Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.7, No.9, 2017

Application of Genetic Markers for Plant Genetic Diversity Study

Tadesse Abate
Department of Animal and Range Science, College of Agriculture and natural resources, Madda Walabu University, P.O. Box 247, Bale robe, Ethiopia

Abstrat
This paper is an overview of the major genetic markers, used in assessing plant genetic diversity studies. Detection and analysis of genetic variation by genetic markers can help us to understand the molecular basis of various biological phenomena in plants. Various genetic markers are available for the genetic diversity study of plants. Phenotypic and biochemical markers tend to have the disadvantages of a low degree of polymorphism, relatively few loci and environmentally variable expression. DNA-based methods are widely used. Some of the common methods based on polymerase chain reaction (PCR) are amplified fragment length polymorphism (AFLP), in which restriction enzymes and non-random primers are used, and simple sequence repeats (SSRs), in which primers based on known sequences are employed. In recent years random amplified polymorphic DNAs (RAPDs), in which random primers are used, have been employed for cultivar identification of many crops. The review details account of the application of different genetic markers for plant genetic diversity study.
Keywords: Genetic diversity, Morphological markers, Molecular markers, Biochemical markers

Introduction

The conservation of genetic diversity both within and among natural populations is a fundamental goal for conservation of biological science. Henceforth, the knowledge utilizing different biotechnological methods are prioritized and also an essential component of plant and animals (as well as insects) resource management and they are becoming increasingly important for the conservation strategies utilizing different markers. A genetic marker is any character that can be measured in an organism which provides information on the genotype of that organism. Markers are used for genetic diversity study, construction of linkage maps and in the tracking of individuals or lines carrying particular genes. The development in biochemistry and molecular biology for the last 40 years was used for the emergence of marker systems to evaluate genetic variation at the intraspecific and interspecific levels (Kalendar *et al*., 2010). It is important, however, to understand that different markers have different properties and will reflect different aspects of genetic diversity (Karp and Edwards, 1995). Phenotypic and biochemical markers tend to have the disadvantages of a low degree of polymorphism, relatively few loci and environmentally variable expression. The advent of the PCR was a breakthrough for molecular marker techniques to describe the genetic constitution of an individual and also be used to determine the genetic polymorphism, diversity and relationship, *etc* existing in a population. Random Amplified Polymorphic DNA (RAPD) is one of the first developed molecular markers that have been studied widely in all genetic programs. RAPD was desirable marker for many years (Williams *et al.,* 1990) because of it uses short primer, no need of priori genome sequence information, high speed, low cost and low technical requirement. On the other hand, presenting many potential priming sites for these sequences, low annealing temperature make the invention for new methods such as Amplified fragment length polymorphism (AFLP) and Simple sequence repeat (SSR) which solved RAPD problems, thus eliminating this system from molecular markers today (Kalendar *et al*., 2011).

Genetic markers

Genetic diversity refers to any variation in nucleotides, genes, chromosomes or whole genomes of organisms (Wang *et al*., 2009). An adequate knowledge of existing genetic diversity, where-in plant population is found and how to best utilize it, is of fundamental interest for basic science and applied aspects like the efficient management of crop genetic resources. Genetic diversity can be assessed among different accessions /individuals within same species (intraspecific), among species (interspecific) and between genus and families (Mittal and Dubey, 2009) by using different types of genetic markers. A genetic marker is any character that can be measured in an organism which provides information on the genotype of that organism. Determining genetic diversity can be based on morphological, biochemical, and molecular types of information (Mohammadi and Prasanna, 2003; Sudre *et al*., 2007; Goncalves *et al*., 2009).

Morphological markers

Morphological markers are usually visually characterized phenotypic characters such as flower color, seed shape, growth habits or pigmentation (Sumarani *et al*., 2004). Morphological markers reflect variation of expressed regions of genome. Morphological traits were among the earliest markers used in germplasm management and they are the strongest determinants of the agronomic value and taxonomic classification of plants. Especially, if

the traits are highly heritable, morphological markers are one of the choices for diversity studies because the inheritance of the marker can be monitored visually (Yoseph, 2005). Despite these advantages, morphological features have a number of limitations including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith, 1992; Konarev, 2000; Muthusamy *et al.,* 2008), which, in turn limits their utility for assessing real genetic diversity. In addition, performing breeding experiments with these markers is time consuming, labour intensive and the large populations of plants required need large plots of land and/or greenhouse space in which to be grown (Stuber *et al.,* 1999).

