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## DNA fingerprinting and its applications in crop improvement: A review

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### Abstract

Crop DNA fingerprinting is becoming more popular in plant breeding as a result of its uses in variety protection, dispute settlement, and forensic science research. The varieties were distinguished based on the morphological markers before the development of proteomic and genomic technology. For genetic diversity analysis in crops, protein based markers were discovered and used in mid 20<sup>th</sup> century. For crop fingerprinting DNA markers are used mostly in the genomic era. Crop fingerprinting with DNA markers began with RFLPs (non-PCR based markers) and progressed to polymerase chain reaction (PCR) based markers that are RAPDs (Randomly Amplified Polymorphic DNA), SSRs (Simple Sequence Repeat), AFLPs (Amplified fragment Length Polymorphisms, ISSRs (Inter Simple Sequence Repeats), SNPs (Single Nucleotide Polymorphism), DArT (Diversity Array Technology). The development of cost effective whole genome sequencing techniques is critical to the future of crop fingerprinting. Distinction of highly similar varieties, mutants, certain clones, and vegetatively propagated crops might be possible with such technology. This review paper gives an idea about different markers used for DNA fingerprinting and also its applications for crop improvement.

**Keywords:** Non PCR based markers, DUS test, genotyping, morphological markers, and heterosis

### Introduction

The use of molecular marker techniques to identify cultivars is referred to as plant DNA fingerprinting. DNA fingerprinting began in 1985, when Alec Jeffreys and colleagues published a series of papers describing tandem-repetitive sections of DNA (also known as minisatellites) can produce somatically stable DNA fingerprints that are fully unique to an individual. During plant breeding programs, seed production, marketing, and product inspection, identification of variety, classification, and sustainability are crucial. The study of genetic variation and relatedness is an important part of the protection and utilization of biodiversity as well as food security (Nybom *et al.*, 2014) [33]. Based on morphological markers i.e., using DUS (Distinct, Uniformity and Stability) test the plant varieties and species were identified (Tiwari *et al.*, 2013) [55]. Due to multigene nature and environment dependent the morphological markers were unreliable, less informative and less effective for identification of variety (Korir *et al.*, 2013) [24]. To know the genetic diversity among plant genotypes, DNA markers are cost effective and are most reliable approach as they are environment independent. The use of molecular markers to characterize hybrids and their parental lines has numerous benefits over morphological and biochemical markers (Sharma *et al.*, 2014) [48]. The most promising technologies for identifying plant genotypes are molecular methods, particularly DNA fingerprinting (Nybom *et al.*, 2014) [33]. Paul Hebert coined the term "DNA fingerprinting" in 2003 as an alternative to conventional morphological-based classification and it is now a generally acknowledged approach for determining genetic differences and relatedness (Hebert *et al.*, 2003). DNA fingerprinting is a technique that uses DNA markers to allocate breeding lines to heterotic groups and to identify varieties (Jamil *et al.*, 2020) [19]. Non PCR based DNA marker is RFLP (Restriction Fragment Length Polymorphism), PCR based markers are mostly used now a days like SSR (Simple Sequence Repeat), RAPD (Randomly Amplified Polymorphic DNA), AFLPs (Amplified fragment Length Polymorphisms), ISSR (Inter Simple Sequence Repeats), SNPs (Single Nucleotide Polymorphism), DArT (Diversity Array Technology), GBS (Genotyping by Sequencing) (Nadeem *et al.*, 2018) [32].

### DNA fingerprinting in crops using different markers

1. Morphological markers
2. Biochemical markers or protein markers (Isozyme)

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### 3. DNA markers:

- a) Non PCR based marker or probe based: RFLP
- b) Amplification based: RAPD, SSR, ISSR, SCAR, CAPs, STS, VNTRs, SPLAT
- c) Probe and PCR based: AFLP, rDNA- ITS
- d) New generation: SNP, EST, SSCP

#### Morphological markers

For its unique identification, a distinctive trait found in a genotype was termed as fingerprint. For identification of cultivars, morphological markers like grain color, presence or absence of awns, plant height, and leaf sheath coloration were used earlier. Using morphological descriptors crops like sugarcane (Selvi *et al.*, 2003) [47], grapevine (Royo *et al.*, 1997) [44], peas (Taran *et al.*, 2005) [54], napier grass (Bhandari *et al.*, 2006) [6] were fingerprinted. Recessive genes influence morphological features, which can only be expressed in homozygous form. Because these traits are quantitative, estimating and genetic mapping them is a difficult task (Bhandari *et al.*, 2006) [6].

