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Review Article

Molecular Markers in Maize Improvement: A Review

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Abstract

Maize is one of the most important cereal crops with diverse adaptation and end-uses. Maize possesses enormous diversity owing to its dynamic genome (repetitive elements). Conventional breeding contributed significantly to maize improvement during the area of the pre-molecular marker, however, has certain limitations. Molecular markers address the limitations of conventional breeding through indirect selection, and growth-stage and environment insensitive nature. Furthermore, molecular markers-based selection is cost-effective and efficient over phenotypic selection. The introduction of molecular markers in maize breeding added new avenues to the maize improvement, especially for complex traits related to quality, agronomic, abiotic, and biotic stresses. Molecular markers contributed essentially to the germplasm characterization, genetic diversity assessment, heterotic grouping, heterosis prediction, construction of highly dense genetic maps, gene mapping and tagging, and marker-assisted and genomic selections. Besides, the molecular markers can also serve as useful criteria for DUS characterization of the new varieties. Furthermore, the markers will also likely be harnessed in the memory stress breeding for abiotic and biotic stresses. Therefore, with the evolving high-throughput sequencing platforms, molecular markers will continue to serve as a boon in the future for maize improvement and hence safeguarding the food and nutritional security globally.

Keywords: Markers; Maize; Diversity; Breeding; QTL

Introduction

Maize (*Zea mays* L.) belongs to Poaceae and is adaptable under versatile agro-climatic conditions. The wide adaptation of maize can be witnessed from its cultivation ranging from 58° N to 40° S, from below sea level to elevations above 3000 m, and in regions having rainfall from 250 mm to over 5000 mm of rainfall per year [1,2]. Modern maize was domesticated from the *Zea mays ssp. parviglumis*, its closest progenitor [3] occurred 6,000 years ago in the tropical valley of the Balsas River in Mexico [4-7]. Hence, Mexico and Central America are considered to be the center of origin of maize [8]. The leading producers of maize are mainly restricted to the world's temperate regions like the USA, Brazil, and Mexico due to its origin from Mexico [9]. Maize is mostly used in poultry feed but it is also used for different purposes such as food, animal feed, value-added products like starch, maltodextrins, maize oil, maize syrup, biofuels, *etc.* [10-12]. It is the choice of food and industries, and as such has achieved the highest compound annual growth rate in last decade, surpassing even wheat and rice. Maize is a diploid

crop with a haploid set of 10 chromosomes and a genome size of 2.3 to 2.7 Gb [13,14]. But unlike other crops, the maize genome is mainly composed of non-genic, repetitive fractions punctuated by distinctive or low-copy DNA clusters that harbor single genes or small gene groups. These repetitive elements comprising transposable elements, ribosomal DNA, high-copy short-tandem repetitions in telomeres, centromeres, and heterochromatin knobs imparts enormous species diversity [15,16]. The enormous cytogenetic variations in maize are supposed to be responsible for imparting huge genetic variation in maize germplasm [17,18].

The beginning of the era of maize improvement focused upon the identification and exploitation of existing variation in maize germplasm along with tapping the unique variability of landraces. The selection of elite cultivars was based upon the phenotypic level, depending upon the heritability of the desired trait. The trait with high heritability was easy to improve through phenotypic selection but the traits with low heritability such as yield were difficult to select through visual selection. Although conventional breeding strategies contributed immensely to maize improvement providing elite cultivars possessing higher yields, good quality, agronomic traits, etc., at the cost of time, economic resources, and labor-consuming practices [19,20]. Hence with the discovery of environmentally insensitive molecular marker technology in the 1980s, the paradigm of selection shifted from phenotypic selection to selection at the genotypic level.

Molecular breeding in maize relies mainly upon the utilization of different types of molecular markers owing to their use for germplasm characterization, genetic diversity studies, genetic purity and identity of lines, heterotic grouping and prediction of heterosis, development of dense genetic maps, gene identification (gene mapping and tagging), marker-assisted selection (MAS), and genomic selection (GS) [21,22]. Molecular markers help in the improvement of complex traits through identification and mapping of the number of Quantitative Trait Loci (QTLs)/genes associated with different traits of economic importance and thereby providing plenty of marker-trait associations to plant breeders (Table 1). These mapped QTLs assist the maize breeders in the rapid development of elite maize cultivars having desirable traits. Furthermore, biotechnologists can explore the QTLs for cloning and characterization of genes to develop genetically modified plants [62-65].