Biochemical Marker

Biochemical markers are differences in enzymes that are detected by electrophoresis and specific staining (Pillai *et al*., 2000). Storage protein and isozymes are the main biochemical markers used for characterization of plant genetic resources, relationships at lower taxonomic levels as well as to detect geographic origin. Isozymes are common enzymes expressed in the cells of plants. The enzymes are extracted, and run on denaturing electrophoresis gels. The denaturing component in the gels (usually SDS) unravels the secondary and tertiary structure of the enzymes and they are then separated on the basis of net charge and mass. Polymorphic differences occur on the amino acid level allowing singular peptide polymorphism to be detected and utilized as a polymorphic biochemical marker. The technique is rapid and economical, and codominant nature of allozyme data makes it useful for the characterization of genetic variation in plant species (Weising *et al.,* 2005). Although protein markers circumvent environmental effects, the number of detectable isozymes are limited and they are typically tissue and developmental stage-specific (Park *et al*., 2009). For this reason, most researchers began to focus on the use of DNA marker systems for genetic and ecological analyses of plant populations.

DNA based markers

Molecular genetic markers are specific fragments of DNA that can be identified within the genome of the organism under study using a broad variety of techniques. Molecular markers are the most recent to be developed and have proven to be the powerful tools for genotype characterization and estimation of genetic variation both within and between plant population by analyzing large numbers of loci distributed throughout the genome (Powell *et al*., 1995; Treuren *et al*., 2005). Molecular or DNA- based markers have many advantages over morphological and biochemical markers. The primary advantages include their availability in potentially unlimited number and the property that they generally are not affected by developmental differences or environmental influences. Nowadays, molecular marker technologies are increasingly being used to complement traditional methods because of their ability to measure diversity directly at the DNA level (Brown and Kresovich, 1996; Karp *et al*., 1997; Spooner *et al*., 2005).

Different types of molecular markers are utilized to evaluate DNA polymorphism and can be classified based on the method of detection as hybridization (non PCR) based markers and polymerase chain reaction (PCR)-based markers. In hybridization based markers DNA profiles are visualized by hybridizing the restriction endonuclease digested DNA fragment, to a labelled probe, which is a DNA fragment of known sequence, while PCR based markers involve *in- vitro* amplification of particular DNA sequences with the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermo stable DNA polymerase enzyme. The amplified DNA fragments are separated by electrophoresis and banding patterns are detected by different methods such as staining (using ethidium bromide dye) and autoradiography.

Non PCR-based techniques

Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) was the first reported molecular marker technique developed in the 1980s (Saiki *et al*., 1985) for use in human genetic applications and was later applied to plants. RFLP analysis is based on the ability of restriction enzymes (also called restriction endonucleases) to cleave DNA in to pieces (digested) at specific target nucleotide sequences consisting usually of a four or six nucleotide pairs. Changes within these sequences which can be caused by point mutations, insertions or deletions, result in DNA fragments of differing length and molecular weights. The resulting restriction fragments are resolved by gel electrophoresis and then blotted (Southern, 1975) on to a nitrocellulose membrane. Specific banding patterns are then visualized by hybridization with labeled probe.

The advantages of RFLPs are the co-dominant nature of the markers, their reliability and specificity. Furthermore, cDNA-based RFLP have the potential to detect homologous sequences in distantly related genomes, which makes them especially useful to construct consensus maps (Isobe *et al*., 2003). RFLP is limited by the relatively large amount of high quality DNA required for restriction digestion, probes need to be developed, the technique is labour and time consuming (Staub *et al*., 1996). However, it is still used in marker assisted selection procedures. These limitations led to the conceptualization of a new set of less technically complex methods known as PCR-based techniques.

