Molecular Breeding in Maize Improvement

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A B S T R A C T

From the initial stages of maize improvement, several techniques are being used and developed to overcome the challenge of nutritional food supply. The discoveries from the era of Darwin and Mendel genetics for the evolutionary changes in the crops have guided the development of molecular breeding. Molecular breeding deals with the manipulation of genes, employing different techniques like Quantitative trait loci (QTL) mapping, marker assisted selections, genetic transformations, etc. The foundation of molecular breeding has revolutionized the crop improvement by providing a better understanding about the genetic makeup of desired traits, thereby supplementing the conventional methods to a greater extent. Use of molecular markers, genomic selection (GS), gene discovery and genetic transformations have now become a topic of keen interest for the plant breeders and the crop scientists. The development of varieties with the help of marker-assisted selection (MAS) along with the conventional methods of plant breeding makes us capable to promote the desired gene pool of the cultivars and enhances their agronomic, nutritional and genotypic characters. These genomic selection leads to rapid and lower cost gains in breeding. Genetic transformation is another approach of molecular breeding to transfer desired genes in plants for making them resistant to biotic and abiotic stress, hence increasing the overall crop advancement. Molecular approach of breeding is holding greater importance for the developing countries after accounting successful results in developed ones with similar procedure in its application and use. Some QPM hybrids and resistant cultivars of maize like CM-137, CM-138, CM-139, CM-140, *etc.* are derived by the use of specific molecular markers, genomic selection of parents and recombination, however, these improved cultivars accounts very less in number.

Introduction

Maize (*Zea mays* L., 2n=20) domains a unique rank in agriculture owing to its varied uses *viz.*, human consumption, poultry feed, green fodder, value added products and industrial usage. It ranks third in staple crops after wheat and rice. These three crops together provides nearly 30% of the food calories to billions of people worldwide. Its role in human consumption is expressed in terms of calorie share and varies significantly across countries (Shiferaw *et al.,* 2011). Maize possesses the highest genetic potential for production among the cereals with a well-defined genomic system. In India, it covers

9.34 million hectares (ha) area contributing to 25 million tonnes of production with a productivity of 2.6 tonnes/ha. A typical maize kernel contains starch (73%), protein (9%) and oil (4%) (Prasanna *et al.,* 2001). Its oil has a higher Poly Unsaturated Fatty Acids (PUFA) accounting for high nutritional quality. Although the developed nations contribute predominantly to the maize production, still the demand for this is expected to increase in developing nations in coming years (Pingali and Pandey, 2001).

The seven parts of Asian belt *i.e.*, China, India, Indonesia, Nepal, Philippines, Thailand and Vietnam are major producing countries of maize and have shown progress in becoming the net exporters of maize from previously the importing one in the last decade (Gerpacio and Pingali, 2007). Consequently, the demand is expected to increase with the change in diet and other uses in the middle-east countries over the next decades.

Cconventional maize breeding methods like population improvement schemes, inbred development, hybridization and backcross selection has not only directed to improve the quantitative and qualitative characters but also assisted to handle the significant constraints in maize production. Despite the huge advancement in maize breeding till datewith an interestto enhance productivity, agronomic fitness, and adjustment to environmental change now becomes so difficult to continue the agricultural development, improvement in nutritional quality, and also to make sure about harvests.

Molecular breeding techniques like Quantitative trait loci (QTL) mapping, Marker assisted breeding and Genomic selection (GS) speed up this process of crop improvement (Ribaut *et al.,* 2010). Marker assisted breeding further includes Marker assisted Selection (MAS), Marker assisted

backcrossing (MABC) i.e., selection and transfer of alleles in between different genetic backgrounds and Marker assisted recurrent selection (MARS)(Bernardo and Yu, 2007).

In this review, we discuss the application of molecular breeding approaches to improve maize cultivars and considers the path line to integrate the new genomic tools with the basic breeding techniques to combat various challenges that will potentially accelerate genetic gain.

Molecular breeding approaches

Quantitative trait loci (QTL) Mapping

Quantitative trait loci (QTL) mapping is a significant way to deal with the hereditary design underlying heterosis in genes of interest, which distinguishes molecular markers statistically and very significantly associated with heterotic changes in segregating populations (Li *et al.,* 2007; Lu *et al.,* 2010).

Recently, the power of QTL mapping has been improved by utilizing high-density linkage maps and new measurement techniques (Li *et al.,* 2007; Liu *et al.,* 2016b). For instance, an important QTL'qTBN5' for the maize tassel branch number was mapped on chromosome 5.05 in a little physical interim ~800 kb dependent on a linkage map including 6,533 bin markers(Chen *et al.,* 2014).