Marker	Mapping Population	Crosses	QTLs	Trait	References
Abiotic Stresses					
RFLPs	$F_{3:4}$ (80)	Os420 × IABO78	16 QTLs	Leaf ABA content	[23]
SSRs and AFLP	F_2	B64 × *Zea mays* ssp. h*ue-huetenangensis*	QTLs (chromosomes 3, 7, and 8)	Adventitious root formation under waterlogging	[24]
SSRs	F_2	B73 × *Zealuxurians*	8 QTLs	Nodal root angle	[25]
SSRs	BC_3F_1 (317)	Mi29 × *Zeanicaraguensis*	3 QTLs	Adventitious root formation (waterlogging)	[26]
SSRs	RILs	Zong3 × 87-1	9 QTLs	Leaf temperature responses to drought	[27]
SSRs	F_2	A150-3-2 × Mo17	22 QTLs	Stay green traits	[28]
SNPs	RILs	Oh43 × W64a (OhW), Ny821 × H99 (NyH)	15 QTLs	Root architectural traits	[29]
SNPs	Two $BC_1F_{2:3}$ backcross	LPSpop and DTPpop Populations	105 QTLs	Early vigour and stay green under drought	[30]
SNPs	DH (240 lines)	PH6WC × PH4CV	6 QTL (qSPH1, *qSPH5–1, qPHI1, qPHI4, qPHI9, qPHI10)*	Plant heigh and plant height-based salt tolerance index	[31]
SNPs	RILs (204)	DH1M × T877	364 QTLs	Primary, seminal, and crown root length, seminal root number	[32]

SNPs	$F_{2:3}$ (650)	220 × PH4CV	19 QTLs	Emergence rate, germination index, total length, root length, and shoot length	[33]
SNPs	$F_{2:3}$ (650)	220 × Y1518	13 QTLs	Emergence rate, germination index, total length, root length, and shoot length	
SNPs	F2:3 (650)	P9-10 × PH4CV	11 QTLs	Emergence rate, germination index, total length, root length, and shoot length	
SNPs	DH n	DHpop1 × DHpop2	26 QTLs	Grain yield, Days to anthesis and silking, ASI under nitrogen deficit condition	[34]
Biotic Stresses					
SSRs	$F_{2:3}$ (193)	CA00106 × CM140	Three QTLs (chromosomes 6, 8 and 9)	BLSB	[35]
SSRs	F_2	L14-04B × L08-05F	6 QTLs	Reaction to Phaeosphaeria leaf spot	[36]
RFLP and SSRs	F_3 progeny	P345C4S2B46-2-2-1-2-B-B-B (yellow), (resistant) × SC-TEP5-19-1-3-1-4-1-1 (white)(Susceptible)	3 putative QTLs	Resistance to sorghum downy mildew	[37]
SSRs and AFLPs	$F_{2:3}$ families	Mo17 × Huangzao4	5 QTLs	Head smut resistance	[38]
SSRs	Seven different backcross populations	CM137, CM138, CM139, CM140 and CM212	TLB resistant genes *Ht-n1*and *Ht2* along with a QTL (*RppQ*) for Polysora rust	TLB and Polysora rust	[39]
SSRs	185 F_2 progeny	CM500-19 × MAI105	3 QTLs	Resistance to Sorghum downy mildew	[40]
SSRs	Inbred line	Y32 × Q11	4 QTLs	Gray leaf spot resistance	[41]
RAPD, ISSR, SSRs and STS	Segregating population (170)	Gm1021 ×Gm1002	8 QTLs	Maize stalk rot disease resistance	[42]
DArTseq	198 BC_1S_1 families	TZEEI 29 (*Striga* resistant) × TZEEI 23 (Striga susceptible)	14 QTLs	Grain yield, ears per plant, and striga damage	[43]
DArT	$F_{2:3}$	KU-R × GT-S	18 QTLs	MSV resistance	[44]
SNPs	170 and 163	B73×B97 and B73×CML322	12 QTLs	NCLB resistance	[45]
Quality Traits					
SNPs	RIL	By804 ×B73	*ZmcrtRB*3 gene	α-carotene content	[46]
SNPs	RIL	CI7 × K22	6 QTLs	Starch content in maize kernels	[47]

