Open Access

OBM Genetics

LIDSEN Publishing Inc.

Review

Molecular Marker Analysis of Genetic Diversity in Maize: A Review

Anand Kumar [1], Ningthai Longmei [2], Pardeep Kumar [2], Prashant Kaushik [3, 4 *]

1. Department of Genetics and Plant Breeding, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, India; E-Mail: anandkumar002014@gmail.com
2. ICAR-Indian Institute of Maize Research, Ludhiana, India; E-Mails: algalomics@gmail.com; ch.pk23@gmail.com
3. Kikugawa Research Station, Yokohama Ueki, 2265, Kamo, Kikugawa City, Shizuoka 439-0031, Japan; E-Mail: prakau@alumni.upv.es
4. Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain

* **Correspondence:** Prashant Kaushik; E-Mail: prakau@alumni.upv.es

Academic Editors: Ioannis N. Xynias and Theano. B. Lazaridou

Special Issue: Genetics, Genomics, and Breeding Crop Cultivars for the Novel Changing World

OBM Genetics
2022, volume 6, issue 1
doi:10.21926/obm.genet.2201150

Received: July 25, 2021
Accepted: March 15, 2022
Published: March 21, 2022

Abstract

Maize is consumed as a food and used as an industrial product in the form of starch, pharmaceuticals, alcoholic beverages, oil, cosmetics, and textiles. In ancient times, landraces were more popular due to the presence of high genetic variability, resistance to biotic and abiotic factors, and the heterogeneous nature of maize; however, it has been replaced by improved and uniform cultivars with a higher yield. Modern maize has more homogeneity that enhances its vulnerability to biotic and abiotic stresses. Thus, we need to study the diversity in maize to successfully use its characteristics. Several markers such as morphological, biochemical, and DNA-based markers/molecular markers are utilized to study the germplasm diversity. Although extensively used, morphological and biochemical markers were highly sensitive to the environment because of which, these are being replaced by molecular markers. In the current era of molecular markers, DNA markers play an important role in

identifying diverse germplasms because of high precision and accuracy. In this review, we focused on the types of markers utilized to accelerate the knowledge of maize genetic diversity and the prospect of genetic diversity.

Keywords
Maize; genetic diversity; molecular marker

1. Introduction

Maize (*Zea maize* L.) is one of the important economic and staple food crops and an energy plant among cereals that is cultivated globally for fulfilling the requirements of human beings [1]. It is a vital source of the income-overwhelming population [2]. With a high yield potential, it has become a model crop among cereals and is therefore called the queen of cereal crops [3]. In addition, it is utilized as an industrial resource for the production of starch, pharmaceuticals, alcoholic beverages, oil, cosmetics, and textiles [4]. Due to the diverse uses of maize and its products, its demand has been increasing continuously globally [5]. In ancient times, landraces were more popular, and maize was highly resistant to biotic and abiotic factors due to its heterogeneous nature, although the yield was low [6]. Now, the landraces are being replaced with hybrid maize, which has a higher yield as compared to landraces [7]. The present cultivated form of maize is originated from its wild relative teosinte (*Zea mays* ssp. parviglumis), but cultivated maize is considerably different from teosinte in terms of morphology and several other characteristics [8]. The production of high-yielding maize cultivars has always been the primary objective of breeding. For increasing the production of maize, several varieties, including sweet corn, popcorn, and high-quality protein corn, are being developed globally [9]. In spite of huge work on developed varieties of maize, its yields are below their potential because of abiotic and biotic stresses, indicating the need to assess the genetic diversity. Knowledge of genetic diversity in maize crop, especially of germplasm and inbred lines, have significantly impacted crop improvement [10]. Maize is characterized by tremendous genetic diversity [4].

Genetic diversity is defined as the total variability present in an individual or organism/population [11]. Genetic diversity is an important aspect of breeding programs to develop high-yielding varieties [12]. The potential of genetic diversity of maize has reduced drastically due to the continuous use of homogeneous varieties [10]. To conserve the diversity, breeders need to study its huge germplasm [13] and inbreds [14]. To obtain the knowledge of genetic diversity in maize, that is, which lines have more diversity than others, several studies have been conducted to study the morphological, biochemical, and molecular characteristics of maize [15]. Although morphological and biochemical methods are being extensively used [16], these approaches are highly sensitive to environmental effects [17]. To address the challenges of morphological and biochemical methods, a new approach based on molecular markers is being utilized to study the relationship among lines and varieties [15]. The expression of DNA markers/molecular markers is rarely influenced by the environment and avoid genotypic × environmental interactions; hence, these markers could reveal the actual level of different population analysis. Thus, these markers are being utilized in populations (Table 1), resulting in huge progress [18-20]. In this review, we focused

on the potential role of genetic diversity for starting breeding programs and the kinds of markers being used to assess genetic diversity.

Table 1 Various markers used in maize for genetic diversity, genetic purity, and DNA fingerprinting.

S. No.	Markers	Population	Purpose	References
01	SSR (120)	94 inbreds	Genetic diversity	[21]
02	SSR (60)	65 inbreds	Genetic diversity	[22]
03	SSR (44)	6 inbred lines	Genetic diversity	[23]
04	SSR (20)	61 diverse array maize	Genetic diversity	[24]
05	SSR (15)	54 inbreds	Genetic diversity	[25]
06	SSR (55)	Parental population	Genetic diversity	[26]
07	SSR (27)	56 inbreds	Genetic diversity	[27]
08	SSR (24)	275 landraces	Genetic diversity	[28]
09	SSR (25)	25 accession	Genetic diversity	[29]
10	SSR (25)	25 accession	Genetic diversity	[30]
11	SSR (27)	56 inbred lines	Genetic diversity	[31]
12	SSR (28)	41 Inbred lines	Discriminate data	[32]
13	SSR (96)	350 landraces	Genetic diversity	[33]
14	SSR (25)	23 landraces, 23 OPVs and 261 inbreds	Genetic diversity	[34]
15	SSR (6)	8, Inbred lines	Genetic diversity	[35]
16	SSR (200)	97 inbred lines	Genetic relationship	[36]
17	SSR (21)	4 synthetic variety	Genetic diversity	[37]
18	SSR (41)	4 F_1 and 4 inbreds	Genetic purity	[38]
19	SSR (36)	7 Hybrids	DNA fingerprinting	[39]
20	SSR (25)	41 inbreds	Genetic diversity	[40]
21	SSR (79)	155 inbred lines	Genetic diversity	[41]
22	SSR (20)	29 inbred lines	Genetic diversity	[42]
23	SSR (42)	96 landraces	Evaluation	[43]
24	30 SSR	233 inbreds	Genetic diversity	[44]
25	18 SSR	56 landraces	Genetic diversity	[45]
26	24 SSR	69 Hybrids	Genetic diversity	[46]
27	21 SSR	24 inbred	Divergence	[47]
28	SNP (1536)	Inbred lines	Golden gate	[48]
29	SNP1000000	6 inbred lines	Genetic variation	[49]
30	SNP (1536)	95 inbred lines	Genetic diversity	[50]
31	137 SNP	63 inbred lines	Genetic diversity	[40]
32	15670 SNP	115 s3 lines	Genetic diversity	[51]
34	50 SNP	89 DH lines	broadening the genetic base	[52]
35	238,772 SNPS	327 inbreds	Genome wide analysis	[53]
36	899,784 SNP	942 inbreds	Genome-wide association	[54]
37	27 SNP	2,815 maize inbreds	Genome-wide association	[55]

38	SNP (56,000)	263 RILs	Genome assembly	[56]
39	AFLP (9)	96 tropical inbreds	Genetic diversity	[57]
40	RFLP	Maize population	Genetic diversity	[58]
41	RFLP (32)	218 inbreds	Genetic characterization	[34]
42	RAPD (32)	79 landraces and two improved varieties	Genetic diversity	[59]
43	SSR (359)	1537, inbreds	Genetic diversity	[60]
44	SSR (82)	155, inbreds	Genetic diversity	[61]
45	RFLP (135)	13 inbreds	Genetic diversity	[62]
46	RAPD (07)	21 landraces	Genetic diversity	[63]
47	SSR (33)	40 tropical inbreds	Genetic diversity	[64]
48	SSR (50), AFLP 569	85, inbreds	Genetic diversity	[65]
50	AFLP	18 S3 inbreds	Genetic diversity	[66]
51	SNP	Inbred lines	Maize fingerprint	[67]
52	SSR (84)	3, inbred	Genetic diversity	[68]
53	RFLP (1132)	51 inbred lines	Genetic diversity	[69]
54	AFLP (731)	77, inbred lines	Genetic diversity	[70]
55	RAPD (262)	18, s_3Inbred lines	Genetic diversity	[58]

2. Classification of Molecular Markers

In the case of plant breeding, several molecular markers have been utilized for the analysis of genetic diversity [18, 19]. Molecular markers can highlight the differences (polymorphisms) within a nucleic acid sequence between different individuals. These differences include insertions, deletions, translocations, duplications, and point mutations. They do not, however, encompass the activity of specific genes [71]. To aggregate the knowledge of molecular markers is a difficult but necessary task. Several molecular markers have been classified for maize.

