

## Designing Maize Crop through Biotechnological Interventions: An Overview

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### ABSTRACT

Tools of molecular biology and genetic engineering have provided humankind with unprecedented power to manipulate and develop novel crop genotypes towards a safe and sustainable agriculture in the 21<sup>st</sup> century. Maize (*Zea mays* L.) being an important food, feed and industrially important grain crop has been genetically engineered for agronomically desirable traits i.e. incorporation of genes for protection against insect pests, resistance to herbicides and nutritional enhancement. Since biotechnology deals with specific alterations and modifications of living organisms through novel techniques of tissue culture and genetic engineering, the present review emphasises on the utilization of these techniques for the improvement of maize crop.

**Key words:** Genetic engineering, *Zea mays*, insect and disease resistance, abiotic stress tolerance

### INTRODUCTION

Maize or corn (*Zea mays* L.) is a crop plant belonging to the family of grasses (*Poaceae*). It is cultivated globally, being one of the most important cereal crops. Due to versatile nature of crop it is grown over a range of agro climatic zones making its suitability to diverse environments that is unmatched by any other crop. It is grown from 58°N to 40°S, from below sea level to altitudes higher than 3000 m (amsl), and in areas with 250 mm to more than 5000 mm of rainfall per year (Shaw, 1988; Dowsell *et al.*, 1996) and with a growing cycle ranging from 3 to 13 months (CIMMYT, 2000). However, the major maize production areas are located in temperate regions of the globe. The United States, China, Brazil and Mexico account for 70% of global maize production while India has 5% of corn acreage and contributes 2% of world production only.

Maize is the third most important food grain in India after wheat and rice. The major maize growing states are Uttar Pradesh, Bihar, Rajasthan, Madhya

Pradesh, Punjab, Andhra Pradesh, Himachal Pradesh, West Bengal, Karnataka and Jammu and Kashmir, jointly accounting for over 95% of the national maize production (Anonymous, 2009). In India, about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (for example starch and oil production) and 1% as seed (AICRP, 2007). In the last one decade, it has registered the highest growth rate among all food grains including wheat and rice because of newly emerging food habits as well as enhanced industrial requirements.

With the advent of plant biotechnology and molecular biology, scientists can take advantage of genes that are derived from various sources, including related and unrelated species, those identified via genetic mapping experiments and most recently from the efforts of functional genomics (the area aimed at understanding the function of all genes in an organism) (Sofi *et al.*, 2007). By application of molecular genetics and genetic engineering coupled with conventional plant breeding approaches, these genes can be efficiently incorporated into modern plant varieties to make them more sustainable and profitable for resource poor farmers. Use of biotechnology during the last one and half decade has emerged as one of the important tools to alter the genetic architecture of maize plant for increasing its productivity by controlling the major insect pests as well as herbicide tolerance (James, 2009). Besides, various other traits such as nutritional enhancement (high lysine content, amylase enzyme, phytase enzyme), drought tolerance etc. are also being explored and incorporated in maize (Stein and Rodriguez-Cerezo, 2010) to make it commercial and environment friendly. Two main biotechnological interventions (molecular markers and genetic transformation) have been successfully exploited to a great extent for the improvement of maize crop and an attempt has been made to review some of these salient details in subsequent section of this article.

## **A. ROLE OF MOLECULAR MARKERS IN MAIZE IMPROVEMENT**

Molecular genetics, or the use of molecular techniques for detecting differences in the DNA of individual plants, has many applications of value to crop improvement. Such Molecular markers, when very tightly linked to genes of interest, can be used to indirectly select for the desirable allele, and represents the simplest form of marker-assisted selection (MAS), whether used to accelerate the backcrossing of such an allele or in pyramiding several desirable alleles. Markers can also be used to dissect polygenic traits into their Mendelian components or quantitative trait loci (QTL), thus increasing our understanding of the inheritance and gene action for such traits, and allowing us to use MAS as a

complement to conventional selection procedures.

The Asian Maize Biotechnology Network (AMBIONET), which was coordinated by CIMMYT and financially supported by the Asian Development Bank (ADB) during 1998–2005 in six Asian countries (China, India, Indonesia, Philippines, Thailand and Vietnam), provided an impetus for application of molecular markers for maize improvement by public sector institutions in Asia. AMBIONET helped to strengthen the capacity of partner institutions, and aided in undertaking collaborative research on (i) DNA fingerprinting and analysis of genetic diversity in important inbred lines; (ii) assigning inbred lines to heterotic groups; (iii) QTL analyses of some important biotic and abiotic stresses; and (iv) initiating molecular MAS projects. Several AMBIONET partner institutions intensified their MAS breeding efforts in maize by attracting funding from their host organizations as well as through external funding (Pray, 2006). We shall highlight here salient applications of molecular markers in the maize genetics and breeding programmes.