#### Isozymes

Because of their speed, accuracy, and relative independence from environmental factors, isozymes were used for fingerprinting after 1960 (Nybom *et al.*, 2014) [33]. Sample collection, enzyme extraction, gel electrophoresis, gel staining, visualization and evaluation of fingerprinting were all included of the isozyme analysis (Sumarani *et al.*, 2004) [53]. Isozymes are used in napier grass (Bhandari *et al.*, 2006) [6], cassava (Sumarani *et al.*, 2004) [53], grapevine (Royo *et al.*, 1997) [44] for fingerprinting and characterization. Protein extraction from the collected plant sample is time consuming and difficult (Nybom *et al.*, 2014) [33]. Protein degradation during sample collection is main issue. The results of isozyme analysis are strongly influenced by differences in sample time and tissue type (Johnson *et al.*, 2010) [20].

#### DNA markers

Several DNA marker methods are widely used in plant diversity research. The genetic variety and relatedness of species may be studied using sequence information because each individual's DNA sequence is unique. RAPD, RFLP, AFLP, SSR markers are first and second generation markers. RFLPs, RAPDs, AFLPs, and SSR are examples of first and second-generation molecular markers, while SNPs, DArT tests, and GBS are examples of third and fourth-generation markers (Paux *et al.*, 2012) [36]. Molecular markers are commonly regarded as potentially useful technologies for improving pulse crop yields (Kelly *et al.*, 2003) [23]. DNA markers, particularly RAPD, AFLPs, and SSRs are proposed to be an appropriate tool for identifying clones (Devarumath *et al.*, 2002) [8], somaclonal variations (Rahman & Rajora 2001) [40], breeding lines and hybrids (Bastia *et al.*, 2001), and cultivars (Mohanty *et al.*, 2001) [31], as well as monitoring introgression, mapping QTLs (Paterson *et al.*, 2003) and to study genetic diversity in maize crop (Kantety *et al.*, 1995) [21]. RFLP is probe based DNA marker or non PCR based marker. PCR based markers includes RAPD, AFLP, SSR, ISSR, GBS, DArT.

#### Non PCR based DNA marker

##### Restriction Fragment Length Polymorphisms (RFLPs)

Earliest used DNA markers are RFLP. RFLPs are a hybridization based polymorphism technique that relies on

restriction enzymes to cleave genomic DNA before hybridizing to DNA-labeled probes to detect DNA fragments of identical size that differ in one base pair. As RFLP markers are co-dominant, they are used for detection of recessive traits (Uddin and Cheng, 2015; Ben-Ari and Lavi, 2012) [56, 5]. In different crops, such as lentils, oats, tomatoes, peanuts, and *Brassica napus*, to understand the species relationship and for taxonomic studies RFLPs were used (Wang *et al.*, 2011) [58]. RFLP genotyping is a time-consuming, expensive, and sophisticated method of genotyping. For many plant species, DNA probes are not available. For RFLP it is difficult to identify more than one base pair change because of the single-locus nature. Hybridization of oligonucleotide probes is a difficult process that is sensitive to minute temperature fluctuations (Ben-Ari and Lavi, 2012) [5].

#### PCR based DNA markers

##### Simple Sequence Repeats (SSR)

SSRs are short nucleotide sequences (1-6 bp) that are found throughout the genome in tandem repeats (Kelkar *et al.*, 2010) [22]. Because of their superior reproducibility, increased polymorphism levels, and high mutation rates, these markers are often utilized in population genetics, functional genomics, association mapping, DNA fingerprinting, diversity analysis, comparative mapping, and gene tagging research. As SSR markers are dominant in nature, they can distinguish both homozygous and heterozygous locus. Many crops like *Helianthus*, barley, soybean, wheat, date palm, rice, and maize, utilize SSRs for fingerprinting (Jamil *et al.*, 2020; Wang *et al.*, 2011) [19, 60]. The creation of SSR markers from genomic DNA is a major difficulty with SSR-based fingerprinting systems since it takes a lot of effort to isolate nuclear microsatellites from plants so expressed sequence tags. The EST-SSR markers were developed using the EST-Database of several species (Nybom *et al.*, 2014; Squirrell *et al.*, 2003) [33]. SSR markers can be developed quickly and cheaply using EST databases (Gupta *et al.*, 2003).

