

# Chapter 14

## Genomics of Tropical Maize, a Staple Food and Feed across the World

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**Abstract** Tropical maize is a major staple crop providing food and feed across the developing world. Genomics of maize is very well advanced but heavily focused on temperate germplasm. Tropical maize germplasm is substantially more diverse than temperate maize with a wide range of landraces and types of varieties. Thus, diversity analysis at genetic, molecular, and functional levels is important for underpinning translational genomics from temperate to tropical maize. Virtually all types of markers have been used for molecular linkage mapping in maize over the past decade. However, single nucleotide polymorphic markers are now very well developed in maize and are becoming the marker of choice for most applications. Both linkage and association-based mapping has been used for identifying marker-trait associations. Maize genome sequencing is now well advanced but focused on gene-rich regions due to its high density of repetitive elements. Functional genomics activities have made use of insertional mutation-based cloning as well as expressed sequence tags and map-based cloning. A wide range of genomic databases and tools have been developed, of which MaizeGDB features a wealth of data and resources facilitating the scientific study of maize. Genomics-assisted breeding is at an advanced stage in temperate, especially in private sector breeding programs, and applications in tropical maize are also common. Marker-assisted selection has been used in maize for yield, grain quality, abiotic and biotic stresses. Using these approaches, commercial maize breeding programs have reported twice the rate of genetic gain compared with phenotypic selection. However, reports in the literature from public breeding programs are inconsistent and generally less promising. Applied maize genomics in the tropics should in the future focus on tropical maize fingerprinting, haplotype establishment, allele mining, gene discovery, understanding genotype-by-environment interactions, and development of decision support tools and networks for developing countries to facilitate effective applications of genomics in maize breeding.

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## 14.1 Introduction

Maize is primarily a cross-pollinating species, a feature that has contributed to its broad morphological variability and geographic adaptability. Maize is also a C4 plant with high photosynthetic efficiency and high biomass production potential. Maize is classified into two distinct types depending on the latitude and the environment in which it is grown. Maize growing in warmer environments located between 30°N and 30°S latitudes is referred to as tropical maize, while that grown in cooler climates beyond 34°N and 34°S is classified as temperate maize. An intermediate type, subtropical maize, is grown between the 30° and 34° latitudes. Here we will only focus on tropical maize which is further classified into three subclasses: lowland (sea level to  $\leq 1000$  masl [meters above sea level]), mid-altitude (1000 to 1600 masl) and highland ( $\geq 1600$  masl).

Tropical maize occupies about 60% of the area harvested and represents 40% of the world production. It is grown in over 60 countries. The average grain yield of a maize crop in the tropics is 1.8 tons/ha, as compared to the global average of 4.2 tons/ha. Although the average crop yield under temperate conditions is over twice that under tropical conditions, temperate maize varieties have a longer crop cycle than do most tropical maize varieties. Thus, the relative daily yield is not so different between tropical and temperate maize (Paliwal 2000). Maize grain is an important cereal for human consumption, particularly in tropical Africa and Latin America. The FAO estimates that an additional 60 Mt of maize production will be needed by 2030. In addition, the demand for maize as animal feed will continue to grow at a very fast rate, particularly in Asian countries, which are estimated to increase production from 165 Mt today to almost 400 Mt by 2030 (Paliwal 2000). Finally, it is likely that the demand for maize by the biofuel industry will also continue to dramatically increase, in some cases to the detriment of food and feed needs (Ortiz et al. 2006; Rosegrant et al. 2006).