### **1. DNA fingerprinting and genetic diversity analysis of inbred lines**

DNA fingerprinting and genetic diversity analysis using molecular markers is useful in effective management of germplasm collections and breeding materials. Accurate assessment of the levels and patterns of genetic diversity using molecular markers is particularly helpful in maize breeding for (i) maintenance and broadening of the genetic base of the elite germplasm, (ii) assignment of lines to heterotic groups; (iii) selection of appropriate parental lines for hybrid combinations; and (iv) generation of segregating progenies with maximum genetic variability for further selection (Mohammadi and Prasanna, 2003). SSR markers have been successfully used for DNA fingerprinting and analysis of genetic diversity in China (Xie *et al.*, 2008), India (Mohammadi *et al.*, 2008), Indonesia (Pabendon *et al.*, 2007) and Thailand (Phumichai *et al.*, 2008).

Marker studies suggest that there is much more diversity in tropical than temperate lines (Tarter *et al.*, 2004). Many useful alleles for improving temperate maize may be hidden in the tropical germplasm and should be uncovered for continued future improvement. For example, a recent study identified a gene, lycopene epsilon cyclase (LCYE), related with provitamin A content in the maize kernel. The diversity of alleles of this gene was investigated using an allele mining strategy, demonstrating that the favourable allele is more common in the tropical lines (Harjes *et al.*, 2008). However, the favourable allele for CrtR-B1 gene, another important gene

influencing provitamin A content, is more common in the temperate germplasm (Yan *et al.*, 2009). Thus, the study also indicates the value of judiciously using tropical germplasm in temperate maize breeding programmes and vice versa.

While significant diversity exists in the maize genotypes of different Asian countries, molecular characterization of the Asian maize inbred lines revealed a relatively narrow genetic base for the CIMMYT lines developed for the Asian region (George *et al.*, 2004). In contrast to the case in southern China where 95% of the lines clustered separately from the CIMMYT lines, lines in the Indonesian breeding programme showed a closer relationship with the CIMMYT lines, reflecting a long history of germplasm exchange. Core collections of inbred lines and landraces in the Gene Bank have been formulated based on geographic origin, phenotypic data and molecular analyses in China (Wang *et al.*, 2008) and in India (Prasanna, 2009).

Molecular markers can also play an important role in plant varietal protection, specifically as tools to distinguish an EDV (Essentially Derived Variety) from an initial (protected) variety, as these markers allow tracing of chromosomal segments from the parent to their progeny and make possible empirical evaluation of genetic relatedness. In addition, DNA fingerprinting (and thus, distinguishing) open-pollinated varieties (OPVs) is possible using SSR markers based on a population bulk DNA fingerprinting technique developed at CIMMYT.

## 2. Assigning inbred lines to heterotic groups

The utility of SSRs for assigning lines to heterotic groups and relating the SSR-based genetic distance with hybrid yield or heterotic performance in maize has been explored by a few research groups in Asia (Xu *et al.*, 2005; Mohammadi *et al.*, 2008; Xie *et al.*, 2008). Based on the planting areas of hybrids in 1992–2001 in China, Teng *et al.* (2004) selected 84 parent lines of 71 widely used hybrids and analysed their heterotic groups and patterns using SSR data. The study led to identification of seven heterotic groups, and also indicated that to a certain extent, a change of position for major heterotic groups of maize took place during the past decade in China. The major heterotic groups were Lancaster, Reid, TangSPT, Zi330 and E28 in the early 1990s, while they were Reid, Tem-tropic I, M30, TangSPT and Lancaster in the early twenty-first century. Temtropic I was a new heterotic group of elite germplasm used widely in China, which contained tropical maize germplasm. In another study, 187 commonly used maize inbreds in

China were defined into six subpopulations, namely PA, BSSS (includes Reid), PB, Lan (Lancaster Sure Crop), LRC (Luda Reb Cob, a Chinese landrace, and its derivatives), and SPT (Siping-tou, a Chinese landrace and its derivatives), based on polymorphic data from 70 loci. Forty of the 187 lines, which formerly had unclear and/or miscellaneous pedigree records, were assigned to one of the six groups inferred via structure analysis (Xie *et al.*, 2008).

The genetic diversity grouping data of the Asian maize inbreds could provide valuable information for improving the efficiency of hybrid breeding. Breeding lines developed from natural populations or non-hybrid material or those lines derived from pools or breeding materials with no clear information about their constitution of germplasm, may not separate into clear-cut heterotic groups. This could be one of the major reasons why many of the tropical maize lines could not be delineated to distinct heterotic groups. A situation was also observed in a regional diversity study of representative inbred lines from several Asian countries (George *et al.*, 2004). Studies using more informative and functional markers based on polymorphic sites in a large number of agronomically important/yield-related genes based on modern genotyping approaches and genomic information may help to define heterotic groups where it has been difficult to do so.