2.1 Analysis of Maize Diversity through Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) was the first genetic marker, developed by Botstein et al. in 1980 [72] to detect DNA polymorphism. RFLP technique depends on the short southern blot technique. In this technique, DNA is digested using a restriction endonuclease enzyme, which cleaves the DNA into different fragments to detect the polymorphism [72]. For example, 148 American maize populations [73] and 488 European maize populations [74] were characterized at the molecular level using RFLP (for more detail, see paper). RFLP is highly reproducible, codominant, highly inherited [75], locus-specific, and highly heritable. Armstrong and Romero-Severson used it to determine the locations and effects of the introgressed A188 chromosomal segments. Five segments were retained through at least the fifth backcross generation. The hypothesis that one or more of these five regions contain genes controlling somatic embryogenesis in maize was tested using an F2 population of the cross A188 × Mo17. A set of five DNA markers (three of them linked) explained 82% of the observed phenotypic variance for the percentage of immature embryos forming the embryogenic callus. Four of the five markers were located in or near introgressed A188 chromosome segments were studied [76]. Bentolila et al. (1991) identified a tight linkage of an RFLP marker to the *Htl* gene of maize that confers resistance to the fungal pathogen *Helminthosporium*

turcicum race 1. This was accomplished by using four pairs of near-isogenic lines (NILs; B73, A619, W153R, and CM105), each differing by the presence or the absence of the gene *Htl* [77]. Moreover, the mean phenotypic performances of F3 families were compared based on genotypic classification at each of the 70 RFLP marker loci. The genetic linkage map assembled from these markers was in good agreement with the previously published maps. In the combined analyses, genomic regions significantly affecting the tolerance to drought were found on chromosomes 1, 3, 5, 6, and 8. For yield, 50% of the phenotypic variance could be explained by five putative QTLs [78].

2.2 Analysis of Maize Diversity through Random Amplified Polymorphism DNA (RAPDs)

Random amplified polymorphism DNA (RAPDs) was the first PCR-based molecular marker to be employed in genetic variation analyses [79, 80]. RAPD markers are generated through the random amplification of genomic DNA using short primers (decamers), separation of the obtained fragments on agarose gel in the presence of ethidium bromide, and, finally, visualization under ultraviolet light [80]. The use of short primers is necessary to increase the probability; although the sequences are random, they can find homologous sequences suitable for annealing [80]. DNA polymorphisms are subsequently produced by "rearrangements or deletions or between oligonucleotide primer binding sites in the genome" [80]. Moreover, earlier knowledge of the genome is not required in this approach; hence, it could be employed across the species using universal primers. RAPD has been extensively used due to its high efficiency, speed, and density for the genetic diversity of landraces and inbred lines in maize [63, 81]. RAPD is used for the analysis of genetic distance; it has been found useful to assess the genetic diversity of 81 landraces and 2 improved varieties by southern Brazilian farmers [82]. Carvalho et al. (2004) studied 32 highly informative RAPD primers amplified 255 markers, of which 184 (72.2%) were polymorphic [59]. A comparative characterization of 17 flint maize landraces (*Zea mays* L.) was carried out using RAPD markers. Fourteen primers provided reliable and consistent polymorphic bands, amplifying 125 fragments (89%). Moreover, previously the genetic variation in 17 maize landraces was characterized based on dissimilarity matrix using the UPGMA based clustering approach[83]. Bruel et al. (2007) analyzed the genetic diversity between 16 corn lines. Twenty-two primers were used, resulting in the amplification of 265 fragments, of which 237 (84.44%) were polymorphic [84].

2.3 Analysis of Maize Diversity through Amplified Fragment Length Polymorphism (AFLPs)

To address the challenge of RAPD and RFLP, amplified fragment length polymorphism (AFLP) was introduced [85]. It is considered an intermediate between RFLPs and RAPDs methodologies as it combines the power of RFLP with the flexibility of PCR-based technology. This method is based on the combination of the two primary analysis techniques: digestion of DNA through restriction endonuclease enzymes and PCR Diversity [71]. The AFLP may produce 51 to 100 bp per assay, in primers pairs. This technique distinguishes the closely related individual of the species [86]. For instance, 96 tropical inbreds [57], 77 inbred lines [71], and 183 inbreds [87] were analyzed using AFLP. To improve the knowledge of the genetic diversity in tropical maize inbred lines, Laborda et al. (2005) fingerprinted 85 lines with 569 AFLP bands and 50 microsatellite loci. These markers revealed substantial variability among lines, with high rates of polymorphism [65]. The 21 inbred lines were fingerprinted, including parents and 210 F1 progeny were evaluated in the field. Joint data analysis mostly revealed a close association between GD and the F1 performance or mid-parent

heterosis in the intergroup than in the intragroup crosses [88]. Several studies were investigated to map the QTLs of *Fusarium moniliforme* ear rot resistance; 230 F2 individuals, derived from a single cross between inbred maize lines R15 (resistant) and Ye478 (susceptible), were genotyped for genetic map construction. They used 63 combinations of AFLP primers to detect the polymorphisms between parents, R15 and Ye478 [89]. The various AFLP marker used for the diversity analysis in maize are mentioned in Table 1.

2.4 Analysis of Maize Diversity through Simple Sequence Repeats (SSRs)

Simple sequence repeats (SSR) are found in both prokaryotic and eukaryotic genomes [90, 91]. These are known by several others names, such as short tandem repeat markers, microsatellite markers, and sequence-tagged microsatellite (STMS) markers. These consist of sequences of repetitions, comprising basic short motifs generally between two and six bp long. Polymorphisms associated with a specific locus are due to diversity variation in the length of the microsatellite, which in turn depends on the number of repetitions of the basic motif. Variations in the number of tandemly repeated units are largely attributed to strand slippage during DNA replication, where the repeats allow matching via excision or addition of repeats [92]. It is a hypervariable marker available in nature [93]. Microsatellites are differentiated based on inter individuals that have unique loci called polymorphism and could be analyzed by PCR. SSR or microsatellite is codominant, highly abundant, with an enormous extent of allelic diversity and ease of assessing SSR size variation due to locus specificity [94]. SSR markers are used for the analysis of genetic diversity and characterization of the germplasm, investigating the genomic diversity of maize landraces, inbred lines, and open pollination. SSR has been employed for characterizing 94 inbreds [21], 65 inbreds [22], 6 inbreds [23], 61 diverse array maize [24], and 54 inbreds [25]. Sharopova et al. (2002) developed 1,051 novel SSR markers for maize from microsatellite-enriched libraries and by identification of microsatellite-containing sequences in public and private databases. Three mapping populations were used to derive map positions for 978 such markers [95]. Stagnati et al. (2022) studied the genetic characterization in 455 individuals using 10 SSR markers and revealed 62 different alleles ranging from four for markers phi127, phi076, and phi084 to nine for marker p-bnlg176 [96]. Adu et al. (2019) assessed the diversity of 70 white maize populations for resistance/tolerance to drought and low soil nitrogen (low-N) using 31 SSR markers. A total of 288 alleles were detected among the germplasm used with a range of 4 to 17 alleles per locus and an average allele number of 9.60 alleles per locus. Polymorphic information content values for the SSR markers ranged from 0.32 to 0.85, with a mean value of 0.68 [97]. Andejelkovic et al. (2018) studied 22 SSRs for phenotypic characterization of 2,217 maize landraces. According to the collecting site and kernel type, 14 samples from the Faculty of Agricultural Sciences and Food, Republic of Macedonia, were selected to compare and identify possible duplicates through coupling with 16 MRIZP gene bank accessions from the same area and kernel characteristics. Phenotypic characterization was performed for 21 traits according to the International Board for Plant Genetic Resources descriptors for maize. The principal component analysis (PCA) identified five PCs with Eigenvalue > 1, explaining 80% of the total phenotypic variation [98]. SSR is a commonly used marker for genetic diversity in maize [99, 100]. Recently, SSR markers have become commonly used in plant breeding programs due to their capability to differentiate desirable genotypes from undesirable ones [101].