##### Amplified Fragment Length Polymorphisms (AFLP)

The AFLP marker approach combines RFLP and PCR to produce a more reliable banding pattern. AFLP is similar to RFLP in that it detects restriction fragments in the genome. For the detection of genomic restriction fragments, PCR amplification is employed instead of southern hybridization, and it merely represents the presence and absence of restriction fragments rather than length differences. AFLPs have been used to observe genetic variation in mango, sorghum, wheat, and sweet potato. In cotton, sweet potato, Bt rice, and soybean, tagging of major agronomic traits, fiber quality traits, and fingerprinting studies have been conducted (Zargar *et al.*, 2017) [64]. AFLPs are dominant markers, so they can't differentiate between homozygous and heterozygous individuals and also require high quality and quantity of DNA.

##### Randomly Amplified Polymorphic DNA (RAPD)

William and colleagues developed RAPD markers (Williams *et al.*, 1990) [61]. For fingerprinting studies RAPD markers were mostly used (Nybom *et al.*, 2014; Gomes *et al.*, 2008) [33, 12]. RAPD markers are useful for analyzing the diversity of many plant species (Sinha *et al.*, 2013). RAPD utilizes a small sample size, produces speedy results in a shorter time period, is less expensive, and does not require prior information of the genomic sequence. PCR fragments are generated from

genomic DNA, which are then analyzed electrophoretically to produce multi locus banding patterns which are later seen under UV trans illuminator. For genotype characterization and fingerprinting, differences in the size range of PCR products are examined (Iqbal *et al.*, 2021) [18]. Several studies have employed RAPD, a dominant marker, as effective methods for identifying markers associated to agronomically important traits (Kotresh *et al.*, 2006) [25]. Crops like corn, rice, wheat, barley, sorghum, oats, and rye are fingerprinted using RAPD markers (Iqbal *et al.*, 2019; Salem *et al.*, 2007) [17, 45]. In RAPD the use of non-specific primers with random sequences may result in an improper hybridization between target DNA and primer. As RAPD markers are dominant it is not possible to distinguish between homozygous and heterozygous locus. Interspecific hybridization was also verified using RAPD markers (Mei *et al.*, 2004) [29].

### Single Nucleotide Polymorphism (SNPs)

In 1996, SNPs was first introduced by Lander (AI Samarai and AI-Kazaz, 2015) [1] which is a common and effective method of DNA fingerprinting. SNPs are stable genetically and numerous, and genotyping chips can be automated to allow for high throughput analysis. The basis of SNPs is based on the hybridization of DNA fragments with SNP chips (high density DNA probe arrays), after which the SNP allele is designated based on the hybridization results (Yang *et al.*, 2013) [63]. In sugar beet, grapevines, soybean, chickpea, olive, mango, cotton, datepalm and common bean SNP markers are widely employed as an important tool for linkage mapping, QTL analysis, DNA fingerprinting, and genetic diversity (Fu *et al.*, 2020; Raatz *et al.*, 2019; Faqir *et al.*, 2017) [39, 9]. SNPs, in comparison to microsatellites, lack information per locus and are therefore less informative per locus (Wang *et al.*, 2017) [57].

### Inter Simple Sequence Repeats (ISSRs)

Since 1994, SSRs have been routinely utilized in DNA fingerprinting as PCR-based multi-locus molecular markers. This technique amplifies inter-specific SSR sequences of various length using selected 16-20 base pair long microsatellite sequences as primers in polymerase to make multi locus markers. ISSR primer sequences are often longer than RAPD primer sequences, allowing for a higher annealing temperature, resulting in more repeatable bands than RAPD primers. ISSR markers have some drawbacks, such as limited reproducibility when compared to other markers, and are dominant in nature. However, because of their high polymorphism, these markers are frequently utilized in genome mapping, genetic diversity, linkage studies, gene tagging, phylogeny and evolutionary biology research (Reddy *et al.*, 2002) [43].

### DNA fingerprinting applications in crop improvement

Markers are employed in crop variety identification, crop protection, prediction of heterosis, seed purity analysis, plant germplasm resource conservation and evaluation, genetic map construction, genotyping, cloning of essential agronomic characteristic genes, and molecular marker assisted breeding (MAB).