SNPs	Two $BC_1F_{2:3}$ backcross	LPSpop and DTPpop Populations	105 QTLs	Early Vigour and Stay-Greenness	[30]
PSY-SNP7 and PSY1-IDI (InDel)	130 diverse tropical adapted yellow maize inbred lines	Inbred lines developed from eight bi-parental crosses of tropical inbred lines, four broad-based populations, and 28 backcrosses involving temperate lines as donors	*PSY1* gene	Provitamin A content	[48]
LCYE-SNP and LCYE-3'InDel			*LCYE* gene		
crtRBI-5' TE, crtR-BI-InDel4 and crtRBI-3' TE			*crtRB1* gene		
SNPs and InDel	155 inbred lines	91 inbreds, 35 high-oil lines, 25 inbred lines derived from Chinese landraces, and four high provitamin A from the University of Illinois	*DGAT1-2* gene	Oil content	[49]
SNPs	F_7	High folate (GEMS31) × low folate (DAN3130)	2 QTLs	Folate content	[50]
InDel	RILs	B73 × Mo17 (IBM population)	8 QTLs	Starch content	[51]
Agronomic Traits					
SSRs	$F_{2:3}$ population	X178 × B73	7 QTLs under non-stress and 7 under stress conditions	Grain yield, 100-kernel weight, kernel number per ear, cob weight per ear, kernel weight per ear, ear weight, and ear number per plant	[52]
SSRs	F_2	LH200 ×LH216	28 QTLs	Grain yield	53
SSRs	200 $F_{2:4}$ lines	cross R15 × 478	14 distinct QTLs	Plant and ear height	[54]
RFLPs and SSRs	236 RILs	CML444 × SC-Malawi	223 QTLs	Male flowering, ASI, grain yield, kernel number, 100-kernel fresh weight, and plant height	[55]
SSRs	F_2 (203)	KCB × GBK 032357	Four candidate markers (*p-umc2189, p-bnlg1179, p-bnlg1014 and p-umc1542*)	Female flowering time, ASI, Kernel number, and grain yield	[56]
SSRs and EST	$F_{2:3}$ (247)	HZ32 × K12.	Six QTLs (*ph6-1, sdw4-1, sdw7-1, tdw4-1, tdw7-1 and rl1-2*)	Plant height, shoot and root dry weight, total dry weight, and root length	[57]
SSRs	F_2	02S6140 × KSS22	14 QTLs	Yield and agronomic traits	[58]

InDel and SNP	71 European inbred lines	Lines from 4 pools: flint/Lancaster (L), flint (F), iodent and iodent/ stiff stalk (I), and Stiff stalk (S)	*Dwarf8* gene	Flowering time	[59]
SSRs	161 DH lines	Zheng58 (Z58) × Chang7-2 (C7-2)	49 QTLs	Ear length, Ear diameter, Ear row number, Kernel number per row 100-kernel weight Grain weight per plant	[60]
SNPs	199 F_2 and its $F_{2:3}$ lines	SG5/SG7	10 QTLs (five QTLs each for kernel length and width)	Kernel size	[61]

Table 1: Detailed information on mapped QTLs for various abiotic and biotic stresses, quality, and agronomic traits using different types of markers.