2.5 Analysis of Maize Diversity through Single Nucleotide Polymorphism (SNPs)

Single nucleotide variation in the genome sequence of individuals of a population is known as single nucleotide polymorphism (SNPs). SNPs are produced due to the addition or deletion of single nucleotide base pairs [102]. SNPs vary from individual to individual among species and constitute the most suitable marker in the genome. SNPs are usually more prevalent in the non-coding regions of the genome. Within the coding regions, when an SNP is present, it can generate either non-synonymous mutations that result in an amino acid sequence change [102] or synonymous mutations that do not alter the amino acid sequence. Synonymous changes can, however, modify mRNA splicing, resulting in phenotypic differences [103]. These are widely dispersed throughout genomes with variable distribution among species. In maize, one SNP is found over 60 to 120 base pairs [104], whereas in humans, it is estimated that one SNP is found over 1,000 base pairs [105]. SNP marker is widely accepted by plant breeders because this is a highly rapid method and provides appropriate results; in addition, this is a biallelic and codominant marker [106]. Six inbred lines [49], 95 inbred lines [50], 63 inbred lines [40], and 89 double haploids [52] were analyzed using SNPs (for more detail, see review paper). Boakyewaa et al. (2019) assessed the genetic diversity of 94 early-maturing white and yellow tropical maize inbred lines using SNP markers. The larger number of SNP markers used in this study showed the population structure of the 94 inbred lines. Cluster analysis resolved the inbred lines into different clusters based on their pedigree, selection history, and endosperm color. However, three heterotic groups were revealed by population structure analysis; additional field evaluation could be more informative to confirm the heterotic groups identified [107]. Zhang et al. (2022) studied 30 main waxy maize inbred lines; genetic analysis of the tested waxy corn materials was performed using the high-quality SNP marker technology. A total of 15,111 SNPs were obtained from 30 test materials [108]. Osuman et al. (2022) evaluated 162 tropical maize inbred lines under combined heat and drought (CHD) and terminal drought (TD) conditions. The mixed linear model was employed for the genome-wide association study using 7,834 SNP markers and several phenotypic data, including days to 50% anthesis (AD) and silking (SD), husk cover (HUSKC), and grain yield (GY). In total, 66, 27, and 24 SNPs were associated with the traits evaluated under CHD, TD, and their combined effects, respectively. SNPs and candidate genes identified in the study will provide invaluable information for breeding climate-smart maize varieties under tropical conditions following the validation of SNP markers [109]. SNPs used for genetic diversity analysis in maize are mentioned in Table 1.

3. Application of Molecular Markers

The main aim of a plant breeder is to improve the exotic cultivar that is deficient for one or more characteristics through crossing from the new germplasm having new traits of interest [110]. In conventional breeding, the whole genome can be developed by crossing between traits of interest through recombination; however, conventional breeding takes more time, and it is laborious to select phenotypes for the trait of interest that could be misleading due to environmental effects [9]. To address these problems, conventional breeding is now being replaced with molecular breeding. Molecular breeding has been employed by several plant breeders and researchers. Several molecular markers have been identified to control disease resistance and agronomic characteristics in maize [9, 110, 111].

Other uses of molecular markers are developing backcrossing programs for the characterization of germplasms, disease diagnosis, and phylogenetic relationships to study evolutionary processes [9]. In addition, SSR and AFLP are reliable markers to estimate the substantial polymorphism; however, SSR is more automated than AFLP in the maize crop [112].

3.1 Genetic Diversity

For plant breeders, the characterization of germplasm is a critical step to identify the variations within the natural population and between lines for the conservation of biodiversity and the use of germplasm in the future. The genetic variation could be fully exploited using molecular characterization [113, 114]. Genetic variation is detected using SSR markers in maize that could easily detect the phenotypic variations and desirable biological functions [114]. For example, 94 inbreds lines were investigated using 120 SSRs by Senior et al. [21]. Similarly, 65 inbreds lines were investigated using 60 SSRs by Enoki et al. [22]. Gethi et al. [23] investigated diversity in six inbreds lines using 44 SSRs. Moreover, 95 inbred lines were investigated using 1,536 SNPs by Hao et al. [50]. Sharma et al. (2018) detected 191 alleles showing a range of two to six alleles per marker and an average value of 3.82 alleles per locus. The PIC value showed a range of 0.050 (umc1069) to 0.817 (bnlg1823) with an average value of 0.585, indicating the efficiency of markers to study the level of polymorphism available in the maize inbred lines [115]. Sathua et al. (2018) analyzed the diversity using 40 SSR markers. Among the 40 SSR markers, only 20 were found informative (polymorphic) with a total of 70 alleles, providing a reference for determining the SSR allele number in genetic relationship analysis of maize inbred lines and other crop germplasms [116]. Adeyemo and Omidiji (2019) assayed the flanking markers of previously mapped quantitative trait loci linked with the oil and protein or oil/protein contents with 20 SSRs. All SSR markers were polymorphic with a mean PIC value of 0.75. Based on the SSR genotyping data, 183 alleles, ranging from 3 to 18 and averaging 9.15 were detected. A moderately high number of unique alleles was present in three farmers' varieties (EK04, EK06, and KW11) and a hybrid variety (LW18) [117]. Joshi et al. (2020) used five SSR markers to develop the DNA fingerprints and assess the diversity of 23 Nepalese maize landraces. Five locus-based DNA fingerprints have been used to distinguish the majority of landraces. At the landrace level, the highest gene diversity, heterozygosity, and PIC values were found in Seto Local and Seti Makai-3. Furthermore, 23 maize landraces formed four clusters, which were related to seed color [118]. Shinde et al. (2021) assessed the hybrid purity of maize hybrid Hyd.18227 × Hyd.10306 (Tall) and their parental line Hyd.18227 (Dwarf) and Hyd.10306 (Tall) using RAPD and SSR markers. The DNA was extracted from young leaves, and PCR was conducted using 24 RAPD and 18 SSR markers. Out of these, 15 RAPD markers and five SSR markers showed polymorphism between the maize parental lines, and the remaining markers produced a monomorphic banding pattern [119]. Kamra et al. (2021) assessed general (GCA) and specific (SCA) combining abilities for agronomic traits and resistance to LWD, identifying high-yielding hybrids with high resistance to LWD, determining the parental genetic distance (GD) using SSR markers, and investigating its relationship with hybrid performance and SCA effects. Earliness and agronomic traits were evaluated in two different locations [120]. For more information, see these papers.