Markers Based on Amplification Techniques (PCR-Derived)

With the beginning of studies that led to the development of polymerase chain reaction (PCR) technology (Saiki *et al*., 1985; Mullis and Faloona, 1987), a large number of approaches for generation of molecular markers based on PCR were detailed, relevant mainly to research in genetic diversity, primarily due to its apparent simplicity and high probability of success. A number of PCR-based DNA markers have been developed in recent years to evaluate genetic variation at the intra-specific and inter-specific levels (Wolfe and Liston, 1998).

Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA (RAPD) was the first PCR based molecular marker technique developed and it is by far the simplest one. RAPDs are generated by PCR amplification of random genomic DNA segments using randomly constructed oligonucleotides as primer which is annealed to the template DNA typically at 37°C (Williams *et al*., 1990; Jacobson and Hedrén, 2007), separation of the amplified fragments on agarose gel in the presence of ethidium bromide and finally, visualization under ultraviolet light. The variation in RAPD profile is in the form of presence or absence of a band resulting from variation in primer binding sites. RAPD polymorphisms can theoretically result from several types of events: (1) insertion of a large piece of DNA between the primer binding sites may exceed the capacity of PCR, resulting in fragment loss; (2) insertion or deletion of a small piece of DNA will lead to a change in size of the amplified fragment; (3) the deletion of one of the two primer annealing sites results in either the loss of a fragment or an increase in size; (4) a nucleotide substitution within one or both primer target sites may affect the annealing process, which can lead to a presence versus absence of polymorphism or to a change in fragment size (Weising *et al.,* 2005).

The power of RAPD is that it is a fast technique, easy to perform and comparatively cheap and little amount of DNA quantities are required to detect relatively small amount of genetic variation (Ragot and Hoisington, 1993). Also, there are no requirements for radioactive chemicals and no need of prior knowledge of the genome that is being analyzed; it can be immediately applicable to the analysis of most organisms because universal sets of primers are used (Williams *et al.,* 1990; Hallden *et al*., 1996).

Despite these advantages, RAPD analysis presents some practical problems. RAPDs are dominant markers, i.e. it is impossible to detect heterozygotes and besides being dominantly inherited, RAPDs also suffer from severe reproducibility problems due to low annealing temperature of primers if compared to other techniques (Jones *et al*., 1997a; Rabouam *et al*., 1999), which ultimately limited their use in the past years. Despite a number of drawbacks, RAPDs are still in used. Main application areas include variety identification, determination of genetic variability, relationship among the crop genotypes and construction of linkage maps (Young , 2000; Jaroslava *et al*., 2002).

Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP) technology is the recent DNA fingerprinting technique developed by Vos *et al.,* (1995), to overcome the limitation of reproducibility associated with RAPD and it is equally applicable to all species with no prior sequence knowledge. AFLP is the selective amplification of restriction fragments from a digest of total gnomic DNA using the polymerase chain reaction (PCR), and detects fragment length polymorphisms from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE) (Vos *et al*., 1995; Matthes *et al*., 1998) or by capillary electrophoresis. Possible reasons for AFLP polymorphisms are (1) sequence variations in a restriction site (the same as in RFLPs), (2) insertions or deletions within an amplified fragment (also the same as in RFLPs), and (3) differences in the nucleotide sequence immediately adjoining the restriction site (not detected with RFLPs).

It can be considered an intermediate between RFLPs and RAPDs methodologies as it combines the power of RFLP with the flexibility of PCR-based technology by ligating primer recognition sequences (adaptors) to the restricted DNA (Lynch and Walsh, 1998). The AFLP technology is a powerful tool to distinguish closely related individuals at the sub-species level (Althoff *et al.,* 2007*)* and can also map genes. AFLP assays require no previous sequence knowledge for primer construction, this allows for the use of standard primers and can detect 20-100 loci per assays (Maughan *et al*., 1996; Powell *et al.,* 1996). It generates higher polymorphism which is also largely reliable and reproducible (Powell *et al*., 1996; Jones *et al*., 1997a). Despite its attractiveness, the AFLP method has some detrimental aspects. AFLP markers are mainly dominant and the analysis is technically demanding (Jones *et al*., 1997a). It requires clean and high molecular weight DNA for ensuring complete digestion by enzymes. Partial digestion of DNA results in non-reproducible variation in DNA profiles.