Genetic resources of tropical maize include wild relatives (teosinte, *Tripsicum*), landraces, open-pollinated varieties, synthetic varieties, inbreds, hybrids, germplasm complexes, pools and populations, and various genetic stocks (mutant, permanent populations, near-isogenic lines, introgression lines, etc). Landraces of maize may also be referred to as races, super-races, sub-races, primitive maize types, racial or geographical groups and racial complex. About 300 tropical maize landraces, comprising thousands of different varieties, have been identified worldwide. (see Figs. 14.1 and 14.2)

Maize breeding in the tropics targets a great diversity of environments, a wide range of cropping systems, and varieties that range from high-tech and highly tailored single cross hybrids to open-pollinated improved varieties to farmers' local varieties and landrace selections (Smith and Paliwal 1996). Maize breeding research in developing countries started in agricultural colleges and during the 1960s and 1970s national research institutions became important centers of maize research and improvement. Yield improvement was the most important breeding target across all tropical countries while the importance of other traits varied from continent to



**Fig. 14.1** Native races of maize tend to have lower harvest indexes, producing larger stalks and more foliage than improved varieties, an advantageous quality when they are grown to produce fodder. (photo by CIMMYT, used with permission) (See color insert)

continent (Pandey and Gardner 1992): diseases and drought in Africa, diseases and maturity in Asia, plant height and diseases in Latin America, and maturity and grain type in the Middle East. A comprehensive review of progress in breeding for hybrid maize, biotic and abiotic stress resistance, and special purpose maize has been provided by Paliwal et al. (2000). Hybrid maize is now well established across the tropics with average yields of five to six tonnes/ha over large areas.

The pioneering work of Barbara McClintock revealed maize as a highly important model plant for genetic research. A large body of genetic knowledge and huge amounts of experimental and molecular data are already available, although most of this is derived from temperate maize-based studies. The genetic divergence required for adaptation to significantly different tropical and temperate environments



**Fig. 14.2** The Jala maize landrace from the western coastal state of Nayarit, Mexico, is known for its gigantic ears. This and other native maize races are in danger of extinction, as the smallholder farmers who grow them leave rural areas to seek better fortunes in cities or other countries, and those who remain increasingly sow improved varieties. (photo by CIMMYT, used with permission) (See color insert)

contributes to diverse target traits associated with tropical and temperate maize breeding programs. Maize has a number of characteristics that make it suitable as an experimental model for crop plants, including (1) an intermediate genome size compared to rice and wheat; (2) typical outbreeding system with flexibility for inbreeding; (3) existence of multiple breeding products (inbreds, hybrids, synthetic varieties, open pollinated varieties and improved landraces); (4) wide adaptability, especially for stressed environments; and (5) a multiple-purpose crop that can be used as food, feed, fuel and many industrial products.

This article will present an overview of various aspects in maize genomics, including genetic and molecular diversity, genetic mapping and trait tagging, physical mapping and genome sequencing, functional genomics, genomic databases and

tools, and genomics-assisted plant breeding. Most advances in maize genomics have resulted from researchers working on temperate maize, but tropical maize is only significantly different in terms of its adaptation to different growing environments. Thus, this chapter will review genomic research in both temperate and tropical germplasm but with emphasis on traits important for tropical production systems and highlighting the gaps that need to be addressed in the future by tropical maize improvement programs.

## 14.2 Molecular Diversity Analysis and Mining

Genetic variation and diversity among germplasm accessions provides a pool of novel alleles and genes for plant breeding. Conventional plant breeding has been dependent on the evaluation of genetic diversity at the phenotypic level. Developments in genomics and molecular biology have provided various tools to identify and manipulate genetic variation at the molecular level. As it becomes increasingly feasible to do this in a functional and trait targeted way, genomics will have an increasing impact on plant genetic research and plant breeding.

### 14.2.1 Molecular Cytogenetics

The maize cytogenetic map is based on the pachytene stage chromosome karyotype and on the chromosome's fractional arm length unit referred to as centiMcClintocks (cMC). The cytogenetic map has many translocation breakpoint loci, but relatively few genetic marker loci (restriction fragment length polymorphisms, RFLPs) to define those breakpoints. The rapid and cost-efficient method that can be employed for construction of a cytogenetic map is to use segments of sorghum DNA (maize-marker-selected sorghum bacteria artificial chromosome [BAC] clones) as probes to stain the corresponding regions of maize chromosomes by fluorescence *in situ* hybridization (FISH) (Koumbaris and Bass 2003). A pachytene cytogenetic FISH map of the maize genome is under development using sorghum BACs corresponding to the 90 maize Core Bin Marker (CBM) loci (Figuroa et al. 2006).

