson, J.R., Westman, A.L., Kresovich, S., Application of

isozyme data to the management of the United States national *Brassica oleracea* L. genetic resources collection. *Genet Resour Crop Evol* **41:** 99–108 (1994).

29. Ronning, C.M., Schnell, R.J.S., Allozyme diversity in a germplasm collection of *Theobroma cacao* L. *J Hered* **85:** 291–295 (1994).
30. Manjunatha, B.R., Virupakshi, S., Naik, G.R., Peroxidases isozyme polymorphism in popular sugarcane cultivars. *Curr Sci* **85(9):** 1347-1349 (2003).
31. Erachadi, S., Haberer, M., TorresRuiz, R.A., Estimating genetic diversity of *Arabidopsis thaliana* ecotypes with amplified fragment polymorphism (AFLP). *Theor. Appl. Genet*. **100:** 633-640 (2000).
32. Lerceteau, E., Szmidt, A.E., Properties of AFLP markers in inheritance and genetic diversity studies of Pinus Sylvestris L. *Heridity* **82:** 252-260 (1999).
33. Godt, M.J., and Hamrick, J.L., Population genetic analysis of Elliottia (Ericaceae), a rare Georgia shrub. *Molecular Ecology* **8:** 75-82 (1999).
34. Flachonsky, H., Schumann, E., Weber, W.E., Peil, A., Application of AFLP for the detection of sex-specific markers in hemp. *Plant Breeding* **120:** 305-309 (2001).
35. Kliebenstein, D.J., Gershenzon, J., Mitchell-olds, T., Comparative quantitative trait loci mapping of aliphatic, indolic and benzylic 585ehaviour585ates production in Arabidopsis thaliana leaves and seeds. *Genetics* **159:** 359-370 (2001).
36. Stansfield, W.D., Theory and problem of Genetics, McGraw-Hill Book Company, New Yark. (1986).
37. Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., Arnheim, N., *Science* **230:** 1350– 1354 (1985).
38. Weising, K., Nybom, H., Wolff, K., Meyer, W (1995) *DNA Fingerprinting in Plants and Fungi* (ed.Arbor, A.) CRC Press, Boca Raton, pp. 1–3.
39. Karp, A., Isaac, P.G., Ingram, G.S., Molecular Tools for Screening Biodiversity: Plants and Animals. *Chapman & Hall, Thompson Sci., London*. (1998).
40. Miller, J.C., Tanksley, S.D., RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theor Appl Genet* **80:** 437–448 (1990).
41. Botstein, D., White, R.L., Skolnick, M., Davis, R.W., Construction of a genetic map in man using restriction fragment length polymorphisms. *Amer J Hum Genet* **32:** 314–331 (1980).
42. Neale, D.B., Williams, C.G., Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. *Can J Fors Res* **21:** 545–554 (1991).
43. Lanner, H.C., Gustafsson, M., Falt, A.S., Bryngelsson, T., Diversity in natural populations of wild *Brassica oleracea* as estimated by isozyme and RAPD analysis. *Genet Resour Crop Evol* **43:** 13–23 (1997).
44. Fang, D.Q., Roose, M.L., Krueger, R.R., Federici, C.T., Fingerprinting trifoliate orange germplasm accessions with isozymes, RFLPs, and inter-simple sequence repeat markers. *Theor Appl Genet* **95:** 211–219 (1997).
45. Brubaker, C.L., Wendel, J.F., Reevaluating the origin of domesticated cotton (*Gossypium hirsutum*; Malvaceae) using nuclear restriction fragment length polymorphisms (RFLPs). *Amer J Bot* **81:** 1309–1326 (1994).
46. Jeffreys, A.J., Wilson, V., Thein, S.L., Hypervariable 'minisatellite' regions in human DNA. *Nature* **314:** 67–73 (1985a).
47. Zhou, Z., Bebeli, P.J., Somers, D.J., Gustafson, J.P., Direct amplification of minisatellite-region DNA with VNTR core sequences in the genus *Oryza*. *Theor Appl Genet* **95:** 942–949 (1997).
48. Wolff, K., Rogstad, S.H., Schaal, B.A., Population and species variation of minisatellite DNA in *Plantago*. *Theor Appl Genet* **87:** 733–740 (1994).