### **3. Molecular diversity in landraces/populations**

Although maize hybrids represent the most economically important portion of the species, breeding populations, open pollinated varieties (OPVs), landraces, and wild relatives contain the majority of the allelic diversity, much of which has never been incorporated into improved maize cultivars. The vast genetic resources available in the North Eastern Himalayan (NEH) region as well as other regions in India are interesting from both breeding and evolutionary viewpoints (Prasanna and Sharma, 2005); however, limited efforts have been made to characterize and use these important genetic resources in maize breeding programmes. Using the 'population bulk DNA fingerprinting' strategy, nearly 250 selected maize landraces in India have been characterized using 42 SSR markers. The study revealed significant intra-population and inter-population diversity in the Indian maize landraces, especially those from the NEH region, and highlighted the genetic distinctiveness of 'Sikkim Primitives' (a landrace with high prolificacy) from the rest of the accessions.

#### 4. QTL analysis in Maize

Following the first report on QTLs for yield-related traits in maize (Stuber *et al.*, 1987), maize researchers worldwide have generated numerous reports of molecular markers tagging genes/QTLs for diverse traits of agronomic and scientific interest. QTLs for several important traits affecting maize in Asia have also been mapped, particularly in China and India. These traits include plant height (Zhang *et al.*, 2007), downy mildew resistance (Sabry *et al.*, 2006), MDMV (Maize Dwarf Mosaic Virus) resistance (Liu *et al.*, 2006), common smut resistance (Ding *et al.*, 2008), head smut resistance (Li *et al.*, 2008), *Fusarium moniliforme* ear rot resistance (Zhang *et al.*, 2006), Banded leaf and sheath blight (BLSB) resistance (Garg *et al.*, 2009), drought stress tolerance (Prasanna, 2009), water logging tolerance (Qiu *et al.*, 2007), nutrient components under low nitrogen stress (Liu *et al.*, 2008), high-oil content (Song *et al.*, 2004), popping ability (Babu *et al.*, 2005), and CMS-S (Tie *et al.*, 2006). Such studies have contributed to a greater understanding of the genetic architecture of various traits in maize.

##### 4.1 Downy mildew resistance

A major emphasis in the Asian maize breeding programmes has been the improvement for resistance to downy mildews, specifically *Peronosclerospora sorghi* (sorghum downy mildew; SDM) and *P. heteropogoni* (Rajasthan downy mildew; RDM) in India, *P. maydis* (Java downy mildew) in Indonesia, *P. zea* in Thailand and *P. philippinensis* in the Philippines. Using a set of RILs derived using Ki3 (downy mildew-resistant) and CML139 (downy mildew-susceptible) as parental lines, QTLs conferring resistance to five different downy mildews in tropical Asia, including SDM and RDM in India, Philippine downy mildew in the Philippines, Java downy mildew in Indonesia, and *P. zea* in Thailand were mapped through a collaborative study within the AMBIONET project (George *et al.*, 2004). The study identified QTLs with significant effects for resistance to the five important downy mildews of maize in Asian; of particular significance was a QTL on chr. 6 (bin 6.05) that influenced resistance to all five downy mildews and accounted for nearly 20 and 31% of the phenotypic variance for *P. sorghi* (SDM) and *P. heteropogoni* (RDM) disease resistance, respectively. Two major QTLs (one each on Chr. 6 and Chr. 3) were further validated in India using a backcross population derived from CM139 (SDM-susceptible) and NAI116 (SDM-resistant) (Nair *et al.*, 2005).

#### 4.2 BLSB resistance

The banded leaf and sheath blight (BLSB) disease, caused by *Rhizoctonia solani* in maize, is one of the most destructive and important diseases of maize in South and Southeast Asia. Very few sources of resistance to this disease have been found. In China, a mapping population consisting of 229 F<sub>2</sub> individuals derived from the cross of inbreds R15 (resistant) and 478 (susceptible) were used to map QTLs conferring resistance to BLSB. Of the eleven significant QTLs for resistance, only four (located on chromosomes 2, 6 and 10) were stable across locations, accounting for 3.72–10.35% of the phenotypic variation (Zhao *et al.*, 2006). In another study in India, a F<sub>2:3</sub> mapping population was generated using CA00106 (BLSB-tolerant) and CM140 (BLSB-susceptible). Phenotyping was undertaken using artificial BLSB inoculation at three locations (Delhi, Pantnagar and Udaipur) which are 'hot spots' for the disease. QTL mapping revealed location-specific QTLs for BLSB resistance, with most of the favorable QTL alleles contributed by the resistant parent CA00106. The study also led to identification of three QTLs (on chr. 6, 8 and 9) with significant epistatic interactions (Garg *et al.*, 2009). The studies on BLSB in Asia have so far revealed a high degree of genotype x environment interaction, and complex nature of inheritance of resistance to the disease. It is important to intensify efforts to identify stable and additional sources of resistance to BLSB and improve the disease resistance of present maize hybrids.