3.2 Selection of Early Generation

Breeders could select the trait of interest in early generation based on visual heritability [121]; however, it provides biased results; to overcome this problem, selection should be performed using molecular markers, and simultaneous characteristics should be selected in early generation in maize [122]. Selection can be done using molecular markers is more effective for traits having a low heritability as compared to phenotypic selection [121]. Hence, breeders and researchers are required to develop an authentic strategy to select complex traits such as yield using a marker in early generations. Weldekidan et al. (2022) used a standardized protocol to recurrently select for the early flowering time at eight locations for two generations, resulting in location-specific lineages [123]. Dari et al. (2010) compared the weevil resistance of S1, S2, S3, and S4 inbred lines with that of their testcross hybrids, studied the gene action for resistance, and developed recommendations for weevil resistance breeding in maize. Weevil resistance was evaluated for lines and hybrids from two maize populations by infesting the F_2 grain samples with weevils and incubating the samples in a controlled temperature and humidity laboratory. Useful genetic variation exists, and maize breeding programs can address weevil resistance with strategies that use both additive and non-additive gene actions [124]. Bernardo (1992) calculated the probabilities of retaining genetically superior lines during early testing and examined the selection intensities appropriate for different testing generations and levels of heritability. He found that larger proportions of lines need to be retained earlier than at later selfing generations to prevent excessive loss of genetically superior lines [125]. Eathington et al. (1997) worked on early generation testing and discarded lines with poor combining ability early in the inbreeding process. Molecular markers can be used to locate quantitative trait loci (QTL) and measure marker effects in early generations. For marker-assisted selection to be useful in improving the efficiency of early generation testing, marker effects estimated in early generations must agree with those in later generations. A total of 146 probe-enzyme combinations were used [126]. The selection for early generation could be done using single-seed descent and through double haploids [121].

3.3 Selection of Donor Parent in Backcrossing

The marker helps to select the donor parent of desirable interest from large genetic diversity datasets. In backcrossing, the donor parent is selected to have a lower genetic distance to reduce backcross generation [127]. Genetic distance analyses can help in selecting suitable donor parents of unknown or diverse pedigree or where phenotypic information is limited. The maize genotype HP467-15 was used as the donor for transferring the β-carotene gene, *crtRB1*, into UMI1200 and UMI1230. In the MABB scheme, one gene-specific marker (crtRB1 3 'TE) and 214 SSR markers for foreground and background selection, respectively, were used [128]. Backcross selection is a widely used method for introgressing LWD resistance into elite agronomic genetic backgrounds. LWD-tolerant donor parent, CV138811, and susceptible recurrent parent, CV143587, were used to develop BC_1F_1 plants at the Monsanto breeding station. BC_1F_1 plants were screened for responses to LWD infection under artificial inoculation conditions. Forty-three BC_1F_1 plants significantly different from susceptible parents but similar to the resistant parent for LWD tolerance were selected. BC_1F_2 families derived from 43 selected BC_1F_1 plants differed significantly. Forty-nine percent of BC_1F_2 families and 40% of BC_1F_2 plants transgressed the resistant parent for LWD tolerance [129], a (proA) resource for humans. In this study, one common corn line with favorable

lcyE alleles was used as the donor parental line, and four elite sweet corn lines were employed as recipient lines. The favorable *lcyE* allele was introgressed into four sweet corn lines by marker-assisted selection (MAS) [130]. The molecular markers for mutant opaque2 allele and modifier loci (endosperm/amino acid) have provided an opportunity to apply marker-assisted backcross breeding to enhance essential amino acids (a.a.), lysine (lys) and tryptophan (trp) in the maize endosperm. In the present study, SSR phi057 that lie within opaque2, SSRs flanking opaque2, modifier loci, and genic SSRs unlinked to opaque2 were used for molecular characterization in backcrosses between opaque2 donor, CML142, and the recurrent parents, BML2 and BML15 (parental lines of DHM119). The trp and lys content in converted inbreds was significantly higher than in normal lines [131]. The recessive opaque-2 (o2) mutant has a greater content of lysine and tryptophan in their endosperm proteins, and their bioavailability is better. Therefore, marker-assisted backcross breeding (MABB) was attempted to incorporate the o2 allele from the donor line VQL 1 into the genetic background of UMI 1200 to develop quality protein maize (QPM) lines. Foreground selection for the gene *o2* was effected using a tightly linked molecular marker umc1066, in UMI 1200 VQL 1 backcross series. Further, background selection was performed together with stringent phenotypic selection for agronomic traits to accelerate the recurrent parent phenome recovery. Background analysis using 250 SSRs revealed up to 97% recurrent parent genome recovery [132]. DNA fingerprinting approach could use for the selection of potential parents diverse genetic resources for analytical crosses [133].

3.4 Recovery of the Recurrent Parent

Background selection helps to reduce 50% of the donor parent genome in each backcrossing and provides the maximum recurrent parent. MAS always provides the donor parent whose closeness is the same as the recurrent parent to avoid other genomes of the donor parent [134]. MAS reduces the time of backcross generation as compared to conventional breeding. BC_1 lines derived through MAS found the proportion of recurrent parent genome not significantly different as compared to BC_3 from conventional backcross. The selection of individuals through MAS reduces the cycle of backcrossing. This result was in agreement with the studies conducted by Visscher et al. (1996) and Frisch et al. (1999) [135, 136]. In addition, Singh et al. (2021) studied the introgression of favorable alleles of *crtRB1* and *lcyE* genes into opaque2 (o2)-based four inbreds, namely, QLM11, QLM12, QLM13, and QLM14 using marker-assisted backcross breeding [137]. Awata et al. (2021) used marker-assisted backcross (MABC) and doubled haploid (DH) methods to transfer genes for resistance to maize lethal necrosis (MLN). Two maize panels consisting of four BC_3F_2 and six DH populations, separately developed through marker-assisted selection from crosses between susceptible CIMMYT lines and MLN-resistant donor parent (KS23-6), were used. The study identified genotypes with reduced MLN infections in both populations; however, lower means for MLN severity and area under disease progress curve (AUDPC) values, and higher heritability estimates were obtained in the DH populations than in the BC_3F_2 populations. Additionally, the DH populations showed higher relative genetic gains for resistance to MLN compared with the BC_3F_2 populations [138]. Sagare et al. (2019) analyzed and identified a promising β-carotene donor to introgress into QPM inbreds employing molecular breeding. Maize inbred MGU23379 (6.31 µg/g of βcarotene) was used as the β-carotene donor to cross with recurrent parents (RPs), CB6-36 (CBML6) and CB7-28 (CBML7). In conversion program, F_1, BC_1F_1, BC_2F_1, BC_2F_2, BC_2F_3, and BC_2F_4 materials were generated. In each generation, foreground selection was performed with crtRB1-3'TE and umc1066. The

crtRB1-3'TE segregated as per the expectation [139]. Sumathi et al. (2020) illustrated the transfer of two QTLs for SDM on chromosomes 3 and 6 from UMI 936 (w) into an elite maize inbred line UMI79 through MABC. For foreground selection, plants carrying the heterozygote allele for QTLs for SDM were selected from F_1 and three back cross (BC_1-BC_3) generations using SSR markers linked to the targeted QTL region of SDM. Consequently, foreground positive plants from the BC_3F_1 generation selfed to produce BC_3F_2, and plants carrying the favorable alleles for the QTLs for SDM were selected, followed by stringent phenotyping against SDM and advanced to BC_3F_3. Thus, two improved lines possessing QTLs for SDM were obtained. Background selection using 146 SSR markers distributed evenly across the maize genome revealed 92.45% and 89.68% recovery of recurrent parent genome (RPG) in two improved lines. In conclusion, the newly developed SDM resistant inbred lines are a potential source for improving SDM resistance in maize breeding programs [140]. Chandran et al. (2019) improved the lysine and tryptophan content by transferring the *o2* gene from donor HKI163 to β-carotene-rich inbred lines, namely, UMI1200β+ and UMI1230β+. These improved lines can be used as genetic resources for maize improvement [141].

3.5 Mapping and Tagging of Genes

Numerous genes have been manipulated by the force of natural selection or the force of plant breeders to create novel genetic variations and select the desirable alleles for existing variations [142]. Several markers are available to trace the new allele of genes from the segregating population. Genome map in plants was first reported in maize [143], followed by rice [144] using RFLP markers. Davis et al. (1999) [145] constructed a 1,736-locus maize genome map containing 1,156 loci probed by cDNAs, 545 probed by random genomic clones, 16 by SSRs, 14 by isozymes, and 5 by anonymous clones [145]. Isolation and mapping of genome-wide resistance (R) gene analogs (RGAs) are of importance in identifying candidate(s) for a particular resistance gene/QTL. The study revealed the mapping of 228 genome-wide RGAs in maize. By developing RGA-tagged markers and subsequent genotyping, a population consisting of 294 recombinant inbred lines (RILs), 67 RGAs were genetically mapped on the maize genome. Moreover, *in silico* mapping was conducted to anchor 113 RGAs by comparing all 228 RGAs to those anchored EST and BAC/BAC-end sequences via tblastx search (E-value < 10¡20). All RGAs from different mapping efforts were integrated into the existing SSR linkage map. After accounting for redundancy, the resultant RGA linkage map was composed of 153 RGAs that were mapped onto 172 loci on the maize genome, and the mapped RGAs accounted for approximately three-quarters of the genome-wide RGAs in maize [146].