Molecular marker system

Molecular markers are considered to be identifiable sequences (landmarks), found at specific locations in the genome and transmitted by the standard laws of inheritance from one generation to the next. These should not be considered as normal genes as they usually do not have any biological effect and they rely on a DNA assay, in contrast to morphological and biochemical markers that are based on visible traits and proteins produced by genes, respectively. Molecular markers have been classified based on technique and principle involved into three different categories *viz.* A) Hybridization-based markers namely restriction fragment length polymorphism (RFLP), B) Polymerase chain reaction (PCR)-based markers like random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR), *etc*. and C) Sequence-based markers *viz.*, single nucleotide polymorphism (SNP) [66]. The desirable properties of molecular markers are that they should be polymorphic so that able to differentiate among the genotypes in a population of parents. Also, properties such as abundance in the genome, neutrality in nature, easyaccessibility, and highly reproducibility make an ideal marker.

RFLP was the first-generation hybridization-based (restriction enzyme digestion) molecular marker system used for the detection of DNA polymorphism r [67]. RAPD marker is PCR based (DNA segment amplification in PCR by using short oligonucleotides of 4-10 bp) marker [68,69]. In comparison to RFLP, it is a simple, quick, and cost-effective method to detect polymorphism based on presence or absence of band but with the limitations of dominant nature and low reproducibility [69-71]. In 1995, Vos., *et al.* [72] developed an amalgam of RAPD and RFLP technology known as AFLP for DNA fingerprinting through combining the techniques of selective PCR amplification of restriction and fragments restriction digestion. The SSRs also known as microsatellites, tandem arrangement of 10-100 repeated sequences of di, tri, and tetra nucleotides [73,74], and due to their co-dominant nature, these have become the markers of choice for germplasm characterization [75,76]. The advantage of SSRs is that different repeat numbers can serve as separate "alleles" and the site can be recognized as a highly polymorphic site with an opportunity to detect multiple alleles (variations) in populations [77]. However, there has been a gradual shift from the use of PCR-based markers to SNPs owing to the availability of cheaper sequencing platforms.

Molecular markers serve as an effective toolbox to explore the plant genome, *viz.*, genetic variability, DNA profiling, comparative mapping, gene mapping, the evolution of the genome, population genetics, diagnostics, *etc*. The next-generation sequencing (NGS) platforms in combination with restriction site associated DNA (RAD)-tag sequencing are now assisting the modern breeding strategies like Genome-wide selection (GWS) and Genome-wide association studies (GWAS) by providing highly dense genomic markers [78,79]. In addition, these play a vital role in the distinctness, uniformity, and stability (DUS) characterization [60].

Application of molecular markers in maize improvement

In maize, numerous DNA markers have been identified through the advanced genome analysis, *viz*., thousands of SSRs, SNPs,

insertion-deletion (InDel) markers and successful utilization of them can be cited from the cloning and characterization of genes for regulation of plant development, resistance to biotic and abiotic stresses and quality attributes in maize [80]. It also helps to dissect polygenic traits into their responsible QTLs along with the understanding of gene action and inheritance patterns [81].

DNA profiling of maize germplasm for analyzing genetic variability

The method to identify an individual's DNA features, which are as distinctive as fingerprints, is DNA profiling (also called DNA fingerprinting). The impact of studying the genetic variations through molecular markers is stable [82] at different growth stages and not confused with environmental, pleiotropic, and epistatic as phenotypic level. RFLPs were first used in phylogenetic analysis and diversity studies between and within species, due to their high abundance throughout the genome, hybridization, and introgression studies [83]. A set of 46 RFLP markers were used to cluster all the inbred lines into the two major heterotic groups and further into subgroups within the major heterotic groups [84]. Another group analyze the 145 released maize inbreds in France and concluded that RFLP markers could serve as tools to discriminate between closely related individuals from different breeding sources [85] Dillman. The RAPDs markers have been widely used in diverse plant species for assessment of genetic variation in populations and species, fingerprinting, and study of phylogenetic relationships among species and subspecies [86]. AFLP markers have vast applications in fingerprinting, gene identity, phylogenetic studies, identification of clones and cultivars [72]. This technique differentiates the individuals at the subspecies level [87]and is also used to explore the genetic similarity among different accessions of maize [88,89].