MAS (Marker Assisted Selection)

MAS (Marker Assisted Selection) include identification of the desirable genes with the use of linked markers for the selection of genotypes. With specific markers, it permits finding of the ideal plant phenotype by analyzing the marker linked DNA banding pattern on a gel or autoradiogram. The pattern

of DNA banding reveals the pedigree of segregants in bands and configures the availability of desired chromosome segment (Jhansi *et al.,* 2014).

MAS is holding significant importance as it helps to enhance the proficiency of maize breeding by inspecting the desirable genes in breeding populations. This can be achieved by transfer and recovery of desired ones and the parental alleles *i.e.,* foreground selection and background selection respectively. This is widely utilized for traits which are governed by single gene i.e., monogenic traits than for traits which are governed by numerous genes comprising small cumulative effects i.e., the polygenic traits.

Marker assisted backcross breeding (MABB)

MABB (Marker assisted backcross breeding) is the least complex type molecular approach of breeding, wherein the objective is consolidating a significant gene from adisease resistant plant (the donor parent) into a susceptible best performing cultivar or breeding line (the recurrent parent).Most of the time the recurrent parent used for backcrossing has almost all the desirable genes, lacking only few characters/traits (Allard, 1999).

DNA markers in backcross breeding i.e., marker-assisted backcrossing (MAB) can be utilized (Holland, 2004) at three levels-

'Foreground selection' includes markers linked with the target genes and substitute screening for the target gene or QTL is done (Hospital and Charcosset, 1997).

'Recombinant selection' includes selection of progenies with target genes after back crossing and events of recombination between the target genes and markers linked to the genes. It decreases the introgression size and 'linkage drag' (Hospital, 2005).

Background selection' includes selection of backcross progenies having the best extent of recurrent parent genome with the help of the markers not linked with the target loci (Hospital and Charcosset, 1997; Frisch *et al.,* 1999).
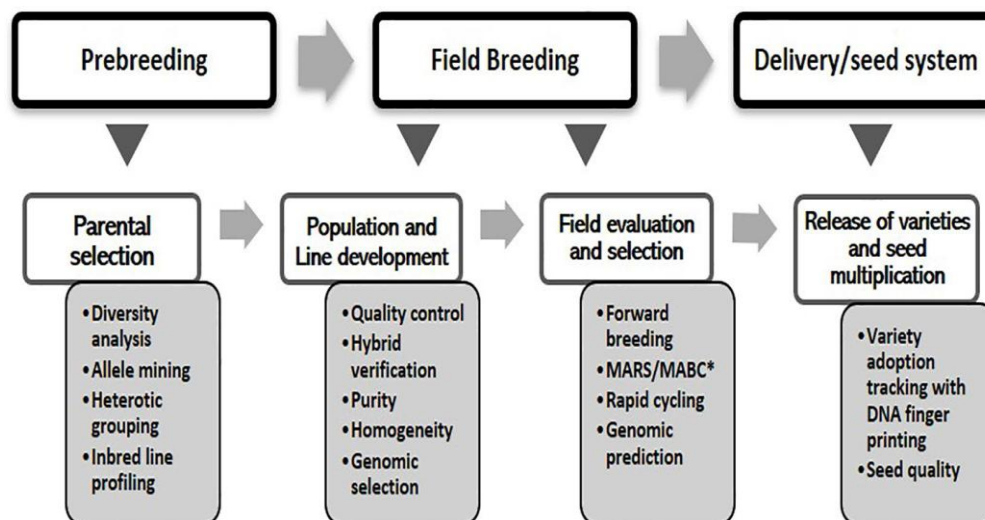
Marker Assisted Backcross Breeding (MABB) permits the selection of desired genotypes at the seedling stage under any environment and effective selection of heterozygous individuals using codominant makers like SSR, SNP.

Marker Assisted Recurrent Selection (MARS)

MARS scheme-allows the selection of multiple genomic regions (within the chromosomes) expressing complex traits using markers in order to identify the best-performing genotypes within a population (Ribaut *et al.,* 2010). MARS permits genotypic selection followed by crossing the selected individuals in one selection cycle (Jiang *et al*., 2007a). Thus MARS can upgrade the proficiency of recurrent selection and accelerates the advancement in the selection process especially assisting to coordinatemultiple favourable genes or loci from various sources (Asima Gazal *et al.,* 2015).

Forward breeding and Pyramiding multiple QTLs of desired genes have been proposed in MARS for traits such as yield, resistance to stress, etc. (Eathington, 2005; Crosbie *et al.,* 2006). Gene mapping is required in MARS to integrate multiple QTLs expressing for the complex traits and are subjected to develop lines with genes of interest following controlled pollination.

Fig.1 General scheme of maize cultivar development

Genomic selection (GS)

Meuwissen (2007) defined genomic selection as synchronous use of markers (10 or 100 or 1000) covering complete dense genome expected to be involved in linkage. To predict the genotypic information of complex traits, genetic markers are used in particular OR to particularly predict the genotypic information of complex traits, genetic makers are used. Desired individuals are selected on the basis of genomic estimated breeding value (GEBV) (Nakaya and Isobe, 2012), calculated by using strategy dependent on genome-wide dense DNA markers (Meuwissen *et al.,* 2001).