### Crop protection

Varietal protection and germplasm characterization are two applications for modern fingerprinting technology. The International Union for the Protection of New Varieties of

Plants (UPOV) is working hard to develop and implement fingerprinting techniques for DUS testing (He *et al.*, 2020; Archak, 2000) [2]. Crop fingerprinting is a technique that can be used in forensic botany. Fingerprinting is regarded as a superior method of detecting adulteration in plant-based foods and pharmaceuticals. Various markers have been proposed to resolve adulteration issues. Different food items, juices, and drugs were subjected to DNA analysis is to determine the plant variety from which they were derived (Nybom *et al.*, 2014) [33]. SSR markers with a distinct amplification pattern can be employed as diagnostic genetic markers for specific hybrids, while DNA fingerprints can be used as a quick reference for comparing the genetic purity of different seed batches, preventing the sale of illegitimate hybrids (Sharma *et al.*, 2014) [48]. The plant variety DNA fingerprinting is critical for securing the rights of plant breeders (Kumar *et al.*, 2001) [26]. Diversity of parental lines can be easily identified by using DNA fingerprinting technique (Ijaz, 2011) [16]. For marker-assisted selection, fingerprinting techniques are utilized, which is beneficial to plant breeders because it minimizes the number of generations required for evaluating different traits (Jamil *et al.*, 2020) [19].

### Prediction of heterosis

To improve the breeding efficiency and process, heterosis prediction is important. Furthermore, DNA markers remove the drawback of isozyme-based heterosis prediction, which is too limited to be widely used. The genetic distance of the molecular marker was related to the heterosis of boll number and weight in single cotton (Percy *et al.*, 2006) [37].

### Identification of cultivar and Seed purity analysis

For identifying molecular markers for DNA fingerprinting, previous researchers considered three criteria i.e., codominance, polymorphism and allele uniqueness (Lukman *et al.*, 2008) [28]. One of the most essential quality control components in hybrid seed development is determining the genetic purity. The traditional field purity test, which examines a variety of plant morphological features, is time-consuming, difficult and also results are obtained after the growing period (Asif *et al.*, 2006) [3]. Because DNA molecular markers have excellent specificity, selectivity, simplicity, precision, and genetic stability, they may detect changes in DNA levels without causing environmental affects, and hence have significant advantages in seed purity detection (Korir *et al.*, 2013) [24]. Bio-security and quality issues to the farm industry can be reduced by assessing genetic purity. Seed purity of maize, cotton, wheat, grape and rice has been identified using DNA molecular marker technique (Zang *et al.*, 2012). RAPD analysis would be beneficial in breeding for rapid and early hybridity verification in large seedling populations, as well as purity testing of different seed lots, allowing true hybrids to be detected and parentage of hybrids and lines/cultivars to be verified (Asif *et al.*, 2006) [3]. Grapevine and pomegranate were identified using the RAPD molecular marker (Zhang *et al.*, 2012; Zhao *et al.*, 2011) [65, 66]. For hybrid identification, DNA fingerprinting techniques which are based on molecular markers are considered effective genomic tools (Salgado *et al.*, 2006; Perry 2004). Two SSR primer pairs are needed to separate two maize hybrids that are unrelated, whereas at least three to four SSR primer pairs are needed to distinguish hybrids that have only one parental line (Lukman *et al.*, 2008) [28]. In terms of plant variety protection, DNA fingerprinting can be used to

estimate hybrid performance and for precise identification (Xu *et al.*, 2004) [62]. For cultivar identification, restriction fragment length polymorphism (RFLP) has also been proposed (Pagnotta *et al.*, 1966) [34].

### Germplasm Resource Evaluation and conservation

For germplasm identification, evaluation and preservation, the DNA molecular marker technology plays a very important role. To screen the important germplasm, preserve and to maintain the breeding population DNA markers are used. The information regarding their DNA level diversity, as well as their origin and evolution relationships would substantially assist us in making better use of the germplasm resources available to us, as well as providing an important source for their protection.

### Genetic diversity assessment

To know the gene flow, parentage analysis is done. The purpose of DNA fingerprinting is to investigate genetic relatedness between genotypes/species. As the data is collected from many ecological zones, genetic relatedness also provides useful information on the domestication process (Raj *et al.*, 2019) [41]. For marker-assisted selection, fingerprinting techniques are utilized, which is beneficial to plant breeders because it minimizes the number of generations required for testing different traits (Chukwu *et al.*, 2019) [7]. The hybrid/parental line DNA fingerprinting data could be successfully used for a genetic purity test that examines diverse seed samples to true-to-type control and parental lines. Because two randomly selected alleles from the population were shown to be different among the hybrids/parental lines, the SSR markers were able to detect genetic diversity (Sharma *et al.*, 2014) [48]. Plant breeders can use DNA fingerprinting and genetic profiling of breeding material to allocate inbred lines/purelines to distinct heterotic groups and determine the best crossing plan to maximize heterosis (Silva *et al.*, 2020) [49]. Morphology, protein and isozyme analysis, RAPDs, and RFLP markers were used in early research on pigeon pea phylogenetic studies (Ratnaparkhe *et al.*, 1995; Sivaramakrishnan *et al.*, 2002) [42, 51].