Microsatellites

Microsatellites , also known as simple sequence repeats (SSRs) (Tautz *et al*., 1986; Litt and Luty, 1989), are DNA stretches, consisting of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units that are widely dispersed through eukaryotic nuclear genomes (Powell *et al*., 1996). They also appear in prokaryotic and eukaryotic organellar genomes, e.g., chloroplast (Powell *et al*., 1995) and mitochondria (Soranzo *et al*., 1999). In

microsatellite analysis, sequence information of the regions flanking the repeats is used for creating locus specific PCR primer pairs. Therefore, specific primers are used to amplify microsatellites by the polymerase chain reaction (PCR). The products generated have been found to be highly reproducible and cost effective (Jones *et al.,* 1997a) and the resulting amplification products are separated on polyacrylamide gels and visualized. Variation on the number of repetitions of the basic motif is mainly due to strand slippage during DNA replication where the repeats allow matching via excision or addition of repeats (Schlotterer and Tautz, 1992).

The proven advantages of SSR markers is due to their high variability resulting from high mutation rates that affect the number of repeating units, exhibit co-dominant inheritance, locus specificity, extensive genome coverage and simple detection using labelled primers that flank the microsatellite and hence define the microsatellite locus (Graham *et al*., 2004; Selkoe and Toonen, 2006). SSRs demonstrate a high degree of transferability between species, as PCR primers designed to an SSR within one species frequently amplify a corresponding locus in related species, enabling comparative genetic and genomic analysis. These features provide the foundation for their successful application in a wide range of fundamental and applicable fields (Chistiakov *et al*., 2006). SSR markers have become quite useful in various aspects of molecular genetic studies, including assessment of genetic diversity (Amsellem *et al*., 2001; Ashley *et al*., 2003), finger printing (Rongwen *et al*., 1995), ecological genetic studies (Li *et al*., 2000), marker-assisted selection (Fazio *et al*., 2003), and genetic linkage mapping (Broun and Tankley, 1996; Graham *et al*., 2004).

Inter Simple Sequence Repeat (ISSR)

Inter simple sequence repeat (ISSR) technique was first described by Zietkiewicz *et al.,* (1994) and Kantety *et al.,* (1995). ISSR involves amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellites as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly inter simple sequence repeats of different sizes. The primer can be based on any of the SSR motifs (di-, tri-, tetra- or penta-nucleotides) found at microsatellite loci giving a wide array of possible amplification products (Blair *et al*., 1999). The potential use of ISSR markers depends on the variety and frequency of microsatellites, which changes with the species and with the targeted SSR motifs (Morgante and Olivieri, 1993). In addition, the number of bands produced by an ISSR primer with a given microsatellite repeat should reflect the relative frequency of that motif in the genome and would provide an estimate of the motif's abundance as an alternative for library hybridization (Blair *et al*., 1999).

The amplification and data-scoring protocols used for ISSR markers are similar to those used for random amplified polymorphic DNA (RAPD) markers with the exception that ISSRs use longer primers (15–30 mers) as compared to RAPD primers (10 mers), which permit the subsequent use of high annealing temperature leading to higher stringency (Wolfe and Liston, 1998). The annealing temperature depends on the GC content of the primer used and ranges from 45 to 65℃. The amplified products are usually 200–2000 bp long and amenable to detection by both agarose and polyacrylamide gel electrophoresis. ISSR is a dominant marker like RAPD in which visible bands are assigned to genetic loci with two alleles: 1= presence and 0= absent but greater robustness in repeatability and generates larger number of polymorphisms per primer because variable regions in the genome are targeted (Ila *et al.,* 2003; Kafkas *et al.,* 2006). The sources of variation in ISSR markers could be: (1) mutations at the priming site (SSR), which could prevent amplification of a fragment as in RAPD markers and thus give a presence/absence polymorphism. (2) An insertion/deletion event within the SSR region or the amplified region would result in the absence of a product or length polymorphism depending on the amplifiability of the resulting fragment size. This method has a wide range of uses, including the characterization of genetic relatedness among populations (Ammiraju *et al.,* 2001), genetic fingerprinting (Blair *et al.,* 1999), gene tagging (Ammiraju *et al.,* 2001), detection of clonal variation (Leroy and Leon, 2000), cultivar identification (Wang *et al.,* 2009), phylogenetic analysis (Gupta *et al.,* 2008), detection of genomic instability (for example, it was used in human quantification of genomic instability to estimate prognosis in colorectal cancer (Brenner, 2011), and assessment of hybridization (Wolfe *et al.,* 1998).