4. Conclusion

Over the last 10,000 years, humans have used the enormous genetic diversity of the maize genome as the raw material for domestication and subsequent crop improvement, a process that took thousands of years. Molecular markers represent a class of molecular tools that are particularly sensitive to new genome-based discoveries and technological advancements and are, therefore, subjected to continuous evolution. Most molecular marker techniques are employed to evaluate the genetic diversity. This review focused on the maize genetic diversity, and several molecular markers such as RFLP, AFLP, SSR, and SNP have been used to detect varying degrees of genetic diversity; SSRs and SNPs are the most prevalent. Due to the bright future of maize research, we must continue to conduct in-depth genetic diversity studies.

Author Contributions

All authors contribute equally.

Competing Interests

No competing interests.

References

1. Kamara MM, Rehan M, Ibrahim KM, Alsohim AS, Elsharkawy MM, Kheir A, et al. Genetic diversity and combining ability of white maize inbred lines under different plant densities. Plants. 2020; 9: 1140.
2. Banda CM. Genetic analysis for heat tolerance in tropical maize (Zea mays) (Doctoral dissertation). Zambia: University of Zambia; 2020.
3. Stanley A, Menkir A, Paterne A, Ifie B, Tongoona P, Unachukwu N, et al. Genetic diversity and population structure of maize inbred lines with varying levels of resistance to striga hermonthica using agronomic trait-based and SNP markers. Plants. 2020; 9: 1223.
4. Kumar P, Hossain F, Singh NK, Choudhary P, Gupta M, Singh V, et al. Nutritional quality improvement in maize (Zea mays): Progress and challenges. Indian J Agric Sci. 2019; 89: 895-911.
5. Wada N, Feng C, Gulati A. Introduction, and overview. In: Maize in Asia: Changing markets and incentives. New Delhi: Academic Foundation; 2008.
6. Prasanna BM, Sharma L. The landraces of maize (Zea mays L.): Diversity and utility. Indian J Plant Genet Resour. 2005; 18: 155-168.
7. Rebourg C, Chastanet M, Gouesnard B, Welcker C, Dubreuil P, Charcosset A. Maize introduction into Europe: The history reviewed in the light of molecular data. Theor Appl Genet. 2003; 106: 895-903.
8. Doebley J. The genetics of maize evolution. Annu Rev Genet. 2004; 38: 37-59.
9. Wani SH, Samantara K, Razzaq A, Kakani G, Kumar P. Back to the wild: Mining maize (Zea mays L.) disease resistance using advanced breeding tools. Mol Biol Rep. 2022. doi: 10.1007/s11033-021-06815-x.
10. Antony BJ, Kachapur RM, Naidu GK, Harlapur SI. Genetic diversity study among maize (Zea mays L.) inbred lines. J Farm Sci. 2021; 34: 352-356.
11. Esquinas-Alcázar J. Protecting crop genetic diversity for food security: Political, ethical and technical challenges. Nat Rev Genet. 2005; 6: 946-953.
12. Platt A, Horton M, Huang YS, Li Y, Anastasio AE, Mulyati NW, et al. The scale of population structure in Arabidopsis thaliana. PLoS Genet. 2010; 6: e1000843.
13. Dubreuil P, Charcosset A. Genetic diversity within and among maize populations: A comparison between isozyme and nuclear RFLP loci. Theor Appl Genet. 1998; 96: 577-587.
14. Dubreuil P, Charcosset A. Relationships among maize inbred lines and populations from European and North-American origins as estimated using RFLP markers. Theor Appl Genet. 1999; 99: 473-480.

15. Govindaraj M, Vetriventhan M, Srinivasan M. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. Genet Res Inter. 2015; 2015: 14.
16. Franco MM, Antunes RC, Silva HD, Goulart LR. Association of a PIT1, GH and GHRH polymorphism with performance and carcass traits in Landrace pigs. J Appl Genet. 2005; 46: 195-200.
17. Beyene A, Gibbon D, Haile M. Heterogeneity in land resources and diversity in farming practices in Tigray, Ethiopia. Agric Syst. 2006; 88: 61-74.
18. Smith OS. Measurement of genetic diversity among maize hybrids: A comparison of isozymic, RFLP, pedigree and heterosis data. Maydica. 1992; 37: 53- 60.
19. De Faria SV, Zuffo LT, Rezende WM, Caixeta DG, Pereira HD, Azevedo CF, et al. Phenotypic and molecular characterization of a set of tropical maize inbred lines from a public breeding program in Brazil. BMC Genomics. 2022; 23: 54.
20. Szalma SJ, Hostert BM, LeDeaux JR, Stuber CW, Holland JB. QTL mapping with near-isogenic lines in maize. Theor Appl Genet. 2007; 114: 1211-1228.
21. Senior ML, Murphy JP, Goodman MM, Stuber CW. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci. 1998; 38: 1088-1098.
22. Enoki H, Sato H, Koinuma K. SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan. Theor Appl Genet. 2002; 104: 1270-1277.
23. Gethi JG, Labate JA, Lamkey KR, Smith ME, Kresovich S. SSR variation in important US maize inbred lines. Crop Sci. 2002; 42: 951-957.
24. Labate JA, Lamkey KR, Mitchell SE, Kresovich S, Sullivan H, Smith JSC. Molecular and historical aspects of corn belt dent diversity. Crop Sci. 2003; 43: 80-91.
25. Vaz Patto MV, Satovic Z, Pêgo S, Fevereiro P. Assessing the genetic diversity of Portuguese maize germplasm using microsatellite markers. Euphytica. 2004; 137: 63-72.
26. Clark TL, Foster JE, Kamble ST, Heinrichs EA. Comparison of Bt (Bacillus thuringiensis Berliner) maize and conventional measures for control of the European corn borer (Lepidoptera: Crambidae). J Entom Sci. 2000; 35: 118-128.
27. Legesse BW, Myburg AA, Pixley KV, Twumasi-Afriyie S, Botha AM. Relationship between hybrid performance and AFLP based genetic distance in highland maize inbred lines. Euphytica. 2008; 162: 313-323.
28. Franco J, Crossa J, Warburton ML, Taba S. Sampling strategies for conserving maize diversity when forming core subsets using genetic markers. Crop Sci. 2006; 46: 854-864.
29. Reif JC, Warburton ML, Xia XC, Hoisington DA, Crossa J, Taba S, et al. Grouping of accessions of Mexican races of maize revisited with SSR markers. Theor Appl Genet. 2006; 113: 177-185.
30. Warburton ML, Brooks TD, Windham GL, Williams WP. Identification of novel QTL contributing resistance to aflatoxin accumulation in maize. Mol Breed. 2011; 27: 491-499.
31. Legesse WB, Myburg A, Pixley VK, Botha MA. Genetic diversity of African maize inbred lines revealed by SSR markers. Hereditas. 2007; 144: 10-17.
32. Gunjaca J, Buhinicek I, Jukic M, Sarcevic H, Vragolovic A, Kozic Z, et al. Discriminating maize inbred lines using molecular and DUS data. Euphytica. 2008; 161: 165-172.