Application of molecular markers in maize breeding

International Maize and Wheat Improvement Center (CIMMYT) successfully coordinated the Asian Maize Biotechnology Network (AMBIONET) with the funds provided by the Asian Development Bank (ADB) from 1998 to 2005 in six countries of the Asian belt i.e., China, India, Indonesia, Philippines, Thailand and Vietnam focusing on application of molecular markers in crop improvement program by public sector foundations (Pray

2006).

AMBIONET worked collaboratively on:-

DNA fingerprinting
Analysing the genomic diversification in inbred lines
Allocating heterotic matches to inbred lines
Analysing genes/QTLs resistant to biotic and abiotic stresses and
Implementation of marker assisted approaches in maize breeding program.

Molecular breeding for resilience in maize

Different biotic and abiotic stresses have negative consequences on maize yield (Gerpacio and Pingali, 2007). The molecular breeding approach includes identification of quantitative trait loci (QTLs) and associated markers, introgression in elite lines through marker-assisted backcrossing (MABC). Linkage mapping (Varshney *et al.,* 2007) and association mapping (Gupta *et al.,* 2005) are used to identify the QTLs associated with biotic and abiotic stresses in maize. Association mapping is likely to be favoured over linkage mapping in future in terms of

precise resolution with less expensive approach.

Small and several epistatic QTLs effect are the difficulties in developing superior genotypes for resilience (Messmer *et al.,* 2009). Although, integration of QTLs with MABC has been constrained as a result of the large population size for backcross. To overcome this, marker assisted recurrent selection (MARS) and genomic selection (GS) are used in one genetic background (Varshney *et al.,* 2007).

Breeding achievements

Drought tolerance

MABC was used to incorporate several QTL alleles from Ac7643, a drought tolerant line to CML-247 maize cultivar for shortening the time interval of ASP (Anthesis-Silking period) in elite maize cultivar (Ribaut and Ragot, 2007).

Downy mildew resistance

QTLs were mapped on 6th chromosome RIL set by crossing the resistant line Ki3 with the susceptible CML-139 maize cultivar (George *et al.,* 2003).

In India, QTLs were mapped each on 3rd and 6th chromosome and validated from backcross mapping population obtained by making cross between the resistant NAI-116 cultivar with susceptible CM-139 maize cultivar (Nair *et al.,* 2005) and by using MABC, a resistant line (CM-139) to downy mildew was developed (Prasanna, 2009a).

Polysora Rust (PR) and Turcicum leaf blight (TLF) resistance

CM-137, CM-138, CM-139, CM-140 and *CM-212* lines of maize were validated as PR and TLF resistant by use of MAS at IARI, India (Prasanna *et al.,* 2010a;Prasanna *et al.,* 2009b).

NAI-147, SKV-21, NAI-112 and *SKV-18*lines of maize were validated for TLF resistant genes (*Htn1and Ht2*) along with mapping QTL 'RppQ' for PR resistance in 7 backcross populations.

Banded leaf and sheath blight (BLSB) resistance

Causal organism - *Rhizoctonia solani* Kuhn

In India, 2nd and 3rd segregating populations were mapped to obtain the BLSB- resistant line by using tolerant line *i.e.,* CA00106 and susceptible line *i.e.,* CM-140 in maize (Garg *et al.,* 2009).

Success in advanced molecular breeding

Development of quality protein maize (QPM) with enhanced nutritional value is achieved by utilization of opaque2-specific SSR markers (Prasanna *et al.,* 2001; Morris *et al.*, 2003; Babu *et al.,* 2005).

'Vivek QPM Hybrid 9' is another example, developed by transferring marker linked opaque2 gene into the parental lines *i.e.,* CM-145 and CM-212 and phenotypic screening for the endosperm mmodifiers in Vivek Hybrid 9 (Babu *et al.,* 2005; Gupta *et al.,* 2009) recently released by VPKAS, Almora, India.

In conclusion the application of molecular breeding for maize improvement has significantly increased in recent years and has helped in enhancing the systematic understanding of germplasm, identification of QTLs/genes by high resolution mapping and the introgression of desired alleles in cultivars/varieties/genotypes. Molecular breeding provides an opportunity for plant

breeders to precisely develop superior genotypes with resilience to biotic and abiotic stress in a less time duration as discussed above. Cost and availability of genomic tools are the bottle necks which hinder molecular breeding strategies especially in developing nations. To counter these challenges platforms need to be developed to access genomic tools with public and private support services across the globe .It is important to focus on the constraints in maize improvement and wise use of genomic tools to develop superior cultivars.

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