The RFLP Full Length Insert Sequencing (FLIS) Project aims to determine and submit to GenBank a high-quality (bidirectional), full length insert sequence for maize RFLP markers including ~ 90 CBMs and potentially as many as 500 other markers from the UMC 98 map ([http://gremlin3dev.gdcb.iastate.edu/prj/RFLP\\_FLIS/](http://gremlin3dev.gdcb.iastate.edu/prj/RFLP_FLIS/)). In addition to being useful for the Cytogenetic Map of Maize Project ([http://www.maizegdb.org/CMM\\_protocols.php](http://www.maizegdb.org/CMM_protocols.php)), these sequences are useful in that they can anchor assemblies of the maize genome to the genetic and cytogenetic maps. A cytogenetic map of the maize genome using RFLP marker-selected sorghum BACs as FISH probes is under construction ([www.cytomaize.org](http://www.cytomaize.org)). The

results can be integrated with other genome maps and released immediately into GenBank and MaizeGDB for public access.

More recently, PCR-based FISH probes for identification of maize mitotic chromosomes were developed (Danilova et al. 2006). Maize centromeres were mapped using telosomes and isochromosomes produced by spontaneous chromosome breaks, radiation-induced chromosome breaks recovered in T-B translocation lines or in oat-maize radiation hybrid lines, single-locus FISH, and half-tetrad analysis (Okagaki et al. 2006a).

### 14.2.2 Genetic Diversity Analysis

Over the past 10,000 years, man has used the rich genetic diversity of the maize genome as the raw material for domestication and subsequent crop improvement. Structural diversity appears to be largely mediated by *helitron transposable elements*. Patterns of diversity are yielding insights into the number and type of genes involved in maize domestication and improvement, and functional diversity experiments are helping develop allele mining protocols that may identify novel genetic variation for use in future crop improvement (Buckler et al. 2006). Molecular markers have been used in genetic diversity studies of tropical maize for diverse purposes including:

1. Examination of genotype frequencies for deviations from Hardy-Weinberg equilibrium at individual loci (Reif et al. 2004)
2. Test for linkage disequilibrium (LD) between pairs of loci (Reif et al. 2004)
3. Construction of “phylogenetic” trees or classification of germplasm accessions based on genetic distance (Warburton et al. 2002; Betrán et al. 2003; Liu et al. 2003; Reif et al. 2004; Xia et al. 2004, 2005)
4. Characterization of molecular variation within populations and/or between populations (Warburton et al. 2002; Reif et al. 2004)
5. Determination of heterotic groups (Warburton et al. 2002; Xia et al. 2004, 2005)
6. Analysis of correlation between the genetic distance and hybrid performance, heterosis, and special combining ability (Betrán et al. 2003)
7. Comparison of genetic diversity among different groups of maize germplasm including those from temperate and tropical areas (Liu et al. 2003; Tarter et al. 2004; Xia et al. 2005)

It has been shown that tropical and subtropical inbreds possess a greater number of alleles and greater genetic diversity than their temperate counterparts. Comparison of diversity in equivalent samples of inbreds and open-pollinated landraces revealed that maize inbreds capture less than 80% of the alleles in the landraces, suggesting that landraces are likely to provide important additional genetic diversity for maize breeding. In addition, tropical highland germplasm is poorly represented in maize inbreds (Liu et al. 2003). The incorporation of a substantial percentage of tropical germplasm in an inbred line does not necessarily negatively impact its combining ability for grain yield or other agronomic traits (Tarter et al. 2004).

Thus, tropical maize accessions represent a valuable source of exotic germplasm to broaden the genetic base of temperate maize without hindering agronomic performance.

Maize has been shown to possess 88% of the gene diversity found in teosinte and 76% of the number of alleles, indicating a modest genome-wide deficit of diversity in maize relative to teosinte (Vigouroux et al. 2005). The pattern of genetic diversity at maize microsatellite or simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) loci can be explained largely by a bottleneck effect with a variable effect from artificial selection depending on the germplasm (Wright et al. 2005).