#### 4.3 Drought tolerance

Drought is the most important constraint to maize production and breeding for drought tolerance is the topmost research priority for maize in South and Southeast Asia. Maize is particularly sensitive to water deficit stress during the reproductive stages. Substantial progress has been made, particularly by CIMMYT and its collaborators in Africa and Asia, in improving, via selection under managed stress, tolerance to drought at flowering. Simultaneously, advances in genotyping and phenotyping, QTL mapping, and gene expression analyses, has significantly improved the prospects for identifying alleles with major effects on drought tolerance and using them in breeding programmes. QTL mapping experiments on drought stress have been undertaken in China (Xiao *et al.*, 2005; Hao *et al.*, 2008), India (Prasanna, 2009).

QTL mapping for drought tolerance of maize in India identified major QTLs on chr. 1, 2, 8 and 10, based on evaluation of a set of 230 CIMMYT developed RILs at two locations (Hyderabad and Karimnagar) (Prasanna, 2009). Analyses of the RIL datasets identified QTLs influencing specific traits under drought stress that co-localised on chr. 1, 2, 8, and 10. Similarly, analysis of an F2:3 population derived from the cross X178 (a widely planted, drought-tolerant line in China) 9 B73 at different locations in central and southern China (Xiao *et al.*, 2005; Hao *et al.*, 2008) resulted in detection of a major QTL for ASI (anthesis-silking interval) and ear number per plant under drought stress on chr. 1 (bin 1.03) and chr. 9 (bins 9.03–9.05), which correspond to some major QTLs identified in different experiments on drought stress worldwide (Tuberosa *et al.*, 2007). The 'consensus QTLs' for drought tolerance in maize identified through different experiments worldwide, including India and China, using different mapping populations, could serve as good candidates for use in marker-assisted breeding to improve maize production under water-limited conditions.

#### 4.4 Low nitrogen stress tolerance and nitrogen use efficiency

Developing maize varieties with tolerance to low soil nitrogen stress and with high nitrogen use efficiency is gaining importance in Asia. Association analysis in this regard has been recently undertaken in China (Wu *et al.*, 2009; Xie *et al.*, 2008). The natural variations of genes encoding two cytosolic members of the glutamine synthetase gene family, Gln1-3 and Gln1-4, were analyzed in a structured population panel of 187 Chinese maize inbreds with phenotyping tests in two environments each at three locations in 2 years. A list of beneficial haplotypes among the lines was identified through this analysis. The most favourable allele accounted for a phenotypic difference of 42.3% of grain yield under low N versus well-fertilized. In another study in China, Liu *et al.* (2008) identified several QTLs that specifically expressed under different nitrogen conditions and could therefore help understand the genetic basis of nitrogen-use efficiency. Powerful analytical techniques are now available to scan the genome for significant marker-trait associations, to estimate epistatic effects among QTLs, and to study QTL 9 environment interactions. The importance of epistasis and QTL 9 environment effects on trait expression has been demonstrated for plant height (Zhang *et al.*, 2007), common smut



resistance (Ding *et al.*, 2008), drought tolerance (Prasanna, 2009), BLSB resistance (Garg *et al.*, 2009), and other traits.

Meta-analyses to integrate results from QTL experiments undertaken in various environments/ locations assumes importance in understanding the genetic basis of complex traits and devising suitable strategies to utilize the information in breeding programmes. Wang *et al.* (2006) constructed an integrated QTL map, based on 1,201 published maize QTLs affecting 68 traits, and showed that maize QTLs for various traits are clustered in all ten chromosomes. Twenty-two plant height QTLs of maize were co-linear with 64 plant height QTLs of rice, and 43 grain yield QTLs of maize were co-linear with seven grain yield QTLs of rice.

The information on QTLs for resistance to various biotic and abiotic stresses as well as for other agronomically important traits using Asian maize germplasm has steadily increased in the last one decade, mainly using biparental mapping populations. However, many of the QTL-marker associations remain unvalidated, and also as elsewhere, not much translation of this information into products using MAS has taken place. This could be attributed to various reasons, which have been well elaborated by Xu and Crouch (2008). Nevertheless, the information still holds significance, and new possibilities to validate QTLs include corroboration of the results by association mapping studies which some institutions in China and India have initiated, and creation of Near Isogenic Lines (NILs) to test the effect of individual QTLs, which should now be done. From the trait perspective, there is still a need for genetic dissection and QTL analysis of traits, such as nutrient use efficiency, waterlogging tolerance and resistance to post-flowering stalk rots, which are important in several Asian countries.