SSRs markers have been utilized to a greater extent for diversity assessment and DNA profiling of maize germplasm globally [80,90]. Twenty EST-SSRs used to conduct the diversity assessment in 80 progenies (40 each of Piranão and CIMMYT population) of Full Sib Reciprocal Recurrent Selection exhibited relatively greater diversity for the Piranão population. The study concluded that genetically diverse populations can significantly contribute to high genetic gains and hence generating promising high-yielding hybrids [91]. Genetic diversity study performed on 108 Benin's maize accessions using three SSR markers grouped the accessions into four clusters related to the regions of collection [92]. Microsatellite markers have been also utilized for evolutionary studies in maize [5,93]. Matsuokas they tend to show extensive variations owing to repeated occurrence in the genome [77]. SSRs were used to assess the genetic diversity for the yield and quality traits [94]. The SNPs are the most abundant molecular markers throughout the genome [87,95]. The development of several high throughput genotyping technologies boosted the adoption of SNPs for diversity studies in wheat [96]and maize [68,79,97,98]. SNP markers are likely to become the marker of choice for breeding in the near future, especially as the full sequences of more plant genomes will become available with the advantage of NGS technologies [99]. However,[98] genotyped 1,537 elite maize inbred lines with 359 SSRs and 8,244 SNPs and proposed that around 7 to 11 times more SNPs should be used for analyzing population structure and genetic diversity as compared to SSRs.

Genetic analysis of traits

The process of constructing linkage maps followed by the QTL analysis *i.e.*, to identify genomic regions associated with traits, is known as QTL mapping. It is based on linkage disequilibrium in segregating populations and involves testing DNA markers throughout the genome for their likelihood of association with QTL. Various genes/loci of several important traits have been successfully tagged using different molecular markers (Table 1). Initially, RFLPs were used in gene mapping and introgression studies in maize [83,80,100] firstly reported QTLs for yield-related attributes in maize. The major factor responsible for yield loss in maize is biotic stresses generally caused by diseases and insect pests [101]. Northern Corn Leaf Blight (NCLB), Turcicum Leaf Blight (TLB), Banded leaf and sheath blight (BLSB), Downy Mildew, Maize Streak Virus (MSV), and aflatoxin contamination are the most common diseases of maize. Maize is also plagued by pests, including European, Mediterranean, and tropical maize borers, as well as pest weevil storage [102]. Several QTLs for downy mildew resistance were mapped on chromosome 6 using recombinant inbred lines (RILs) derived from the cross of Ki3 (resistant) and CML139 [103]. Later under a multi-institutional project, Asian Maize Biotechnology Network (AMBIONET), different downy mildew reactions were tested in RILs evaluated at Mandya (Southern India); at Farm Suwan (Thailand) for sorghum downy mildew; at Maros (Indonesia) for Java downy mildew; at Udaipur

(Western India) for Rajasthan downy mildew and southern Mindanao (Philippines) for Philippine downy mildew. This study reported the presence of tight linkage between *umc11*, *umc23a*, and *umc113* SSR markers and QTL on chromosome 6 for efficient use in MAS studies [104,105] developed a backcross population by crossing NAI116 (resistant to sorghum downy mildew) with CM139 and identified the QTLs for sorghum downy mildew resistance on chromosomes 3 and 6. [40]mapped 3 putative QTLs for sorghum downy mildew (SDM) resistance on chromosomes 2, 3, and 6 in maize.[106] screened a total of 115 RILs for mapping QTL related to MSV disease resistance using 52 SSR markers and identified three SSR primers; *bnlg1811, umc1917,* and *umc1144* on chromosome 1 which could help to differentiate resistant lines from susceptible lines. In China, [107] reported 4 stable QTLs on chromosomes 2, 6, and 10 out of a total of 11 QTLs mapped in the F_2 population of R15 (resistant) × 478 (susceptible) for BLSB resistance in maize. Similarly, [35] identified three QTLs in the $F_{2:3}$ population for BLSB resistance on chromosomes 6, 8, and 9. Recently, [44,45] mapped QTLs using DArT and SNP markers for MSV and NCLB resistance, respectively.