Genetic, molecular and functional diversity analysis will play a key role in translating temperate germplasm-based genomics outputs into valuable information for tropical germplasm research and enhancement. Tropical maize improvement will benefit from translational genomics through the methodologies and technologies developed in temperate germplasm-based genomics, including molecular markers, genotyping system, transformation protocols, marker-trait association, etc. On the other hand, novel genes and alleles, gene and linkage blocks, distinct haplotypes and heterotic patterns can be identified from tropical germplasm and characterized for improvement of both temperate and tropical maize. As the tropical germplasm hosts a large number of open-pollinated varieties, landraces and wild relatives, which are adapted to more diverse environments and serve as a reservoir of genes for wide adaptation, genomics-assisted breeding systems have to be modified for more effective application in tropical maize genetic resources. For example, molecular and functional diversity of the maize genome can be characterized through allele mining, identification of distinct haplotypes for different inbred lines, single feature polymorphism analysis, and discovery of nearly identical paralogs and their evolutionary implications. Novel or unique alleles may not ever have been found via simple phenotypic screens, either because it is not possible to grow and measure every plant in a large germplasm collection under all possible environmental conditions, because its effect may be masked in an unsuitable genetic background, or because its effect may be so small that it will not be found unless specifically sought in carefully controlled phenotypic screens (not generally possible on a very large scale). Therefore, a combination of various methods is required to identify those alleles to ascertain their function.

### ***14.2.3 Allele Mining***

In general, there are two approaches that have been elaborated for allele mining: re-sequencing and eco-tilling (Comai et al. 2004). However, eco-tilling is not being widely used in maize at this time, due to the very high numbers of sequence differences found between different maize accessions, which confound interpretation. Whole genome genotyping using sequence-based markers can be carried out for the re-sequencing method. Allele discovery from germplasm collections is in its infancy, currently constrained by the difficulty of establishing which of the various alleles present is functionally different from the wild type in an agronomically

beneficial way. Methods to ascertain allele function include marker-assisted backcrossing, transformation, transient expression assays, and association analysis using an independent association mapping set from the one that was used to identify the original allele (Sheen 2001).

There is a growing awareness that levels and patterns of allelic diversity contribute to the chromosomal context of a locus. “Diversity maps” showing the distribution of allelic diversity across the chromosomes and genomes of a variety of organisms suggest that in certain well-defined gene pools there is an association between chromosome structural features such as centromeres and telomeres and with selection in particular well-defined gene pools (Dvorak et al. 1998; Hamblin and Aquadro 1999; Gaut et al. 2000). Diversity analysis of individual genes promises to shed new light on crop productivity and evolutionary processes underlying plant domestication (Wang et al. 1999). As one of the crops with high resolution of genetic maps, maize is an ideal choice to develop a diversity map that promises a whole host of new information about the consequences of natural selection, domestication, and polyploidy formation, and perhaps even heterosis. Clearly, such approaches of relating molecular level variation to phenotypic diversity are an essential backdrop for future studies of diversity in large populations of candidate genes. Using quantitative trait locus (QTL) information together with association approaches can focus the list of candidates to a manageable number that can be directly related to a specific phenotype. Mapping QTL (see section 14.3.2) to the level of individual genes will provide new insights into the molecular and biochemical basis of genetic diversity and allelic variation for maize improvement. Linkage disequilibrium (LD)-based association mapping will help identify alleles associated with wide adaptation. A range of new alleles of previously identified genes can be identified based on molecular analysis of germplasm subsets and characterized to determine their relative value (see section 14.3.3).

## **14.3 Genetic Mapping and Trait Tagging**

Understanding genes and their relationship to traits and the influence of their location on chromosomes is highly important for plant breeding. It will facilitate manipulation of genes through more efficient identification, introgression, and selection. There has been great emphasis on elucidating the genetic basis of agronomically important traits. Genetic maps are constructed using molecular markers and then the maps are used to locate genes for the traits of agronomic importance using biometrical tools.