49. Kumar, P., Gupta, V.K., Misra, A.K., Modi, D.R. and Pandey, B.K., Potential of Molecular Markers in Plant Biotechnology. *Plant Omics Journal.* **2(4):** 141-162 (2009).
50. Matthes, M.C., Daly, A., Edwards, K.J., Amplified fragment length polymorphism (AFLP). In: Karp A.; Isaac P.G. and Ingram D.S. (eds): *Molecular Tools for Screening Biodiversity*. Chapman and Hall, Cambridge, Vol. 1, **99:** 183–190 (1998).
51. Jones, C.J., Edwards, K.J., Castaglione, S., Winfield, M.O., Sala, F., Wiel, C., van, de., Bredemeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, A., Marmiroli, N., Aert, R., Volckaert, G., Rudea, J., Linacero, R., Vazquez, A., Karp, A., Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Mol Breed* **3:** 381–390 (1997).
52. Witsenboer, H., Vogel, J., Michelmore, R.W., Identification, genetic localization, and allelic diversity of selectively amplified microsatellite polymorphic loci in lettuce and wild relatives (*Lactuca spp*.). *Genome* **40:** 923–936 (1997).
53. Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee van de, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., AFLP: a new technique for DNA fingerprinting. *Nucl Acids Res* **23:** 4407–4414 (1995).
54. Herselman, L., Genetic variation among Southern African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. *Euphytica* **133(3):** 319-327 (2003).
55. George, U.D.E., Kenworthy, N., William, J., Costa, J.M., Cregan, P.B., Alvernaz, J., Genetic diversity of soybean cultivars from China, Japan, North America, and North American ancestral lines determined by amplified fragment length polymorphism. *Crop Science* **43(5):** 1858-1867 (2003).
56. Lubberstedt, T., Anderson, J.R., Functional markers in plants. *Trends Plant Sci.* **8:** 554–560 (2003).
57. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl Acids Res* **18:** 6531–6535 (1990).
58. Hadrys, H., Balick, M., Schierwater, B., Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molec Ecol* **1:** 55–63 (1992).
59. Monteleone, I., Ferrazzini, D., Belletti, P., Effectiveness of neutral RAPD markers to detect genetic divergence between the subspecies *uncinata* and *mugo* of *Pinus mugo* Turra. *Silva Fennica* **40(3):** 391–406 (2006).
60. Litt, M., Luty, J.A., A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Amr J Hum Genet* **44:** 397–401 (1989).
61. Ghislain, M., Spooner, D.M., Rodríguez, F., Villamon, F., Núñez, C., Vásquez, C., Bonierbale, M., Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. *Theor Appl Genet* **108:** 881–890 (2004).
62. Yu, J.K., Dake, T.M., Singh, S., Benscher, D., Li, W., Gill, B., Sorrells, M.E., Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. *Genome / National Research Council Canada* **47(5):** 805-818 (2004a).
63. Yu, J.K., Rota, M., La., Kantety, R.V., Sorrells, M.E., EST. derived SSR markers for comparative mapping in wheat. and rice. *Mol Genet Genomics* **271:** 742–751 (2004b).
64. Nicot, N., Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). *Theor Appl Genet* **109:** 800–805 (2004).
65. Gao, L.F., Jing, R.L., Huo, N.X., Li, Y., Li, X.P., Zhou, R.H., Chang, X.P., Tang, J.F., Ma, Z.Y., Jia, J.Z., One hundred and

one new microsatellite loci derived from ESTs (EST-SSRs) in bred wheat. *Theor Appl Genet* **108:** 1392–1400 (2004).

66. Hearne, C.M., Ghosh, S., Todd, J.A., Microsatellites for linkage analysis of genetic traits. *Tren Genet* **8:** 288–294 (1992).
67. Morgante, M., Hanafey, H., Powell, W., Microsatellites are preferentially associated with nonrepetitive DNA in plant genome. *Nature Genet* **30:** 194–200 (2002a).
68. Jarne, P., Lagoda, P.J.L., Microsatellites, from molecules to populations and back. *Trends Ecol Evol* **11:** 424–429 (1996).
69. Mohammadi, S.A., Prasanna, B.M., Analysis of genetic diversity in crop plants – salient statistical tools and considerations. *Crop Sci* **43:** 1235–1248 (2003).
70. Ayers, N.M., McClung, A.M., Larkin, P.D., Bligh, H.F.J., Jones, C.A., Park, W.D., Microsatellites and a single-nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germ plasm. *Theor Appl Genet* **94:** 773- 781 (1997).
71. Russell, J., Booth, A., Fuller, J., Harrower, B., Hedley, P., Machray, G., Powell, W., A comparison of sequence-based polymorphism and haplotype content in transcribed and anonymous regions of the barley. *Genome* **47:** 389–398 (2004).
72. Zehdi, S., Trifi, M., Billotte, N., Marakchi, M., Pintaud, J.C., Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism. Hereditas **141:** 278–287 (2004).
73. Zietkietkiewicz, E.A.R., Rafalski, A. and Labuda, D., Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics* **20:** 176-183 (1994).
74. Mitra, C., Kharb, P., Uppal, S., Jain, S., Genetic diversity analysis in date palm (*Phoenix dactylifera* L.): a comparative assessment using ISSR and RAPD marker assays. *J Hortic Sci Biotech* **86:** 398–402 (2011).
75. Arslan, E., Tamkoc, A., The application of ISSR-PCR to determine the genetic relationship and genetic diversity between narrow leaved bluegrass (*Poaangustifolia*) and rough bluegrass (*Poatrivialis*) accessions. *Turk J Biol* **35:** 415–423 (2011).
76. Pasqualone, A., Lotti, C., Bruno, A., De Vita, P., Di Fonzo, N., Blanco, A., Use of ISSR markers for cultivar identification in durum wheat**.** *Option Méditerranéennes, Série A*, **40:** 157–161 (2000).
77. Hayashi, K., PCR-SSCP: a method for detection of mutations. *Genet Anal Techn Appl* **9:** 73–79 (1992).
78. Akopyanz, N., Bukanov, N., Westblom, T.U., Berg, D.E., PCR-based RFLP analysis of DNA sequence diversity in the gastric pathogen *Helicobacter pylori*. *Nucleic Acids Research* **20:** 6221–6225 (1992).
79. Konieczny, A., Ausubel, F.M., A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. *Plant Journal* **4:** 403–410 (1993).
80. Paran, I., Michelmore, RW (1993) Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. *Theor Appl Genet* 85:985–993.
81. Cho, R.J, Mindrinos, M., Richards, D.R., Sapolsky, R.J., Anderson, M., Drenkard, E., Dewdney, J, Reuber TL, Stammers M, Federspiel N, Theologis A, Yang WH, Hubbell E, Au M, Chung E.Y., Lashkari, D., Lemieux, B., Dean, C., Lipshutz, R.J., Ausubel, F.M., Davis, R.W., Oefner, P.J., Genome-wide mapping with biallelic markers in *Arabidopsis thaliana*. *Nature Genet* **23:** 203–207 (1999).
82. Kephart, S.R., Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *Amer J Bot* **77:** 693–712 (1990).
83. May, B., Starch gel electrophoresis of allozymes. In Molecular *genetic analysis of populations: a practical approach* (AR