### Genotyping

Individual cultivars are identified using DNA fingerprints produced using PCR or non-PCR based markers. When compared to morphological markers, DNA markers are more reliable (Iqbal *et al.*, 2019) [17]. DNA fingerprinting is a useful approach for identifying closely related species and varieties, as well as assessing genetic diversity and estimating genetic relatedness (Jamil *et al.*, 2020) [19]. DNA fingerprinting aids in the determination of varietal purity, which aids in the prevention of the sale of impure seed in the market. Plant hybridity testing is another useful application of DNA fingerprinting. The co-dominant character of SSR markers facilitates their use in hybridity testing and, as a result, will be valuable in regulating hybrid seed marketing. DNA markers are also a trustworthy source for identifying the pedigree parentage of new crop varieties, and they will be used to register them under the Plant Breeders Rights Rules to secure plant breeders rights (Jamil *et al.*, 2020) [19]. As SSR markers are highly reproducible, they are used for genotyping asexually propagated cultivars. SNP chips are utilized for high-throughput genotyping, and the data is subsequently used to identify several QTLs in the genome (Fujii *et al.*,

2013) [11]. AFLPs are employed to fingerprint in-vitro produced crops (Kumar *et al.*, 2019) [27]. For chimera clones identification and for somatic mutation genotyping SSR markers are used (Meng *et al.*, 2018) [30].

### Conclusion

With the rapid advancement of molecular biology today, scientists need to apply technologies based on the molecular level to improve the agriculture economy. In the future, DNA fingerprinting has a lot to offer, including variety protection under the Plant Breeders Rights Rules, dispute settlement, and forensic activities to plant sciences, and will aid in the expansion of genetic knowledge a database of several certified crops (Wang *et al.*, 2019) [59]. Variety distinctions were made based on morphological characteristics as far back as the nineteenth century. However, as technology progressed, DNA-based markers became available. With the introduction of next-generation sequencing technology in the twenty-first century, DNA fingerprinting has advanced a step further, and genotyping is now done through sequencing. Because of their superior reproducibility, increased polymorphism levels, and high mutation rates, SSR markers are more commonly utilized markers. The transfer of DNA fingerprints into readily available and useful information that can be utilized directly in cultivar identification is critical in order to properly use DNA markers to cultivar identification.

### References

1. Al-Samarai FR, Al-Kazaz AA. Molecular markers: An introduction and applications. *European journal of molecular biotechnology*. 2015;9(3):118-130.
2. Archak S. Plant DNA fingerprinting: an overview. *AgBiotech Net. Review Article*. 2000;2:ABN046.
3. Asif M, Mehboob-ur-Rahman YZ. Y: Genotyping analysis of six maize (*Zea mays* L.) hybrid using DNA fingerprinting technology. In *Pakistan J Bot*, 2006.
4. Bastia T, Scotti N, Cardi T. Organelle DNA analysis of Solanum and Brassica somatic hybrids by PCR with universal primers. *Theoretical and Applied Genetics*. 2001;102(8):1265-1272.
5. Ben-Ari G, Lavi U. Marker-assisted selection in plant breeding. In *Plant biotechnology and agriculture*. Academic Press, 2012, 163-184.
6. Bhandari AP, Sukanya DH, Ramesh CR. Application of isozyme data in fingerprinting Napier grass (*Pennisetum purpureum* Schum.) for germplasm management. *Genetic Resources and Crop Evolution*. 2006;53(2):253-264.
7. Chukwu SC, Rafii MY, Ramlee SI, Ismail SI, Oladosu Y, Okporie E, *et al.* Marker-assisted selection and gene pyramiding for resistance to bacterial leaf blight disease of rice (*Oryza sativa* L.). *Biotechnology & Biotechnological Equipment*. 2019;33(1):440-455.
8. Devarumath R, Nandy S, Rani V, Marimuthu S, Muraleedharan NRAPD, Raina S. RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micro propagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* ssp. *assamica* (Assam-India type). *Plant Cell Reports*. 2002;21(2):166-173.
9. Faqir N, Muhammad A, Hyder MZ. Diversity assessment and cultivar identification in date palm through molecular markers-a review. *Turkish Journal of Agriculture-Food Science and Technology*. 2017;5(12):1516-1523.
10. Fu XJ, Pei JX, Zheng YT, Guo DD, Yang QH, Jin HX, *et*