Abiotic stresses such as moisture stress, high and low-temperature stress, salinity; nutrient stress, *etc*. frequently limit the growth and productivity of major crop species such as maize. The reproductive stage is the most sensitive affecting maize production during drought stress. Several QTLs related to a low anthesis-silking interval (ASI) were incorporated in CML247 from drought-tolerant line Ac7643 [108,109] mapped a QTL, *Root-ABA1* for abscisic acid levels in leaf and associated with root development under drought stress in maize. Later, [39] 2009b mapped QTLs for drought tolerance associated traits on chromosomes 1, 2, 8, and 10. Major QTLs for ASI and number of ears/plant under drought stress mapped on chromosomes 1 and 9 in X178 × B73 based $F_{2:3}$ mapping population [5,20,110] conducted a meta-QTL (m-QTL) analysis using three bi-parental tropical populations (CML444 × MALAWI; CML440 × CML504; CML444 × CML441) and reported 7 and 1 genomic regions for grain yield (GY) and ASI, respectively. Among these m-QTL for GY and ASI on chromosomes 7 and 3, respectively, were found to be adaptable to drought stress and therefore can be efficiently used in the MARS programme [110]. Significant QTLs for adventitious root formation in progenies of B64 and Teosinte accession of *Zea mays* ssp. *huehuetenangensis* conferring water-logging tolerance have been mapped on chromosomes 3, 7, and 8. Similarly, QTLs for adventitious root formation mapped on chromosomes 1, 5, and 8 in a population derived from the cross of teosinte (*Zea mays* spp. *nicaraguensis*) with B73 and evaluated under waterlogging conditions [24,26]. During the waterlogging period at the seedling stage of 0 to 3 days, 3 to 6 days, and 6 to 9 days, 6 QTLs (*ph6-1*, *sdw4-1*, *sdw7-1*, *tdw4-1*, *tdw7-1,* and *rl1-2*) were reported to be associated with plant height, shoot and root dry weight, total dry weight and root length [57]. The recent past has witnessed the prioritized use of SNPs for QTL mapping for abiotic stress [31,32,34], quality traits [50,51], and agronomic traits [60,61]. Hence, the identified QTLs can serve as a potential source to develop abiotic stress-resilient maize cultivars globally [64,111].

Marker assisted selection

The selection or identification of desirable plants (for the specific trait) possessing a specific gene of interest *via* linked marker is known as marker-assisted selection (MAS). Therefore, indirect selection for the desired trait through MAS accelerates the breeding process and therefore leading to the rapid development of improved cultivars. MAS has great advantages as it leads to the culling of unwanted plants in early generations and thereby retaining a lesser number of high-priority lines in subsequent generations. The greatest efficiency of MAS is exhibited in early generations particularly in the case of loose linkage between the marker and the selected QTL as it leads to an increased probability of recombination between the marker and QTL. The major disadvantage of applying MAS at early generations is the cost of genotyping a larger number of plants which can be overcome by the use of co-dominant DNA markers to fix specific alleles in their homozygous state as early as the F_2 generation. In maize, MAS was first used for the conversion of normal lines to lysine and tryptophan-rich Quality Protein Maize (QPM) using opaque 2-specific SSR markers [112]. The "Vivek QPM hybrid 9" was the first MAS-based product in maize developed by transferring the *O2* gene [113]. The selection through marker system can be performed at the early seedling stage irrespective of genotype × environment (G × E) interactions. Assembling the desired genes into a single parent from multiple parents is known as Marker-assisted gene pyramiding [62]. MAS technique can be further divided as Marker-assisted backcross breeding (MABC), Marker-assisted recurrent selection (MARS), and Genomic Selection (GS).