## 5. MAS for developing improved maize germplasm

Significant progress has been made worldwide in optimizing MAS for improvement of both qualitatively and quantitatively inherited traits using maize as a model system. One successful example of MAS for maize improvement, and of particular use to the developing world, is the utilization of opaque2- specific SSR markers in conversion of maize lines into quality protein maize (QPM) lines with enhanced nutritional quality (Prasanna *et al.*, 2001; Morris *et al.*, 2003; Babu *et al.*, 2005). A MAS-derived QPM hybrid, 'Vivek QPM Hybrid 9' has been recently released by the Vivekananda

Parvatiya Krishi Anusadhan Sansthan (VPKAS) in Almora, India. This QPM hybrid was developed through marker-assisted transfer of the 2 gene and phenotypic selection for endosperm modifiers in the parental lines (CM145 and CM212) of Vivek Hybrid 9 (Babu *et al.*, 2005; Gupta *et al.*, 2008).

The same approach was used to develop QPM versions of several elite, early maturing inbred lines adapted to the hill regions of India (Gupta *et al.*, 2008) and QPM versions of six elite inbred lines (CM137, CM138, CM139, CM150 and CM151), which are the parents of three single-cross hybrids, PEHM2 (CM137  $\times$  CM138), Parkash (CM139  $\times$  CM140) and PEEHM5 (CM150  $\times$  CM151) (Khanduri *et al.*, 2009). Scientists at IARI have pyramided major genes/ QTLs for resistance to turicum leaf blight (*Exserohilum turicum*) and Polysora rust (*Puccinia polysora*) in five elite Indian lines, CM137, CM138, CM139, CM140 and CM212 (Prasanna, 2009). Similar efforts on MAS for generation of QPM lines and transfer of major QTLs for SCMV resistance are being implemented at CAAS, and the MAS products are in pipeline (Shihuang Zhang, personal communication).

Another potential application of MAS in maize could be for improving the provitamin A content of grain. Quantifying the provitamin A carotenoid content of maize samples is difficult, time-consuming and expensive, and breeding programmes will therefore benefit greatly from use of MAS to reduce the need for phenotypic assays. Following the publication of results of association mapping studies (Harjes *et al.*, 2008), sequence-tagged, PCR-based markers were developed and demonstrated for use in selecting favorable alleles of LCYE (lycopene epsilon cyclase), a crucial gene in the carotenoid pathway. More recently, collaborative research between CIMMYT, the University of Illinois, and Cornell University, led to detection of important allelic variation and development of useful markers for favourable alleles of LCYE and another critical gene in the pathway, CrtR-B1 (carotene beta-hydroxylase 1) (Yan *et al.*, 2009). CIMMYT maize breeders are using MAS to develop better source lines by combining favorable alleles for both LCYE and CrtR-B1 in lines with above-average concentrations of provitamin A; however, they are first validating the effect of these two alleles on provitamin A concentrations in various maize populations before investing in widespread use of MAS in breeding programme.

Allele mining and marker development are also underway for other genes of the carotenoid biosynthetic pathway, including PSY (phytoene synthase) and CCD (carotenoid cleavage dioxygenases), giving hope that MAS will soon be possible for several genes which together explain much

of the variation for provitamin A in maize. Despite the complexities of improving polygenic traits through MAS, there have been a few successful examples, including MAS for the improvement of drought tolerance of both tropical inbred lines and populations at CIMMYT (Ribaut and Ragot, 2006).

Recent efforts are focusing on strategies that combine high-density genotyping with index-based selection for drought tolerance. Marker-assisted recurrent selection (MARS) refers to the improvement of an F<sub>2</sub> population by one cycle of marker-assisted selection (i.e., based on phenotypic data and marker scores) followed commonly by two or three cycles of marker-based selection (i.e., based on marker scores only). Bernardo and Charcosset (2006) examined the usefulness of having prior knowledge of QTLs under genetic models that included different numbers of QTLs, different levels of heritability, unequal gene effects, linkage, and epistasis, and concluded that with known QTL, MARS is most beneficial for traits controlled by a moderately large number of QTL (e.g., 40). Bernardo and Yu (2007) further analyzed the prospects for genome-wide selection (GWS) for improving quantitative traits in maize, and concluded that this approach, although more expensive, is superior to MARS for improving complex traits, as GWS effectively avoids issues pertaining to the number of QTL controlling a trait, the distribution of effects of QTL alleles, and epistatic effects due to genetic background.

## 6. Doubled haploid technology and MAS

The use of doubled haploid (DH) techniques to rapidly develop inbred lines is widespread among commercial maize breeding programmes particularly in Europe and USA, and to a limited extent in Asia. Some of the leading public and private institutions in Asia are using or have initiated programmes to develop DH lines in maize using haploidy inducers (Chen and Song, 2003; Zhang *et al.*, 2008), with the exception of Vietnam's National Maize Research Institute (NMRI), which has developed a large number of stable DH lines in different genetic backgrounds using the anther culture technique (Cuong *et al.*, 2007).

Factors making DHs increasingly attractive include the development of better inducer lines, more efficient chromosome doubling methods, and protocols to efficiently introgress transgenes, especially stacked transgenes. Unfortunately, the available inducer lines are of temperate adaptation, so the development of haploidy inducer lines in tropical genetic background, currently ongoing under a CIMMYT collaborative project with the University

of Hohenheim (Germany), promises to be extremely valuable to breeding programmes in tropical and subtropical regions of Asia and elsewhere.