- al.* DNA Fingerprinting of Vegetable Soybean Cultivar 'Zhexian No. 9' using 101 New Developed HRM-Based SNP Markers. *Legume Research: An International Journal*. 2020;43(1):8-17.
11. Fujii H, Shimada T, Nonaka K, Kita M, Kuniga T, Endo T, *et al.* High-throughput genotyping in citrus accessions using an SNP genotyping array. *Tree genetics & genomes*. 2013;9(1):145-153.
  12. Gomes S, Martins-Lopes P, Lima-Brito J, Meirinhos J, Lopes J, Martins A, *et al.* Evidence for clonal variation in 'Verdeal-Transmontana' olive using RAPD, ISSR and SSR markers. *The Journal of Horticultural Science and Biotechnology*. 2008;83(4):395-400.
  13. Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS. Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. *Molecular genetics and genomics*. 2003;270(4):315-323.
  14. He B, Geng R, Cheng L, Yang X, Ge H, Ren M. Genetic diversity and fingerprinting of 33 standard flue-cured tobacco varieties for use in distinctness, uniformity, and stability testing. *BMC plant biology*. 2020;20(1):1-10.
  15. Hebert PD, Cywinska A, Ball SL, DeWaard JR. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*. 2003;270(1512):313-321.
  16. Ijaz S. Microsatellite markers: An important fingerprinting tool for characterization of crop plants. *African Journal of Biotechnology*. 2011;10(40):7723-7726.
  17. Iqbal MZ, Jamil S, Mehmood A, Shahzad R. Identification of seven olive varieties using rapid molecular markers. *J Agric. Res*. 2019;57(1):07-14.
  18. Iqbal M, Shahzad R, Shahzad R, Bilal K, Qaisar R, Nisar A, *et al.* DNA Fingerprinting of Crops and Its Applications in the Field of Plant Breeding. *J Agric. Res*. 2021;59(1):13-28.
  19. Jamil S, Shahzad R, Kanwal S, Yasmeen E, Rahman SU, Iqbal MZ. DNA fingerprinting and population structure of date palm varieties grown in Punjab Pakistan using simple sequence repeat markers. *International Journal of Agriculture and Biology*. 2020;23(5):943-50.
  20. Johnson M, Nanthini AUR, Malar TRJJ. Isozyme variation and genetic relationships among three *Plumbago* species. *Journal of Ecobiotechnology*, 2010.
  21. Kantety RV, Zeng X, Bennetzen JL, Zehr BE. Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Molecular breeding*. 1995;1(4):365-373.
  22. Kelkar YD, Strubczewski N, Hile SE, Chiaromonte F, Eckert KA, Makova KD. What is a microsatellite: a computational and experimental definition based upon repeat mutational behavior at A/T and GT/AC repeats. *Genome biology and evolution*. 2010;2:620-635.
  23. Kelly JD, Gepts P, Miklas PN, Coyne DP. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. *Field Crops Research*. 2003;82(2-3):135-154.
  24. Korir NK, Han J, Shangguan L, Wang C, Kayesh E, Zhang Y, *et al.* Plant variety and cultivar identification: advances and prospects. *Critical reviews in biotechnology*. 2013;33(2):111-125.
  25. Kotresh H, Fakrudin B, Punnuri SM, Rajkumar BK, Thudi M, Paramesh H, *et al.* Identification of two RAPD markers genetically linked to a recessive allele of a Fusarium wilt resistance gene in pigeonpea (*Cajanus cajan* L. Millsp.). *Euphytica*. 2006;149(1):113-120.