Among molecular markers, ISSRs are widely used in genetic diversity studies because amplification in this technique does not require genome sequence information for designing the primer, and the primers are not proprietary and can be synthesized by any one, their development costs are low, and the laboratory procedures can easily be applied to any plant species (Aga *et al.,* 2005; Tesfaye *et al.,* 2013; Zietkiewicz *et al*., 1994). The method provides highly reproducible results and generates abundant polymorphisms in many systems. This technique can rapidly differentiate closely related individuals (Zietkiewicz *et al.,* 1994) and have been successfully used to assess genetic diversity among closely related cultivars which were difficult to distinguish with other molecular marker (Dagani *et al*., 2003; Salhi-Hannachi *et al.,* 2005; Okpul *et al*., 2005).

Conclusion

Genetic diversity being referred to as the sum total of genetic variations found in a species or population is

source for ecological biodiversity. Measuring genetic diversity is significant to examine variations present among the organisms on the basis of genetic markers at phenotypic, biochemical and genotypic level. Morphological markers enable the detection of genetic variation based on individual phenotypic variations. However, there are limitations confined to these types of markers. Morphological markers limitation led the assessment of biodiversity from relying on morphological markers to using isozymes and DNA markers that became known as molecular markers. There are different types of molecular markers which are classified based on variation type at the DNA level, mode of gene action and method of analysis. They are key tools in genome analysis which ranges from localization of a gene to improvement of plant varieties through marker assisted selection . Even though there are several applications of DNA markers, marker assisted selection is the most promising technique for crops cultivar development.

References

Aga, E., Endeshaw Bekele and Bryngelsson, T. (2005). Inter simple sequence repeat (ISSR) variation in forest coffee tree (Coffea Arabica L.) populations from Ethiopia. *Genetica* 124: 213-221.

Althoff, D., Gitzendanner, M. and Segraves, K. (2007). The utility of amplified fragment length polymorphisms in phylogenetics: a comparison of homology within and between genomes. *Syst. Biol.* 56: 477-484.

Ammiraju,J.,Dholakia,B.,Santra,D.,Singh,H.,Lagu.,M.,Tamhankar,S.,Dhaliwal,H.,Rao,V. ,Gupta,V. and Ranjekar, P. (2001). Identification of Inter simple sequence repeat (ISSR) markers associated with seed size in wheat. *Theor Appl Genet*. 102: 726- 732.

Amsellem, L., Dutech, C. and Billote, N. (2001). Isolation and characterization of polymorphic microsatellite loci in Rubus alceifolius Poir (Rosaceae), an invasive weed in La Reunion island. *Molecular ecology notes* 1: 1-2.

Ashley, M., Wilk, J., Styan, S., Craft, K., Jones, K., Feldheim, K., Lewers, K. and Ashman, T. (2003). High variability and disomic segregation of microsatellites in the octoploid Fragaria virginiana Mill (Rosaceae). *Theor. Appl. Genet*. 107: 1201-1207.

Blair, M., Panaud, O. and McCouch, S. (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (Oryza sativa L.). *Theor Appl Genet.* 98:780-792.

Brenner, B., Swede, H., Jones, B., Anderson, G. and Stoler, D. (2011). Genomic Instability Measured by Inter-Simple Sequence Repeat PCR and High-Resolution Microsatellite Instability are Prognostic of Colorectal Carcinoma Survival After Surgical Resection. *Ann Surg Oncol*. 19: 344-50.