Although much has been written about the use of DHs in maize breeding (Forster and Thomas, 2005), there is very little published evidence that DHs and MAS are commonly used together. A likely application of MAS and DNA fingerprinting together with DHs should be to select parents with complementary genotypes to form crosses for use in deriving DH lines. Another application of the combined use of MAS and DHs could be in recurrent selection projects. Bouchez and Gallais, (2000) demonstrated with simulations that use of DH lines will theoretically enhance the efficiency of recurrent selection schemes for traits with low heritability, particularly for breeding programmes without access to offseason nurseries. Similarly, for some traits MAS could be cheaper, faster or more effective than phenotyping DH lines to select parents for subsequent cycles of recurrent selection projects.

A third application in which DH and MAS complement each other is to derive DH lines from bi-parental crosses when the objective is to obtain inbred lines genetically similar to either parent of the cross (Smith *et al.*, 2008) or to identify recombinants at or flanking specific loci. The most frequent application of this approach would likely be the use of DH line conversion protocols instead of slower conventional backcrosses (Forster and Thomas, 2005), and application of MAS to identify the DH lines with closest genetic similarity to one of the parent lines. Finally, the most widespread combined use of DH and MAS is probably for genetic studies such as bulked segregant analysis and developing genetic maps (Chang and Coe, 2009; Forster and Thomas, 2005).

Because DHs offer a fast way to obtain homozygous lines, they can save time and increase the efficiency of projects designed to identify or map marker-trait associations, leading to potential use of markers in MAS breeding projects. A DH population of 83 lines was developed in China from a cross between Nongxi531 and Nongxi110, and was used to map QTLs influencing grain quality; because of the contrasting phenotypes of the parents, this population can also be useful to map QTLs for yield components, especially kernel row number (Zhang *et al.*, 2008).

## B. GENETIC TRANSFORMATION

The development of transgenic technologies that allow introduction and functional expression of foreign genes in plant cells and their regeneration has

been extended during the last two and half decades to the production of transgenic plants with improved insect and disease resistance, herbicide tolerance and seeds with enhanced nutritional qualities and plants that are adopted to adverse environmental conditions (Srivastava, 2003). Transgenic technology allows plants with specific qualities to be developed in a much shorter period of time than when using conventional plant breeding and it also makes possible the introduction of characteristics that cannot be achieved through plant breeding alone (Husaini and Srivastava, 2006). Rapid progress in gene manipulation and the desirability of improving agricultural productivity, both to decrease inputs of pesticides and fertilizers as well as to benefit quality, have fueled the development of genetically modified maize plants.

In total maize is grown in 17 countries worldwide. Major countries growing GE maize are USA, Argentina, Canada, Brazil and South Africa. Other countries include Uruguay, Philippines, Chile, Egypt, Honduras and seven EU countries. On a global basis, in 2008, genetically engineered maize occupied 37.3 million hectares equivalent to 24% of the global maize area of 157 million hectares (James, 2008). There have been a substantial increase in the deployment of stacked traits of Bt and herbicide tolerance, particularly in USA. The triple gene products in GM maize, featuring two Bt genes (one to control the European corn borer complex and the other to control root worm) and one herbicide trait continued to grow in adoption in USA in 2008. The area occupied by maize with insect resistant and herbicide tolerant genes and the two characters stacked together is given as Table 1.

**Table 1. Global area of GM maize in 2008**

S. No.	GM maize containing	Area in million hectares
1.	Insect resistant gene	7.1
2.	Herbicide tolerant gene	5.7
3.	Stacked traits	24.5
	Total	37.3

Maize has been genetically engineered to insert agronomically desirable traits i.e. incorporation of a gene that codes for the *Bacillus thuringiensis* (Bt) toxin, protecting plants from insect pests and resistance to herbicides (Tables 2 and 3).

**Table 2. GM maize with insect resistance trait**

Developer	Product name	Event name/ genes	Trait
<i>Commercialised maize events</i>			
Monsanto	Yield Gard Corn Borer	MON810	Resistance to lepidopterans
Monsanto	Yield Gard Rootworm	MON863	Resistance to coleopterans
Monsanto	Yield Gard VT	MON88017	Resistance to coleopterans
Dow Agro Sciences And Pioneer Hi-Bred	Herculex I	1507	Resistance to lepidopterans
Dow AgroSciences And Pioneer Hi-Bred	Herculex RW	59122	Resistance to coleopterans
Syngenta	Agrisure CB	Bt11	Resistance to lepidopterans
Syngenta	Agrisure RW	MIR604	Resistance to coleopterans
<i>Maize events authorised in at least one country but not yet commercialised anywhere</i>			
Monsanto	Yield Gard VTPRO	MON89034	Resistance to lepidopterans