  26. Kumar LD, Kathirvel M, Rao GV, Nagaraju J. DNA profiling of disputed chilli samples (*Capsicum annum*) using ISSR-PCR and FISSR-PCR marker assays. *Forensic Science International*. 2001;116(1):63-68.
  27. Kumar M, Chaudhary V, Sirohi U, Sharma VR, Naresh RK. Application of molecular markers and their utility in genetic studies of floricultural crops: a review. *International Journal of Agriculture, Environment and Biotechnology*. 2019;12(3):229-247.
  28. Lukman R, Ahmad Afifuddin PS, Thiraporn2 Andi Khaeruni R. A precise means to confirm purity of commercial maize varieties on the basis of SSR analysis. In *Correct citation: Zaidi, PH; Azrai, M. and Pixley, KV, eds. 2010. Maize for Asia: Emerging Trends and Technologies. Proceeding of The 10th Asian Regional Maize Workshop, Makassar, Indonesia, 20-23 October 2008. Mexico DF: CIMMYT, 2008, 255.*
  29. Mei M, Syed NH, Gao W, Thaxton PM, Smith CW, Stelly DM, Chen ZJ. Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). *Theoretical and applied genetics*. 2004;108(2):280-291.
  30. Meng YS, Ning ZHAO, Hui LI, Hong ZHAI, HE SZ, LIU QC. SSR fingerprinting of 203 sweet potato (*Ipomoea batatas* (L.) Lam.) varieties. *Journal of Integrative Agriculture*. 2018;17(1):86-93.
  31. Mohanty A, Martin JP, Aguinalalde I. Chloroplast DNA study in wild populations and some cultivars of *Prunus avium* L. *Theoretical and Applied Genetics*. 2001;103(1):112-117.
  32. Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, *et al.* DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*. 2018;32(2):261-285.
  33. Nybom H, Weising K, Rotter B. DNA fingerprinting in botany: past, present, future. *Investigative genetics*. 2014;5(1):1-35.
  34. Pagnotta MA, D'Ovidio R, Iacono E, Tanzarella OA, Porceddu E. Utilization of non radioactive RFLP probes for durum wheat variety identification. *Journal of Genetics and Breeding*. 1996;50:155-160.
  35. Paterson AH, Saranga Y, Menz M, Jiang CX, Wright R. QTL analysis of genotype × environment interactions affecting cotton fiber quality. *Theoretical and applied genetics*. 2003;106(3):384-396.
  36. Paux E, Sourdille P, Mackay I, Feuillet C. Sequence-based marker development in wheat: advances and applications to breeding. *Biotechnology advances*. 2012;30(5):1071-1088.
  37. Percy RG, Cantrell RG, Zhang J. Genetic variation for agronomic and fiber properties in an introgressed recombinant inbred population of cotton, 2006.
  38. Perry DJ. Identification of Canadian durum wheat varieties using a single PCR. *Theoretical and Applied Genetics*. 2004;109(1):55-61.
  39. Raatz B, Mukankusi C, Lobaton JD, Male A, Chisale V, Amsalu B, *et al.* Analyses of African common bean (*Phaseolus vulgaris* L.) germplasm using a SNP fingerprinting platform: diversity, quality control and molecular breeding. *Genetic Resources and Crop*