33. Vigouroux Y, Mitchell S, Matsuoka Y, Hamblin M, Kresovich S, Smith JSC, et al. An analysis of genetic diversity across the maize genome using microsatellites. Genetics. 2005; 169: 1617-1630.
34. Warburton ML, Reif JC, Frisch M, Bohn M, Bedoya C, Xia XC, et al. Genetic diversity in CIMMYT nontemperate maize germplasm: Landraces, open pollinated varieties, and inbred lines. Crop Sci. 2008; 48: 617-624.
35. Shehata AI, Al-Ghethar HA, Al-Homaidan AA. Application of simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines. Saudi J Biol Sci. 2009; 16: 57-62.
36. Wu CL, Zhang QQ, Dong BX, Li SF, Zhang CQ. Analysis of genetic structure and relationships of maize inbred lines in China. Acta Agron Sinica. 2010; 36: 1820-1831.
37. Valdez-Morales M, Barry K, Fahey Jr GC, Domínguez J, de Mejia EG, Valverde ME, et al. Effect of maize genotype, developmental stage, and cooking process on the nutraceutical potential of huitlacoche (Ustilago maydis). Food Chem. 2010; 119: 689-697.
38. Daniel IO, Adetumbi JA, Oyelakin OO, Olakojo SA, Ajala MO, Onagbesan SO. Application of SSR markers for genetic purity analysis of parental inbred lines and some commercial hybrid maize (Zea mays L.). J Exp Agric Int. 2012; 28: 597-606.
39. 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: 69-75.
40. Sserumaga JP, Makumbi D, Njoroge K, Muthomi JW, Cheminingwa GN, Si-myung L, et al. Molecular characterization of tropical maize inbred lines using microsatellite DNA markers. Maydica. 2014; 2014: 267-274.
41. Xia XC, Reif JC, Hoisington DA, Melchinger AE, Frisch M, Warburton ML. Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: I. Lowland tropical maize. Crop Sci. 2004; 44: 2230-2237.
42. Nikolić A, Ignjatović-Micić D, Kovačević D, Čamdžija Z, Filipović M, Mladenović-Drinić S. Genetic diversity of maize inbred lines as inferred from SSR markers. Genetika. 2015; 47: 489-498.
43. Chen Q, Song J, Du WP, Xu LY, Jiang Y, Zhang J, et al. Identification, mapping, and molecular marker development for Rgsr8. 1: A new quantitative trait locus conferring resistance to Gibberella stalk rot in maize (Zea mays L.). Front Plant Sci. 2017; 8. doi: 10.3389/fpls.2017.01355.
44. Salami HA, Sika KC, Padonou W, Aly D, Yallou C, Adjanohoun A, et al. Genetic diversity of maize accessions (Zea mays L.) cultivated from Benin using microsatellites markers. Am J Mol Biol. 2016; 6: 12-24.
45. Belalia N, Lupini A, Djemel A, Morsli A, Mauceri A, Lotti C, et al. Analysis of genetic diversity and population structure in Saharan maize (Zea mays L.) populations using phenotypic traits and SSR markers. Genet Resour Crop Evol. 2019; 66: 243-257.
46. Li YG, Jiang D, Xu LK, Zhang SQ, Ji PS, Pan HY, et al. Evaluation of diversity and resistance of maize varieties to Fusarium spp. causing ear rot in maize under conditions of natural infection. Czech J Genet Plant Breed. 2019; 55:131-137.
47. Nikolić N, Mitrović J, Karabegović I, Savić S, Petrović S, Lazić M, et al. A comparison between wheat and different kinds of corn flour based on minerals, free phenolic acid composition and antioxidant activity. Qual Assur Saf Crops Foods. 2019; 11: 341-349.

48. Yan J, Shah T, Warburton ML, Buckler ES, McMullen MD, Crouch J. Genetic characterization and linkage disequilibrium estimation of a global maize collection using SNP markers. PloS One. 2009; 4: e8451.
49. Lai J, Li R, Xu X, Jin W, Xu M, Zhao H, et al. Genome-wide patterns of genetic variation among elite maize inbred lines. Nature Genet. 2010; 42: 1027-1030.
50. Hao Z, Li X, Xie C, Weng J, Li M, Zhang D, et al. Identification of functional genetic variations underlying drought tolerance in maize using SNP markers. J Integr Plant Biol. 2011; 53: 641-652.
51. Ajala SO, Olayiwola MO, Ilesanmi OJ, Gedil M, Job AO, Olaniyan AB. Assessment of genetic diversity among low-nitrogen-tolerant early generation maize inbred lines using SNP markers. S Afr J Plant Soil. 2019; 36: 181-188.
52. Brauner PC, Melchinger AE, Schrag TA, Utz HF, Schipprack W, Kessel B, et al. Low validation rate of quantitative trait loci for Gibberella ear rot resistance in European maize. Theor Appl Genet. 2017; 130: 175-186.
53. Hurst P, Liang Z, Smith C, Yerka M, Sigmon B, Rodriguez O, et al. Genome wide analysis of Ga1-s modifiers in maize. bioRxiv. 2019. doi: 10.1101/543264.
54. Mazaheri M, Heckwolf M, Vaillancourt B, Gage JL, Burdo B, Heckwolf S, et al. Genome-wide association analysis of stalk biomass and anatomical traits in maize. BMC Plant Biol. 2019; 19: 45.
55. Owens BF, Mathew D, Diepenbrock CH, Tiede T, Wu D, Mateos-Hernandez M, et al. Genome-wide association study and pathway-level analysis of kernel color in maize. G3 Genes Genomes Genet. 2019; 9: 1945-1955.
56. Yang N, Liu J, Gao Q, Gui S, Chen L, Yang L, et al. Genome assembly of a tropical maize inbred line provides insights into structural variation and crop improvement. Nature Genet. 2019; 51: 1052-1059.
57. Oliveira KM, Laborda PR, Garcia AAF, Zagatto Paterniani MEA, De Souza AP. Evaluating genetic relationships between tropical maize inbred lines by means of AFLP profiling. Hereditas. 2004; 140: 24-33.
58. Garcia AAF, Benchimol LL, Barbosa AMM, Geraldi IO, Souza Jr CL, de Souza AP. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genet Mol Biol. 2004; 27: 579-588.
59. Carvalho VP, Ruas CF, Ferreira JM, Moreira RMP, Ruas PM. Genetic diversity among maize (Zea mays L.) landraces assessed by RAPD markers. Genet Mol Biol. 2004; 27: 228-236.
60. Van Inghelandt D, Melchinger AE, Lebreton C, Stich B. Population structure and genetic diversity in a commercial maize breeding program assessed with SSR and SNP markers. Theor Appl Genet. 2010; 120: 1289-1299.
61. Yang Z, Zhai W. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (Zea mays L.). Innov Food Sci Emerg Technol. 2010; 11: 169-176.
62. Marsan PA, Castiglioni P, Fusari F, Kuiper M, Motto M. Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor Appl Genet. 1998; 96: 219-227.
63. Ristić D, Babić V, Anđelković V, Vančetović J, Mladenović-Drinić S, Ignjatović-Micić D. Genetic diversity in maize dent landraces assessed by morphological and molecular markers. Genetika. 2013; 45: 811-824.