**Table 3. GM maize with herbicide tolerance and quality traits**

Developer	Product name	Event name/ genes	Trait
<i>Commercialised maize events</i>			
Monsanto	RoundupReady Corn 2	NK603	Herbicide tolerance (to glyphosate)
Syngenta	Agrisure GT	GA21	Herbicide tolerance (to glyphosate)
Monsanto	High lysine	LY038	Crop composition (high lysine content)
Syngenta	n/a	3272	Crop composition (amylase content)

Both pest resistance and herbicide tolerant genes/events have also been stacked. As of now several events of the two traits have been approved in different countries and are being extensively cultivated. In early 2009, there were nine different event of GM maize in the varieties cultivated globally and three additional maize events have been authorized in atleast one country worldwide, but not yet commercialized anywhere (Stein and Rodriguez-Cerezo, 2009).

In addition, there are five more GM maize events that have entered the

regulatory system in at least one country but that are not yet authorised anywhere in the world, namely Syngenta's new lepidopteran resistant maize, Pioneer's Optimum GAT maize and three GM maize events from China (Table 4).

**Table 4. GM maize in the regulatory pipeline worldwide**

Developer	Product name	Event name / genes	Trait
Syngenta	Agrisure Viptera	MIR162	Insect resistance (to lepidopterans)
Pioneer Hi-Bred	Optimum GAT	98140	Herbicide tolerance (to ALS inhibitors and glyphosate)
n/a (China)	n/a	Cry1A	Insect resistance
n/a (China)	n/a	n/a	Crop composition (high lysine content)
n/a (China)	n/a	n/a	Crop composition (phytase enzyme)

In addition to the above seven new events containing traits regarding crop composition and drought tolerance are at advanced stages of research and development, as given in Table 5.

**Table 5. GM maize in the advanced R&D pipeline worldwide**

Developer	Product name	Event name / genes	Trait
Monsanto	n/a	MON87754	Crop composition (high oleic content)
Pioneer Hi-Bred	Optimum Acre Max 1	n/a	Insect resistance (to coleopterans)
Monsanto and BASF	n/a	MON87460	Abiotic stress tolerance (to drought)
Dow AgroSciences	DHT	n/a	Herbicide tolerance
n/a (India)	n/a	cry1Ac + cp4epsp4	Insect resistance
Syngenta	n/a	n/a	Abiotic stress tolerance (to drought)
BASF Plant Science	NutriDense	n/a	Crop composition (protein, amino acid and phytase content)

### GM Maize- a case study of Phillipines

The Philippines is the first country to allow the first biotech food crop to be commercially planted in Asia. National Bio-Safety Committee of the Philippines (NBCP) is the highest regulatory body with regard to the introduction, use and transfer of GMOs and Potentially Harmful Exotic Species (PHES) in the Philippines. Governed by the set of guidelines of NBCP, the Department of Agriculture (DA) approved in December 2002 the propagation and importation of Bt corn—the first genetically modified crop approved for fieldtesting in the country.

Bt-corn is a variety of corn where a specific gene of *Bacillus thuringiensis* (*Bt*), a common soil bacterium used safely since the 1950s by organic gardeners and farmers worldwide as biological insecticide, is inserted to produce a protein that protects the corn plant from Asiatic corn borers, the number one enemy of corn. This pest infestation causes up to 80% yield loss as borers feed on the stem, leaves, and corn ears even before the crop matures. According to DA Bt corn may increase corn yields by as much as 40%, and reduce production costs (including labor costs) by eliminating the need for commercial pesticides. Thus, Bt-corn gives promise of better incomes for corn farmers, poverty reduction in rural areas, and enhancement of the country's self-sufficiency in corn.

From 1996 to 2002, Bt corn has been planted in almost 44 million hectares around the globe and has the potential to increase corn production up to 35 million MT per year (5% increase in temperate maize growing areas and 10% in the tropical areas).

Notwithstanding the benefits from Bt corn and the positive experience with GM crops of developing countries, oppositors to the technology insist that GM crops run against the natural evolution of crops and have not yet been proven as safe to humans and the environment. Furthermore, they fear that once GMOs are released to the environment, their possible harmful effects would be irreversible. In addition, they argue that GM crops will foster dependence of farmers on the multinational companies that produce the seeds. Although highly unpopular among some environmentalists and consumers, a clearly defined scientific basis to declare Bt corn as unsafe has not been established. Moreover, more than 50 international organizations, including the Food and Agriculture Organization (FAO), declared GM crops, including Bt corn, as safe and nonthreatening to humans and the environment (Manuzon, Philippine Business Magazine Vol 10. No. 6).

### Status of GMO Approvals in Phillipines

According to data from the Bureau of Plant Industry (BPI) which monitors



importation of GMOs, there were 16 plant and plant-based genetically altered products approved (as of December 22, 2003) for importation, for direct use for food, feed or processing in the country (Table 6).