### ***14.3.1 Linkage Mapping***

The first generation of maize molecular maps was constructed using RFLP markers (Coe et al. 1987; Burr et al. 1988), which have been subsequently saturated

with various types of PCR-based markers (Lee et al. 2002; MaizeGDB database at <http://www.maizegdb.org/>). More recently, linkage mapping has been revolutionized by high density SNP-based maps while candidate gene maps have also become possible providing the opportunity of linking forward and reverse genetics approaches. Several types of mapping panels have been used in maize, including F<sub>2</sub> (Coe et al. 1987; Beavis and Grant 1991), immortalized F<sub>2</sub> (Gardiner et al. 1993; Davis et al. 1999), and recombinant inbred line (RIL) populations (Burr et al. 1988; Causse et al. 1996; Taramino and Tingey 1996). These mapping efforts were based on populations with 48 to 214 individuals screened with 92 to 1736 markers. In addition, composite maps have been constructed from multiple crosses (Causse et al. 1996). The Maize Mapping Project (MMP) has assembled a high-resolution genetic map for the intermated B73 × Mo17 (IBM; I-intermated; B-B73; M-Mo17) population (Lee et al. 2002), consisting of ~1000 RFLP and ~1000 SSR markers (MaizeGDB, [www.maizegdb.org](http://www.maizegdb.org/)). With the second panel of intermated recombinant inbred lines (IRILs) developed from F<sub>2</sub> × F252, the two IRILs were recently used for linkage mapping of 1454 maize candidate genes (Falque et al. 2005).

SNPs are the most abundant type of sequence variation encountered in most genomes and are ideally suited to the generation of high density genetic maps (Cho et al. 1999). A total of 14,832 SNPs have been identified from 102,551 maize ESTs (Batley et al. 2003) and 169 SNPs and indels from 36 maize inbreds (Ching et al. 2002). Most recently efforts have been made to combine many physically and genetically mapped probes and genes onto a single consensus map (Schaeffer et al. 2006) and integrate this into MaizeGDB. This includes: (1) the IDP maps of Pat Schnable ([maizemapping.plantgenomics.iastate.edu](http://maizemapping.plantgenomics.iastate.edu/)); (2) the Genoplante cDNA maps (Falque et al. 2005); (3) Genetic 2005 maps (Ed Coe); (4) SNP maps, incorporated into the community IBM94 maps (Mike McMullen).

### ***14.3.2 Gene Tagging/QTL Mapping***

Molecular marker-facilitated gene mapping in maize started in the early 1990s (Edwards et al. 1992; Stuber et al. 1992). Since then, there have been large numbers of studies for identifying associated major genes (gene tagging) and quantitative traits (QTL) mapping. Although much of this has been focused on temperate maize germplasm for major genes and QTL with large effects, many alleles may be shared between temperate and tropical germplasm as revealed by molecular markers (Tarter et al. 2004; Xia et al. 2005). Thus, the temperate germplasm-based gene tagging and QTL mapping is likely to be valuable for the genetic improvement of tropical maize.

The most intensive mapping study to date was based on a population of 1000 individuals derived from two elite inbred lines, which was phenotyped in 19 environments (Schon et al. 2004) for grain yield, grain moisture, and plant height. In another recent study, the high and low oil and protein content lines derived from 70 generations of long-term selection were crossed, intermated, and used for mapping (Laurie et al. 2004). Both studies identified numerous QTL of very small effect by

using high statistical power and robust innovative analyses. These results support the hypothesis that traits with quantitative phenotypic variation are often the product of numerous QTL of small effect. Conventional QTL mapping of drought tolerance in maize over the past decade has also detected many QTL but all of only minor effect (Ribaut et al. 2004). However, the parental lines used in these studies are probably capturing only a small proportion of maize functional variation (Buckler et al. 2006). The founders of the elite inbred lines used as parental genotypes were the products of intensive breeding selection for more than 50 years, a process that might have eliminated all large-effect QTL. Thus, mapping studies using more diverse maize germplasm should be carried out in order to determine whether QTL of major effect in fact exist. In rice, such a large-effect QTL for grain yield under reproductive-stage drought stress has been identified from upland rice, explaining 51% of genetic variance (Bernier et al. 2007).