64. Menkir A, Melake-Berhan A, The C, Ingelbrecht I, Adepoju A. Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers. Theor Appl Genet. 2004; 10: 1582-1590.
65. Laborda PR, Oliveira KM, Garcia AAF, Paterniani MEAGZ, De Souza AP. Tropical maize germplasm: What can we say about its genetic diversity in the light of molecular markers? Theor Appl Genet. 2005; 111: 1288-1299.
66. da Silva Meyer AD, Garcia AAF, de Souza AP, de Souza Jr CL. Comparison of similarity coefficients used for cluster analysis with dominant markers in maize (Zea mays L). Genet Mol Biol. 2004; 27: 83-91.
67. Vroh Bi I, McMullen MD, Sanchez-Villeda H, Schroeder S, Gardiner J, Polacco M, et al. Single nucleotide polymorphisms and insertion-deletions for genetic markers and anchoring the maize fingerprint contig physical map. Crop Sci. 2006; 46: 12-21.
68. Barcaccia G, Lucchin M, Parrini P. Characterization of a flint maize (Zea mays var. indurata) Italian landrace, II. Genetic diversity and relatedness assessed by SSR and Inter-SSR molecular markers. Genet Resour Crop Evol. 2003; 50: 253-271.
69. Lübberstedt T, Melchinger AE, DuBle C, Vuylsteke M, Kuiper M. Relationships among early European maize inbreds: IV. Genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data. Crop Sci. 2000; 40: 783-791.
70. Frascaroli E, Schrag TA, Melchinger AE. Genetic diversity analysis of elite European maize (Zea mays L.) inbred lines using AFLP, SSR, and SNP markers reveals ascertainment bias for a subset of SNPs. Theor Appl Genet. 2013; 126: 133-141.
71. Mondini L, Noorani A, Pagnotta MA. Assessing plant genetic diversity by molecular tools. Diversity. 2009; 1: 19-35.
72. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980; 32: 314-331.
73. Mumm RH, Hubert LJ, Dudley JW. A classification of 148 U.S. maize inbreds: II. Validation of cluster analysis based on RFLPs. Crop Sci. 1994; 34: 852-865.
74. Gauthier P, Gouesnard B, Dallard J, Redaelli R, Rebourg C, Charcosset A, et al. RFLP diversity and relationships among traditional European maize populations. Theor Appl Genet. 2002; 105: 91-99.
75. Kesawat MS, Kumar BD. Molecular markers: It's application in crop improvement. J Crop Sci Biotechnol. 2009; 12: 169-181.
76. Armstrong CL, Romero-Severson J, Hodges TK. Improved tissue culture response of an elite maize inbred through backcross breeding, and identification of chromosomal regions important for regeneration by RFLP analysis. Theor Appl Genet. 1992; 84: 755-762.
77. Bentolila S, Guitton C, Bouvet N, Sailland A, Nykaza S, Freyssinet G. Identification of an RFLP marker tightly linked to the Ht1 gene in maize. Theor Appl Genet. 1991; 82: 393-398.
78. Agrama HAS, Moussa ME. Mapping QTLs in breeding for drought tolerance in maize (Zea mays L.). Euphytica. 1996; 91: 89-97.
79. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 1990; 18: 7213-7218.
80. Shinde NA, Bharose AA, Sarode DK, Swathi RS, Pimpale PA, Shinde SS. Assessment of hybrid purity in maize (Zea mays L.) using RAPD and SSR markers. Pharma Innov J. 2021; 10: 870-874.

81. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990; 18: 6531-6535.
82. Moeller DA, Schaal BA. Genetic relationships among native American maize accessions of the great plains assessed by RAPDs. Theor Appl Genet. 1999; 99: 1061-1067.
83. Okumus A. Genetic variation and relationship between Turkish flint maize landraces by RAPD markers. Am J Agri Biol Sci. 2007; 2: 49-53.
84. Bruel DC, Carpentieri-Pipolo V, de Fátima Ruas C, Gerage AC, de Souza SGH. Assessment of genetic diversity in maize inbred lines using RAPD markers. Crop Breed Appl Biotechnol. 2007; 7: 173-178.
85. Vos P, Hogers R, Bleeker M, Rijans M, van der Lee T, Hornes M, et al. A new technique for DNA fingerprinting. Nucl Acid Res. 1995; 23: 4407-4414.
86. Althoff DM, Gitzendanner MA, Segraves KA. The utility of amplified fragment length polymorphisms in phylogenetics: A comparison of homology within and between genomes. Syst Biol. 2007; 56: 477-484.
87. da Silva Meyer A, Garcia AAF, de Souza AP, de Souza Jr CL. Comparison of similarity coefficients used for cluster analysis with dominant markers in maize (Zea mays L). Genet Mol Biol. 2004; 27: 83-91.
88. Kiula BA, Lyimo NG, Botha AM. Association between AFLP-based genetic distance and hybrid performance in tropical maize. Plant Breed. 2008; 127: 140-144.
89. Zhang F, Wan XQ, Pan GT. QTL mapping of Fusarium moniliforme ear rot resistance in maize. 1. Map construction with microsatellite and AFLP markers. J Appl Genet. 2006; 47: 9-15.
90. Tóth G, Gáspári Z, Jurka J. Microsatellites in different eukaryotic genomes: Survey and analysis. Genome Res. 2000; 10: 967-981.
91. Abdulazeez SD, Kakarash SA, Ismael NB. Genetic diversity among ten maize genotypes using simple sequence repeat DNA markers. Polytech J. 2021; 11: 32-37.
92. Schlotterer C, Tautz D. Slippage synthesis of simple sequence DNA. Nucleic Acids Res. 1992; 20: 211-215.
93. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nature Biotech. 2013; 31: 233-239.
94. Bocianowski J, Nowosad K, Wróbel B, Szulc P. Identification of associations between SSR markers and quantitative traits of maize (Zea mays L.). Agronomy. 2021; 11: 182.
95. Sharopova N, McMullen MD, Schultz L, Schroeder S, Sanchez-Villeda H, Gardiner J, et al. Development and mapping of SSR markers for maize. Plant Mol Biol. 2002; 48: 463-481.
96. Stagnati L, Soffritti G, Martino M, Lanubile A, Desiderio F, Ravasio A, et al. Morphological and genetic characterization of local maize accessions from Emilia Romagna region, Italy. Sustainability. 2022; 14: 91.
97. Adu GB, Awuku FJ, Amegbor IK, Haruna A, Manigben KA, Aboyadana PA. Genetic characterization and population structure of maize populations using SSR markers. Ann Agric Sci. 2019: 64; 47-54.
98. Andjelkovic V, Nikolic A, Kovacevic D, Mladenovic-Drinic S, Kravic N, Babic V, et al. Conserving maize in gene banks: Changes in genetic diversity revealed by morphological and SSR markers. Chil J Agric Res. 2018; 78: 30-38.
99. Kumar A, Singh VP. Maize Genetic Diversity: Utilization of Molecular Markers in Genetic Diversity. Int J Curr Microbiol App Sci. 2020; 9: 1948-1959.

100. Kumar A, Singh RCVP. Genome mapping: Utilization of molecular markers in genome mapping. IJCS. 2020; 8: 3060-3065.
101. Yao Z, Yang X, Zhang Y, Duan Z, Zhao Z. Screening and diversity analysis of core primers on SSR markers for yunnan maize hybrids. J Nucl Agric Sci. 2021; 35: 796-806.
102. López-Malvar A, Malvar RA, Butrón A, Revilla P, Jiménez-Galindo JC, Souto XC, et al. Identification of single nucleotide polymorphisms (SNPs) for maize cell wall hydroxycinnamates using a multi-parent advanced generation intercross (MAGIC) population. Phytochemistry. 2022; 193: 113002.
103. Sa KJ, Choi IY, Park JY, Choi JK, Ryu SH, Lee JK. Mapping of QTL for agronomic traits using high-density SNPs with an RIL population in maize. Genes Genomics. 2021; 43: 1403-1411.
104. Ching A, Caldwell KS, Jung M, Dolan M, Smith OS, Tingey S, et al. SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genet. 2002; 3: 19.
105. Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature. 2001; 409: 928-933.
106. Srii VS, Nethra N, Umarani K, Lohithaswa HC, Shadakshari YG, Prasad SR. SNP genotyping of maize (Zea mays) hybrids and parental inbred lines for genetic purity testing using double digest restriction site-associated DNA sequencing. Seed Sci Technol. 2021; 49: 193-206.
107. Boakyewaa Adu G, Badu-Apraku B, Akromah R, Garcia-Oliveira AL, Awuku FJ, Gedil M. Genetic diversity and population structure of early-maturing tropical maize inbred lines using SNP markers. PloS One. 2019; 14: e0214810.
108. Zhang C, Zhang S, Chen L, Yu T, Zhang W, Wen S. Analysis of waxy maize germplasm resources in southwest China based on SNP markers. Res Square. 2022. doi:10.21203/rs.3.rs-1248113/v1.
109. Osuman AS, Badu-Apraku B, Karikari B, Ifie BE, Tongoona P, Danquah EY. Genome-wide association study reveals genetic architecture and candidate genes for yield and related traits under terminal drought, combined heat and drought in tropical maize germplasm. Genes. 2022; 13: 349.
110. Phillips RL, Vasil IK. Introduction: Molecular marker maps of major crop species. In: DNA-based markers in plants. Switzerland: Springer; 1st ed.2001. p. page 142-143.
111. Yin ZC, Wang XF, Long Y, Dong ZY, Wan XY. Advances on genetic research and mechanism analysis on maize resistance to ear rot. China Biotechnol. 2022; 41: 103-115.
112. Wang N, Yuan Y, Wang H, Yu D, Liu Y, Zhang A, et al. Applications of genotyping-by-sequencing (GBS) in maize genetics and breeding. Scie Rep. 2020; 10: 16308.
113. Arzhang S, Darvishzadeh R, Alipour H. Evaluation of genetic diversity of maize lines (Zea mays L.) under normal and salinity stress conditions. Cereal Res. 2022. doi:10.22124/CR.2022.21075.1699.
114. Andorf C, Beavis WD, Hufford M, Smith S, Suza WP, Wang K, et al. Technological advances in maize breeding: Past, present and future. Theor Appl Genet. 2019; 132: 817-849.
115. Sharma T, Kumar A, Dwivedi SC, Vyas RP. Molecular characterization and genetic diversity analysis of selected maize inbreds using SSR markers. J Environ Biol. 2018; 39: 228-236.
116. Sathua SK, Gayatonde V. Molecular diversity analysis of maize (Zea maysL.) inbreds using SSR markers. Electron J Plant Breed. 2018; 9: 1122-1129.