**Table 6. Approval Registry for the Importation of Regulated Articles for Direct Use for Food, Feed or Processing**

Transformation Event	Introduced Trait and Gene	Technology Developer
Corn MON 810	Resistance to corn borer Cry 1A (b) gene from Bt	Monsanto Company
Corn Bt 11	Insect protected, herbicide tolerant maize-Bt protein and PAT protein from <i>Streptomyces viridochromogenes</i>	Syngenta Seeds
Soybean 40-3-2	Resistance to herbicide, round-up CP4 EPSPS from <i>Agrobacterium</i> sp. Strain CP4	Monsanto Company
Corn NK 603	Glyphosate tolerance imparted by the CP4EPSPS coding sequence	Monsanto Company
Corn MON 863	Cry3Bb1 for resistance to the corn rootworm, <i>Diatrofica</i> sp.	Monsanto Company
Corn TC 1507/CRY1F	Resistance to certain lepidopterous pests in Maize-Cry1F and PAT genes	Pioneer Hi-Bred
Corn DBT 418	Lepidopteran resistance, phosphinotrin tolerance-Cry1Ac	Monsanto Company
Canola RT 73	Glyphosate (Roundup) Tolerance-CP4EPSPS	Monsanto Company
Corn Bt 176	Insect protected-Bt protein and PAT protein from <i>Streptomyces viridochromogenes</i>	Syngenta seeds
Corn GA 21	Modified EPSPS for tolerance glyphosate	Monsanto Company
Corn DLL25	Phosphinotricin (PPT) herbicide tolerance specifically glufosinate ammonium-bar gene from bacterium <i>Streptomyces hygroscopicus</i>	Monsanto Company
Corn T25	Phosphinotricin (PPT) herbicide tolerance specifically glufosinate-PAT gene from <i>Streptomyces viridochromogenes</i>	Bayer Crop Science
Cotton 1445	Tolerance to round-up herbicide-CP4EPSPS	Monsanto Company
Cotton 15985	Resistance to lepidopteran pests-Cry2Ab2 gene	Monsanto Company
Potato Bt 6 (RBBT02-05) and (SPBT02-05)	Resistance to Colorado potato beetle-CryIIIA	Monsanto Company
Potato RBMT 15-101, SEMT 15-02 & SEMT 15-15	Resistance to Colorado potato beetle; resistance to potato virus Y (PVY)-CryIIIA and PVFcp	Monsanto Company

Source: [www.bpi.da.gov.ph](http://www.bpi.da.gov.ph). Transformation Event refers to the integration of a transgene in the cell of the plant, as a result altering its natural make up. In the case of Bt corn, it refers to the unique insertion of the Bt gene into the corn's DNA.

## CONCLUSIONS

The technological opportunities for implementing molecular marker-assisted breeding in maize have increased tremendously in recent years. We have provided only a glimpse of these advances and the efforts made in the Asian institutions, specifically in the public sector, to utilize molecular markers for diverse purposes in maize genetics and breeding. Significant strides have been made in China and India, particularly with regard to understanding the phenotypic and molecular diversity in the maize germplasm, identification of QTLs influencing diverse traits, especially tolerance to important biotic and abiotic stresses, and MAS for improving disease resistance and nutritional quality. Yet, the application of molecular marker-assisted breeding tools to accelerate gains in maize productivity has barely begun in much of Asia, and there is vast potential and need to expand the scope and impact of such operations. Molecular marker-assisted breeding has special relevance for rapidly improving the low current average yields of maize in Asia by aiding breeders to address the biotic and abiotic stresses that most constrain productivity. Breeders will also want to avail molecular tools to more efficiently add value to new maize cultivars, e.g., by enhancing their nutritional or biochemical qualities for use as food, feed, and industrial material. The scientific and economic capacity in Asia is improving, which in turn, will enhance the rate and efficiency of breeding progress using modern tools as well as utilization of advanced genotyping platforms. Thus, these efforts will have not only regional but international impact in the years to come. A major investment in MAS infrastructure, including year round nurseries, high throughput and precision phenotyping facilities, and dedicated personnel are required for the Asian institutions to effectively deploy MAS, maximize selection gains, and minimize time required for cultivar development. A further challenge is that, as mentioned above, the investment needed to become and remain competent in molecular marker-assisted breeding techniques may require innovative models for resource-pooling, intellectual-property-respecting partnerships. Indeed, our survey indicated that even the largest commercial seed companies regularly engage in partnerships for implementing their molecular maize breeding programmes (e.g., out-sourcing of critical activities, and alliances with other private- and to a lesser extent public-sector institutions). Finally and crucially, scientists must ensure that the tools of molecular marker-assisted breeding are wisely and intensely focused on developing practical solutions—i.e., commercially viable, improved cultivars—to the most important constraints to maize production in Asia.

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