Broun, P. and Tanksley, S. (1996). Characterization and genetic mapping of simple repeat sequences in the tomato genome. *Mol. Gen. Genet.* 250: 39-4

Brown, S. and Kresovich, S. (1996). Molecular characterization for plant genetic resources conservation. In: Genome mapping of plants, Pp. 85 -93, (Paterson, H., ed). Academic press, San Diego.

Chistiakov, D., Hellemans, B. and Volckaert, F. (2006). Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Aquaculture* 255:1-29.

Dagani, C., Deng, J., Beiles, A., El Batsri, R., Goren, M. and Gazit, S. (2003). Identifying Lychee (Litchi chinensis Sonn.) cultivars and their relationships using Inter simple Sequence Repeat (ISSR) markers. *J. Amer. Soc. Hort. Sci*. 128 : 838-845.

Fazio, G., Chung, S. and Staub, J. (2003). Comparative analysis of response to phenotypic and marker-assisted selection for multiple lateral branching in cucumber (Cucumis sativa L.). *Theor. Appl. Genet.* 107: 875-883.

Goncalves, L., Rodrigues, R., Amaral, D., Junior, A. andKarasawa, M. (2009). Heirloom tomato gene bank: assessing genetic divergence based on morphological, agronomic and molecular data using a Ward-modified location model. *Genet. Mol. Res*. 8: 364-374.

Graham, J., Smith, K., MacKenzie, Jorgenson, L., Hackett, C. and Powell, W. (2004). The construction of a genetic linkage map of red raspberry (Rubus idaeus subspp.idaeus) based on AFLPs, genomic-SSR and EST-SSR markers. *Theor. Appl. Genet*. 109: 740-749.

Gupta, S., Souframanien, J. and Gopalakrishna, T. (2008). Construction of a genetic linkage map of black gram, Vigna mungo (L.) Hepper, based on molecular markers and comparative studies. *Genome* 51: 628–637.

Hallden, C., Hansen, M., Nilsson, N., Hejrdin, A. and Sall, T. (1996). Competition as a source of errors in RAPD analysis. *Theor. Appl. Genet*. 93: 1185–1192.

Ila, H., Kafkas, S. and Topaktas, M. (2003). Chromosome numbers of Four (Anacardiaceae) species. *Journal of Horticultural Science Biotechnology* 78: 35– 38.

Isobe, S., Klimenko, I., Ivashuta, S., Gau, M. and Kozlov, N. (2003). First RFLP linkage map of red clover (Trifolium pratense L.) based on cDNA probes and its transferability to other red clover germplasm. *Theor Appl Genet*. 108:105-112

Jacobson, A. and Hedrén, M. (2007). Phylogenetic relationships in Alisma (Alismataceae) based on RAPDs and

sequence data from ITS and trnL. *Plantsystematics and evolution* 265: 27- 44.

Jaroslava, A., Polakova, K. and Leisova, L. (2002). DNA analysis and their application in plant breeding. Czech J. Genet. *Plant Breed*. 38: 29-40.

Jones, C., Edwards, K., Castaglione, S., Winfield, M., Sala, F. and van de Wiel, C. (1997a) .Reproducibility testing of RAPD, AFLP, and SSR markers in plants by a network of European laboratories. *Mol. Breed.* 3:381-390.

Kafkas, S., Ozkan, H., Acar, I., Atli, H. and Koyoncu, S. (2006). Detecting DNA polymorphism and genetic diversity in a wide germplasm: comparison of AFLP, ISSR, RAPD markers. *American Society for Horticultural Science* 131: 522-529.

Kantety, R., Zeng, X., Bebbetzen, J. and Zehr, B. (1995). Assessment of genetic diversity in dent and popcorn (Zea mays L.) inbreed lines using inter-simple sequence repeat (ISSR) amplification. *Mol Breeding* 1: 36-373.

Karp, A. and Edwards, K. (1995). Molecular techniques in the analysis of the extent and distribution of genetic diversity. IPGRI Workshop on Molecular Genetic Tools in Plant Genetic Resources, 9–11 October, Rome, IPGRI.

Karp, A., Kresovich, S., Bhat, K., Ayad, W. and Hodgkin, T. (1997). Molecular tools in plant genetic resources conservation: a guide to the technologies. IPGRI technical bulletin No. 2. International Plant Genetic Resources Institute, Rome.