Marker-Assisted Backcrossing (MABC)

The transfer of one or more genes to an elite cultivar that is deficient in few traits is termed marker-assisted backcrossing (MABC). Foreground selection, recombinant selection, and background selection are key steps of MABC [114]. Initially, "foreground selection" involves the use of markers to screen the target/desired gene in the individual plants at the early seedling stage [62,115]. Secondly, selection of backcross (BC) progeny possessing target gene and recombinant events between target loci and linked flanking markers is performed under "recombinant selection" to reduce the linkage drag [116]. Minimum two BC generations are used because of rare chances of double recombination to occur on both sides of target loci [117]. Lastly, "background selection" is used to select for the progeny with maximum recovery of the recurrent parent (RP) genome using the unlinked genomic markers (to target locus), helping to save time relative to conventional breeding [115,118]. A successful example of MABC in maize breeding can be cited from the development of Vivek QPM 9 through introgression of *opaque 2* from CML176 (donor parent) to V25, high yielding and extra-early maturing normal maize line [112]. Later, [108] developed a drought-tolerant line by crossing the tropical inbred line CML247 (susceptible) and Ac7643 (tolerant line). Later, maize breeders at IARI (Indian Agricultural Research Institute), New Delhi executed the gene pyramiding in five elite lines (CM137, CM138, CM139, CM150, and CM151) to develop TLB (*Htn1*and *Ht2* genes) and Polysora rust resistance (*RppQ* QTL) lines using four resistant donors *viz.*, NAI147, SKV21, NAI112 and SKV18 [119]. Later, the same approach was used to develop CM139 (sorghum downy mildew resistant line) and QTL-NILs [62,120]. APQH9, an EDV of Vivek QPM 9 possessing higher Provitamin A has been developed through introgression of *crtRB1* in the parental lines of Vivek QPM 9 [11,121]. Further efforts have been made on gene pyramiding for quality improvement in maize [122,123]. The MABC limitations lie in its use for improving only a single trait with qualitative nature [124]. The basic strategy of MABC for the transfer of recessive genes has been illustrated in figure 1.

Figure 1: Marker assisted back-crossing (MABC) scheme for transferring target gene from donor parent to recipient parent.

Marker-assisted recurrent selection (MARS)

MARS involves the repeated selection and accumulation of numerous loci/genes governing complex traits to develop elite cultivars. MARS is just an extension of phenotypic recurrent selection focusing on the use of molecular markers for increasing the frequency of favorable alleles within the population. This approach is used to combine several gene/QTL into a single individual/genotype within-population for complex traits like drought tolerance, disease resistance, and yield in maize resilience breeding program population [125]. It involves the genotyping of the F_2 or F_3 population (use of markers linked to minor or major QTLs) and phenotyping of F_2 derived F_4 or F_5 individuals, followed by the use of recombination cycles to evaluate the marker effects [126]. Initially, identification of QTLs in the population is performed followed by pyramiding of superior alleles in the population by crossing lines having superior alleles (favorable QTLs). Later, RILs selected based on accurate phenotypic screening in multi-location field trials are released as varieties [127]. MARS has been exploited in maize to build up stable disease tolerant lines possessing a combination of various genes and therefore conferring tolerance to multiple races of a pathogen [62]. MARS has been used to confer early flowering in agronomically superior but late flowering NSE331 line by crossing with NSE626 (flowers 8 to 10 days earlier) as donor parent [128]. CIMMYT also utilized this approach to develop 10 populations based on SNP-based recurrent selection of crosses between commercial lines CML and drought-

tolerant donor lines CZL or VL [124]. MARS is more advantageous to MABC as it allows capturing of whole genomic regions having minor and major QTLs [127]. A generalized approach depicting the basic steps of MARS is represented in figure 2.

Figure 2: Marker assisted recurrent selection (MARS) scheme for accumulation of favorable QTLs in a population.

Genomic selection

Genomic selection can be defined as the simultaneous selection of densely located whole genomic markers (tens or hundreds or thousands) with anticipation that all genes are in linkage disequilibrium with at least some markers [129]. This strategy is still in the embryonic stage in terms of its use in plants as compared to the rest of the marker-assisted approaches [126,130,131]. This approach involves the prediction of complex traits by using genetic markers covering the entire genome and desirable individuals can be selected based on markers derived genomic estimated breeding value (GEBV). It differs from QTL mapping and is based on the development of the GS model for the estimation of GEBV [129,132]. Initially, a training population is used to train the model (associating the relationship between genotype and phenotype) and estimate GEBVs which are further utilized to select desirable individuals in the breeding program. GS was first demonstrated in maize, barley, and Arabidopsis [133] after witnessing better accuracy as compared to pedigree information alone [134]. The GS scheme has the potential to efficiently utilize off-season nursery and greenhouse facilities and thereby hastening the breeding program for complex traits through efficient selection [135]. NCLB resistance in maize was predicted using a genomic selection approach relying upon extensive phenotyping of 100 dent and 97 flint lines for NCLB resistance and high-density SNP markers genotyping [136]. Furthermore, rapid-cycle GS is an effective strategy for achieving higher genetic gains for abiotic stress tolerance in maize [137].