- Evolution. 2019;66(3):707-722.
40. Rahman M, Rajora O. Microsatellite DNA somaclonal variation in micropropagated trembling aspen (*Populus tremuloides*). Plant Cell Reports. 2001;20(6):531-536.
  41. Raj SM, Pei A, Foll M, Schlamp F, Excoffier L, Fuller DQ, *et al.* Reconstruction of nine thousand years of agriculture-based diet and impact on human genetic diversity in Asia. BioRxiv, 2019, 747709.
  42. Ratnaparkhe MB, Gupta VS, Ven Murthy MA, Ranjekar PK. Genetic fingerprinting of pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives using RAPD markers. Theoretical and applied genetics. 1995;91(6):893-898.
  43. Reddy MP, Sarla N, Siddiq EA. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica. 2002;128(1):9-17.
  44. Royo JB, Cabello F, Miranda S, Gogorcena Y, Gonzalez J, Moreno S, *et al.* The use of isoenzymes in characterization of grapevines (*Vitis vinifera*, L.). Influence of the environment and time of sampling. Scientia Horticulturae. 1997;69(3-4):145-155.
  45. Salem HH, Ali BA, Huang TH, Qin DN, Wang XM, Xie QD. Use of random amplified polymorphic DNA analysis for economically important food crops. Journal of Integrative Plant Biology. 2007;49(12):1670-1680.
  46. Salgado KCPDC, Vieira DGGC, Von Pinho ÉVDR, Guimarães CT, Von Pinho RG, Souza LVD. Genetic purity certificate in seeds of hybrid maize using molecular markers. Revista Brasileira de Sementes. 2006;28:169-175.
  47. Selvi A, Nair NV, Balasundaram N, Mohapatra T. Evaluation of maize microsatellite markers for genetic diversity analysis and fingerprinting in sugarcane. Genome. 2003;46(3):394-403.
  48. Sharma JK, Singh A, Lata S. DNA fingerprinting of commercial maize hybrids and their parental lines using simple sequence repeat markers. Crop Improv. 2014;41(1):69-75.
  49. Silva KJD, Pastina MM, Guimarães CT, Magalhães JV, Pimentel LD, Schaffert RE, *et al.* Genetic diversity and heterotic grouping of sorghum lines using SNP markers. Scientia Agricola. 2020, 78.
  50. Sinha M, Shamim MD, Priya S, Singh KN. DNA Fingerprinting of Pigeon pea (*Cajanus cajan* (L.) Millsp.) Genotypes by RAPD Marker for the Breeding of New Varieties. Indian Journal of Agricultural Biochemistry. 2013;26(2):195-198.
  51. Sivaramkrishnan S, Kannan S, Reddy LJ. Diversity in selected wild and cultivated species of pigeonpea using RFLP of mtDNA. Euphytica. 2002;125(1):21-28.
  52. Squirrell J, Hollingsworth PM, Woodhead M, Russell J, Lowe AJ, Gibby M, *et al.* How much effort is required to isolate nuclear microsatellites from plants? Molecular ecology. 2003;12(6):1339-1348.
  53. Sumarani GO, Pillai SV, Harisankar P, Sundaresan S. Isozyme analysis of indigenous cassava germplasm for identification of duplicates. Genetic Resources and Crop Evolution. 2004;51(2):205-209.
  54. Taran B, Zhang C, Warkentin T, Tullu A, Vandenberg A. Genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on molecular markers, and morphological and physiological characters. Genome. 2005;48(2):257-272.
  55. Tiwari JK, Rastogi N, Chandrakar P, Sarawgi A, Verulkar S. Varietal Identification of Four Rice Varieties from Chhattisgarh through DUS Characterization. Indian J Plant Genet. Resour. 2013;26(3):238-240.
  56. Uddin MS, Cheng Q. Recent application of biotechniques for the improvement of mango research. In Applied Plant Genomics and Biotechnology. Woodhead Publishing, 2015, 195-212.
  57. Wang B, Sun Y, Song N, Zhao M, Liu R, Feng H, *et al.* *Puccinia striiformis* f. sp. *tritici* mi croRNA-like RNA 1 (Pst-milR1), an important pathogenicity factor of Pst, impairs wheat resistance to Pst by suppressing the wheat pathogenesis-related 2 gene. New Phytologist. 2017;215(1):338-350.
  58. Wang FG, Tian HL, Zhao JR, Yi HM, Wang L, Song W. Development and characterization of a core set of SSR markers for fingerprinting analysis of Chinese maize varieties. Maydica, 2011, 56(1).
  59. Wang F, Tian H, Yi H, Zhao H, Huo Y, Kuang M, *et al.* Principle and strategy of DNA fingerprint identification of plant variety. Molecular Plant Breeding, 2019, 10.
  60. Wang J, Lydiate DJ, Parkin IA, Falentin C, Delourme R, Carion PW, *et al.* Integration of linkage maps for the amphidiploid *Brassica napus* and comparative mapping with *Arabidopsis* and *Brassica rapa*. BMC genomics. 2011;12(1):1-20.
  61. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acids research. 1990;18(22):6531-6535.
  62. Xu SX, Liu JIE, LIU GS. The use of SSRs for predicting the hybrid yield and yield heterosis in 15 key inbred lines of Chinese maize. Hereditas. 2004;141(3):207-215.
  63. Yang W, Kang X, Yang Q, Lin Y, Fang M. Review on the development of genotyping methods for assessing farm animal diversity. Journal of animal science and biotechnology. 2013;4(1):1-6.
  64. Zargar M, Romanova E, Trifonova A, Shmelkova E, Kezimana P. AFLP-analysis of genetic diversity in soybean [*Glycine max* (l.) Merr.] cultivars Russian and foreign selection, 2017.
  65. Zhang YP, Tan HH, Cao SY, Wang XC, Yang G, Fang JG. A novel strategy for identification of 47 pomegranate (*Punica granatum*) cultivars using RAPD markers. Genet Mol Res. 2012;11:3032-3041.
  66. Zhao MZ, Zhang YP, Wu WM, Wang C, Qian YM, Yang G, *et al.* A new strategy for complete identification of 69 grapevine cultivars using random amplified polymorphic DNA (RAPD) markers. Afr. J Plant Sci. 2011;5(4):273-280.