Konarev, V. (2000). Cultivar identification and gene pool registration by seed proteins in cultivated plants. St. Petersburg: Vses. Inst. Rastenievod, Russia.

Leroy, X. and Leon, K. (2000). A rapid method for detection of plant genomic instability using unanchored-microsatellite primers. *Plant Mol Biol Rep*. 18 : 283a–283g.

Li, Y., Fahima, T., Korol, A., Peng, J., Roder, M., Kirzhner, V., Beiles, A. and Nevo, E. (2000) . Microsatellite diversity correlated with ecological-edaphic and genetic factors in three microsatellites of wild emmer wheat in north Israel. *Mol. Biol. Evol*. 17: 851-862.

Litt, M. and Luty, J. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet*. 44:397–401

Matthes, M., Daly, A. and Edwards, K. (1998). Amplified fragment length polymorphism (AFLP) In: Molecular Tools for Screening Biodiversity, Pp.183–190, (Karp A., Isaac P.G., Ingram D.S., eds.). Chapman and Hall, Cambridge.

Maughan, P, Maroof, M., Buss, G. and Huesis, G. (1996). Amplified Fragment Length Polymorphism (AFLP) in soy-bean: species diversity, inheritance and near isogenic line analysis. *Theoretical and applied genetics* 93: 392-401

Mittal, N. and Dubey, A. (2009). Microsatellite markers - A new practice of DNA based markers in molecular genetics. *Pharmacognosy Reviews* 3: 235-246.

Mohammadi, S. and Prasanna, B. (2003). Analysis of genetic diversity in crop plants - salient statistical tools and considerations. *Crop Sci*. 43: 1235-1248.

Morgante, M. and Olivieri, A. (1993). PCR amplified microsatellite as markers in plant genetics. *Plant J.* 3: 175-182.

Mullis, K. and Faloona, F. (1987). Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction. *Methods Enzymol.* 155: 335-350

Muthusamy, S., Kanagarajan, S and Ponnusamy, S. (2008). Efficiency of RAPD and ISSR markers system in assessing genetic variation of rice bean (Vigna umbellata) landraces. *Electronic J. Biotech.* 11: 1-10.

Okpul, T., Mace, E., Godwin, I., Singh, D. and Wagih, M. (2005). Evaluation of variability among breeding lines and cultivars of taro (Colocasia esculenta) in 73 Papua New Guinea using ISSR fingerprinting and agro-morphological characterization. PGR Newsletter 143: 8-16.

Park, Y., Lee, J. and Kim, N. (2009). Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops. *Molecules* 14: 4546-4569.

Pillai, S., Sundaresan, P., Harisankar and Sumarani, G. (2000). Molecular characterization of germplasm in tropical tuber crops, DAE-BRNS Symposium, Mumbai

Powell, W., Morgante, M., Andre, C., McNicol, J., Machray, G., Doyle, J., Tingey, S. and Rafalski, J. (1995). Hypervariable microsatellites provide a general source of polymorphic DNA markers for the chloroplast genome. *Current Biology* 5: 1023- 1029.

Powell, W., Morgante, M., Andre, C., McNicol, J., Machray, G., Doyle, J., Tingey, S. and Rafalski, J. (1995). Hypervariable microsatellites provide a general source of polymorphic DNA markers for the chloroplast genome. *Current Biology* 5: 1023- 1029

Rabouam, C., Comes, A., Bretagnolle, V., Humbert , J., Periquet, G. and Bigot, Y. (1999) .Features of DNA fragments obtained by random amplified polymorphic DNA (RAPD) assays. *Mol Ecol*. 8: 493–503.