Hoelzel, ed.). Oxford University Press, Oxford, UK. Pp. 1–27 (1992).

84. Dillon, S.L., Lawrence, P.K., Henry, R.J., The use of ribosomal ITS to determine phylogenetic relationships within *Sorghum*. *Plant System Evolu* **230:** 97–110 (2001).
85. Clegg, M.T., Molecular evaluation of plant genetic resources. In *Gene conservation and exploitation:* Proceedings of the 20th Stadler genetics symposium held at the University of Missouri, Colombia, Missouri, USA. Pp. 67–86 (1993a).
86. Maughan, P.J., Saghai Maroof, M.A., Buss, G.R., Microsatellite and amplified sequence length polymorphisms in cultivated and wild soybean. *Genome* **38:** 715–723 (1995).
87. Michelmore, R.W., Paran, I., Kesseli, R.V., Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. *Proc Natl Acad Sci USA*, **88:** 9828–9832 (1991).
88. Martin, G.B., Williams, J.G.K., Tanksley, S.D., Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. *Proc Natl Acad Sci USA* **88:** 2336–2340 (1991).
89. Riedel, G.E., Swanberg, S.L., Kuranda, K.D., Marquette, K., Pan La, P., Bledsoe, P., Kennedy, A., Lin, B.Y., Denaturing gradient gel electrophoresis identifies genomic DNA polymorphism with high frequency in maize. *Theor Appl Genet* **80:** 1–10 (1990).
90. Riesner, D., Steger, G., Zimmat, R., Owens, R.A., Wagenhofer, M., Hillen, W., Vollbach, S., Henco, K., Temperature-gradient gel electrophoresis of nucleic acids: analysis of conformational transitions, sequence variations, and protein-nucleic acid interactions. *Electrophor* **10:** 377–89 (1989).
91. Perez, J.A., Maca, N., Larruga, J.M., Expanding informativeness of microsatellite motifs through the analysis of heteroduplexes: a case applied to*Solanum tuberosum*. *Theor Appl Genet* **99:** 481–486 (1999).
92. Hauser, M.T., Adhami, F., Dorner, M., Fuchs, E., Glossl, J., Generation of codominant PCR-based markers by duplex analysis on high-resolution gels.*Plant Journal* **16:** 117–125 (1998).
93. Steinmetz, L.M., Mindrinos, M., Oefner, P.J., Combining genome sequences and new technologies for dissecting the genetics of complex phenotypes. *Trend Plant Sci* **5:** 397–401 (2000).
94. Kota, R., Wolf, M., Michalek, W., Graner, A., Application of denaturing highperformance liquid chromatography for mapping of single nucleotide polymorphisms in barley (*Hordeum vulgare* L.). *Genome*, **44:** 523–528 (2001).
95. Caetano-Anolles, G., Fingerprinting nucleic acids with arbitrary oligonucleotide primers. *Agro Food Industry Hi Tech* 7:26–31 (1996).
96. Caetano-Anolles, G., Bassam, B.J., Gresshoff, P.M., DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Biotech* **9:** 553–557 (1991).
97. Welsh, J., McClelland, M., Fingerprinting genomes using PCR with arbitrary primers. *Nucl Acids Res* **18:** 7213–7218 (1990).
98. Staub, J.E., Serquen, F.C., Gupta, M., Genetic markers, map construction, and their application in plant breeding. *Hort Sci* **31:** 729–741 (1996).
99. Heath, D.D., Iwama, G.K., Devlin, R.H., PCR primed with VNTR core sequences yields species specific patterns and hypervariable probes. *Nucl. Acids Res* **21:** 5782–5785 (1993).
100. Somers, D.J., Demmon, G., Identification of repetitive, genome-specific probes in crucifer oilseed species. *Genome* **45:** 485–492 (2002).