Heterotic grouping and DUS characterization

The process by which the diverse inbred lines are classified into various groups for efficient selection of best parents in breeding programs is known as heterotic grouping [138-140] used AFLP and SSR markers to group 40 diverse inbred lines into two groups based on genetic dissimilarity. With the advent of cheaper sequencing technologies, SNP has taken over SSR for heterotic grouping. [141] grouped 450 maize inbred lines using SNP markers into 3 major groups. Recently, [142] also used the SNP markers for heterotic grouping of tropical maize inbred lines. Molecular markers are better alternatives to handle the DUS characterization, an essential criterion for safeguarding the new varieties, and hence it is likely to witness the adoption of a robust genomics-based registration system for new maize varieties [60].

Positional cloning

Positional cloning refers to gene identification where a gene for a specific phenotype is identified only by its approximate chromosomal location. It is very difficult to do positional cloning in maize as its genome contains a huge amount of repetitive DNA. The successful earliest example of positional cloning can be cited from mapping of a QTL for the difference between maize and teosinte to *teosinte branched* 1 gene-containing region. Later various genes such as *vgt1*(vegetative to generative transition 1), *ramosa1* (*ra1*), *lax panicle*, *clavate1* (*clv1*), *ra2, ra3,* and *ts4* have been cloned to different chromosomal regions [144-146]. There are various useful resources for positional cloning in maize *viz.* Maize GDB, Arizona Genomics Institute, Gramene, MAGI, TIGR Rice Genome Annotation, TIGR Maize Database, TIGR Maize Gene Index, TIGR Maize Genomic Blast Search, TIGR Maize Marker Mapping, Maize Genetic Mapping Project, Maize Mapping Project, and Maize Seq [147].

Conclusion and Future Prospects

Molecular markers are always advantageous to morpho-biochemical markers due to their efficient application through better collaborations between breeders and biotechnologists. Different strategies of molecular breeding *viz.*, MAS, MABC, MARS, and GS enabled maize breeders to develop climate and disease pest-resilient elite maize cultivars. But the higher cost of technical equipment in molecular breeding remains the major hindrance to applied at a large scale for developing countries. For example, in developing countries like India, the lack of facilities and the high

cost of molecular marker platform technologies limited its efficient use for maize improvement. However, the last decade witnessed improvement in the infrastructure development and reduced cost of sequencing owing to the development of high throughput sequencing platforms. But still, there is a need to formulate appropriate research policies and promote public-private partnership models to harness the results from complementation of molecular markers and conventional breeding. The availability of reference genome in maize enabled the development of target trait-specific diagnostic markers in the recent past. However, success with molecular markers is limited mostly to qualitative traits but the use of abundant SNP markers can surely help to improve the complex quantitative traits too. The complex traits like abiotic and biotic stresses would likely witness the use of markers for the memory stress-based breeding in maize [148,149].

Molecular markers hold immense importance for yield improvement in maize. The pyramiding of the yield related QTLs via selection through linked markers will help to augment the grain yield in maize. The higher genetic gains in yield is also likely to be achieved in near future through MARS and GS. The use of high density SNP markers can be vital in genomic prediction of hybrid performance through selective crosses among superior inbred lines and hence leading to superior heterotic hybrids. The identification of favourable haplotypes for grain yield will strengthen the haplotype based breeding in maize. Therefore, the use of molecular markers is likely to boost maize productivity to fulfill the food requirement of an ever-growing population.

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