Ragot, M. and Hoisington, D. (1993). Molecular markers for plant breeding: Comparisons of RFLP and RAPD

genotyping costs. *Theoretical and Applied Genetics* 86: 965-984.
Rongwen, J., Akkaya, M., Bhagwat, A., Levi, U. and Cregan, P. (1995). The use of microsatellite DNA markers for soybean genotypes identification. *Theor. Appl. Genet*. 90: 43-48.
Saiki, R., Scharf, S., Faloona, F. and Mullis, K. (1985). Enzymatic amplification of betaglobingenomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230: 1350-1354
Salhi-Hannachi, A., Chatti, K., Mars, M., Marrakchi, M. and Trifi, M. (2005). Comparative analysis of genetic diversity in two Tunisian collections of fig cultivars base on random amplified polymorphic DNA and inter simple sequence repeats fingerprints. *Genet Res and Crop Evol*. 52: 563-573.
Schlotterer, C. and Tautz, D. (1992). Slippage synthesis of simple sequence DNA. *Nucleic Acids Res.* 20: 2211-2215.
Selkoe, K. and Toonen, R. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol. Letters* 9: 615-629.
Smith, J. and Smith, O. (1992). Fingerprinting crop varieties. *Adv. Agron*. 47: 85-140.
Soranzo, N., Provan, J. and Powell, W. (1999). An example of microsatellite length variation in the mitochondrial genome of conifers. *Genome* 42: 158–161.
Southern, E. (1975). Deletion of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol.Biol*. 96 : 503-517
Spooner, D., van Treuren, R. and de Vicente, M. (2005). Molecular markers for genebank management. IPGRI technical bulletin No. 10. International Plant Genetic Resources Institute, Rome.
Staub, J., Serquen, F. and Gupta, M. (1996). Genetic markers, map construction, and their application in plant breeding. *Hortscience* 31:729-741
Stuber, C., Polacco, M. and Senior, M. (1999). Synergy of Empirical Breeding, Marker Assisted selection and Genomics to Increase Crop Yield Potential. *Crop Science* 39: 1571-1583.
Sudre, C., Leonardecz, E., Rodrigues, R. and Júnior, A. (2007). Genetic resources of vegetable crops: a survey in the Brazilian germplasm collections pictured through papers published in the journals of the Brazilian Society for Horticultural Science. *Hortic. Bras*. 25: 496-503.
Sumarani, G., Pillai, S., Harisankar, P. and Sundaresan, S. (2004). Isozyme analysis of indigenous cassava germplasm for identification of duplicates. *Genetic Resources and Crop Evolution* 51: 205-209
Tautz, D., Trick, M. and Dover, G. (1986). Cryptic simplicity in DNA is a major source of genetic variation. *Nature* 322: 652-656.
Tesfaye, K., Govers, K., Bekele, E., and Borsch,T. (2013). ISSR finger printing of coffea Arabica throughout Ethiopia reveals high variability in wild populations and distinguishes them from land races. *Plant Syst Evol*. 1-17
Treuren, R., Bas, N., Goossens, P., Jansen, J. and Soest, L. (2005). Genetic diversity in perennial ryegrass and white clover. *Mol. Ecol*.14: 39-52.
Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407–441.
Wang, H., Wu, Z., Lu , J., Shi, N., Zhao,Y., Zhang, Z. and Liu, J. (2009). Molecular diversity and relationships among Cymbidium goeringii cultivars based on intersimple sequence repeat (ISSR) markers. *Genetica* 136: 391–399.
Weising, K., Nybom, H., Wolff, K. and Kahl, G. (2005). DNA Fingerprinting in Plants: Principles, Methods, and Applications Second edition. Taylor & Francis Group, CRS Press, New York.
Williams, J., Kubelik, A., Livak, K. and Rafalski, J. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*. 18: 6531-6535
Wolfe, A. and Liston, A. (1998). Contribution of PCR based methods to plant systematic and evolutionary biology. In: Molecular biology of plants II DNA sequencing ,Pp. 43-86, (Soltis, D. E., Soltis, P. S., Doyle, J.J., eds.). Kluwer Acad. Publ., Boston, Dordrecht, London.
Yoseph Beyene (2005). Genetic analysis of traditional Ethiopian highland maize (Zea mays L.) using molecular markers and morphological traits: implication for breeding and conservation. Phd Dissertation, University of Pretoria, Pretoria
Young, N. (2000). Constructing a plant genetic map with DNA markers. In: DNA-based markers in plants, Pp. 495-540, (Phillips, R. L, Vasil, J. K., eds.). Kluwer Academic Publishers
Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.