117. Adeyemo OA, Omidiji O. Genetic diversity and population structure of farmers' maize varieties (Zea mays L.) from three selected states in Nigeria using SSR markers and their relationship with standard hybrids. Ife J Sci. 2019; 21: 261-275.
118. Joshi BK, Rawat J, Adhikari B, Pokhrel R. SSR markers based genetic diversity in nepalese maize landraces. SAARC J Agric. 2020; 18: 23-37.
119. Shinde N, Sarode DK, Pimpale P, Shinde SS, Pimpale PA, Shinde SS. Assessment of hybrid purity in maize (Zea mays L.) using RAPD and SSR markers. Pharma Innov J. 2021; 10: 870-874.
120. Kamara MM, Ghazy NA, Mansour E, Elsharkawy MM, Kheir A, Ibrahim KM. Molecular genetic diversity and line× tester analysis for resistance to late wilt disease and grain yield in maize. Agronomy. 2021; 11: 898.
121. Lande R, Thompson R. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics. 1990; 124: 743-756.
122. Stromberg LD, Dudley JW, Rufener GK. Comparing conventional early generation selection with molecular marker assisted selection in maize. Crop Sci. 1994; 34: 1221-1225.
123. Weldekidan T, Manching H, Choquette N, de Leon N, Flint-Garcia S, Holland J, et al. Registration of tropical populations of maize selected in parallel for early flowering time across the United States. J Plant Regist. 2022; 16: 100-108.
124. Dari S, Pixley KV, Setimela P. Resistance of early generation maize inbred lines and their hybrids to maize weevil [Sitophilus zeamais (Motschulsky)]. Crop Sci. 2010; 50: 1310-1317.
125. Bernardo R. Retention of genetically superior lines during early-generation testcrossing of maize. Crop Sci. 1992; 32: 933-937.
126. Eathington SR, Dudley JW, Rufener GK. Marker effects estimated from testcrosses of early and late generations of inbreeding in maize. Crop Sci. 1997; 37: 1679-1685.
127. Babu R, Nair SK, Kumar A, Venkatesh S, Sekhar JC, Singh NN, et al. Two-generation marker-aided backcrossing for rapid conversion of normal maize lines to quality protein maize (QPM). Theor Appl Genet. 2005; 111: 888-897.
128. Natesan S, Duraisamy T, Pukalenthy B, Chandran S, Nallathambi J, Adhimoolam K, et al. Enhancing β-carotene concentration in parental lines of CO6 maize hybrid through marker-assisted backcross breeding (MABB). Front Nutr. 2020; 7. doi:100.3389/fnut.2020.00134.
129. Sunitha NC, Gangappa E, Gowda RPV, Ramesh S, Swamy SD, Hemareddy HB. Effectiveness of one cycle of phenotype-based backcross breeding for resistance to late wilt disease in maize (Zea mays L.). Mysore J Agric Sci. 2020; 54: 47-50.
130. Yang R, Yan Z, Wang Q, Li X, Feng F. Marker-assisted backcrossing of lcyE for enhancement of proA in sweet corn. Euphytica. 2018; 214: 130.
131. Shetti P, Sagare DB, Surender M, Reddy SS. Development of lysine and tryptophan rich maize (Zea mays) inbreds employing marker assisted backcross breeding. Plant Gene. 2020; 23: 100236.
132. Pukalenthy B, Manickam D, Chandran S, Adhimoolam K, Sampathrajan V, Rajasekaran R, et al. Incorporation of opaque-2 into 'UMI 1200', an elite maize inbred line, through marker-assisted backcross breeding. Biotechnol Biotechnol Equip. 2019; 33: 144-153.
133. Njeri SG, Makumbi D, Warburton ML, Diallo A, Jumbo MB, Chemining'wa G. Genetic analysis of tropical quality protein maize (Zea mays L.) germplasm. Euphytica. 2017; 213: 261.

134. Tanksley SD, Medina-Filho H, Rick CM. Use of naturally-occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity. 1982; 49: 11-25.
135. Visscher PM, Thompson R, Haley CS. Confidence intervals in QTL mapping by bootstrapping. Genetics. 1996; 143: 1013-1020.
136. Frisch M, Bohn M, Melchinger AA. Minimum sample size and optimal positioning of flanking markers in marker-assisted backcrossing for transfer of a target gene. Crop Sci. 1999; 39: 967-975.
137. Singh J, Sharma S, Kaur A, Vikal Y, Cheema AK, Bains BK, et al. Marker-assisted pyramiding of lycopene-ε-cyclase, β-carotene hydroxylase1 and opaque2 genes for development of biofortified maize hybrids. Sci Rep. 2021; 11: 12642.
138. Awata LAO, Ifie BE, Danquah E, Tongoona P, Suresh LM, Gowda M, et al. Comparison of non-overlapping maize populations of unequal sizes for resistance to maize lethal necrosis. J Crop Improv. 2021: 1-20.
139. Sagare DB, Shetti P, Surender M, Reddy SS. Marker-assisted backcross breeding for enhancing β-carotene of QPM inbreds. Mol Breed. 2019; 39: 31.
140. Sumathi K, Ganesan KN, Aarthi P, Sruthymenon V, Devasree S, Karthikeyan A, et al. Introgression of QTLs determining sorghum downy mildew (SDM) resistance into elite maize line UMI 79 through marker-assisted backcross breeding (MABC). Australas Plant Pathol. 2020; 49: 159-165.
141. Chandran S, Pukalenthy B, Adhimoolam K, Manickam D, Sampathrajan V, Chocklingam V, et al. Marker-assisted selection to pyramid the opaque-2 (o2) and β-carotene (crtRB1) genes in maize. Front Genet. 2019; 10. doi:10.3389/fgene.2019.00859.
142. Flavell AJ, Jackson V, Iqbal MP, Riach I, Waddell S. Ty1-copia group retrotransposon sequences in amphibia and reptilia. Mol Gen Genet. 1995; 246: 65-71.
143. Gardiner JM, Coe EH, Melia-Hancock S, Hoisington DA, Chao S. Development of a core RFLP map in maize using an immortalized F2 population. Genetics. 1993; 134: 917-930.
144. McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, et al. Molecular mapping of rice chromosomes. Theor Appl Genet. 1988; 76: 815-829.
145. Davis GL, McMullen MD, Baysdorfer C, Musket T, Grant D, Staebell M, et al. A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. Genetics. 1999; 152: 1137-1172.
146. Xiao W, Zhao J, Fan S, Li L, Dai J, Xu M. Mapping of genome-wide resistance gene analogs (RGAs) in maize (Zea mays L.). Theor Appl Genet. 2007; 115: 501-508.

Enjoy *OBM Genetics* by:

1. Submitting a manuscript
2. Joining in volunteer reviewer bank
3. Joining Editorial Board
4. Guest editing a special issue

For more details, please visit:
http://www.lidsen.com/journals/genetics