Tropical maize populations have a broad genetic base with greater variability than temperate germplasm (Lanza et al. 1997). Also tropical growing areas are more prone to environmental stresses caused by precipitation and temperature variability and by different types of soils than are temperate areas (Ribaut et al. 1997). Thus, QTL mapping in tropical maize germplasm is likely to identify QTL not present in temperate germplasm. The traits that have been tagged with tropical germplasm include insect resistance, with focus on sugarcane borer (Bohn et al. 1996, 1997; Grohn et al. 1998; Khairallah et al. 1998); plant height (Khairallah et al. 1998); kernel oil content (Mangolin et al. 2004); flowering parameters (Ribaut et al. 1996; Khairallah et al. 1998); drought tolerance and its secondary traits including anthesis-silking-interval (ASI; Ribaut et al. 1996, 2004; Vargas et al. 2006); and yield components (Ribaut et al. 1997; Lima et al. 2006). Because of the high genotype-by-environment interaction in tropical areas, gene tagging/QTL mapping has focused not only on mapping QTL but also on analysis of QTL-by-environment interaction (Crossa et al. 1999; Lima et al. 2006; Vargas et al. 2006).

Gene tagging for quality traits has received great attention. High essential amino acids and vitamin A content have received particular attention in tropical germplasm. *Opaque 2*, which confers high lysine, is a recessive single gene trait, but a number of modifier genes cause it to behave in a quantitative manner. By bulked segregant analysis of vitreous and opaque seed from a cross of a South African QPM line (K0326Y) and a soft *o2* inbred (W64Ao2), Lizarraga Guerra et al. (2006) found that there are two loci clearly linked to the modified phenotype. The first was found in bin 7.02 and is near the 27-kD gamma-zein locus, which is consistent with prior results from RFLP mapping. The second was found in bin 9.02 and may be associated with starch synthesis genes. Sequencing of starch synthesis genes in isogenic backgrounds showed that four of these genes have sequence differences in a *mo2* compared to *o2* or normal. The mapping of provitamins A and total carotenoids in maize grain has been reported by Stevens et al. (2006), where near-isogenic populations for a mutation in the phytoene synthase (*y1*) gene show a huge range in concentrations of beta-carotene, alpha-carotene, beta-cryptoxanthin, lutein, zeaxanthin, and total carotenoids. QTL mapping has also been reported for starch, protein, and oil concentrations using high-oil maize parental genotypes (Zhang et al. 2006).

Domesticated maize (*Zea mays* spp. *mays*) and its wild progenitor, teosinte (*Z. mays* spp. *parviglumis*), differ dramatically in their overall plant architecture and the morphology of their female inflorescences. However, the major morphological differences between maize and teosinte are conferred by two QTL that have been dissected into single Mendelian loci: *teosinte branched 1* (*tb1*) that suppresses lateral branching leading to apical dominance (Doebley et al. 1995, 1997; Wang et al. 1999) and *teosinte glume architecture* (*tga1*) that affects the hardness of the seed coat in teosinte (Dorweiler et al. 1993; Wang et al. 2005). Other key loci controlling the differences between maize and teosinte have also been identified (Briggs et al. 2006).

### 14.3.3 Association or Linkage Disequilibrium Mapping

Association mapping, also known as linkage disequilibrium (LD) mapping, is a method that relies on LD to study the relationship between phenotypic variation and genetic polymorphism (Flint-Garcia et al. 2003a). The high resolution of association mapping depends on the structure of LD or the correlation between polymorphic loci within the test population. LD decays particularly rapidly in maize, so association studies in landraces and a broad sample of tropical and temperate inbreds will be especially powerful as LD often declines to nominal levels within 1.5 kb (Tenaillon et al. 2001; Remington et al. 2001). In contrast, elite breeding materials have less rapid LD decay and are therefore less valuable for association mapping studies (Ching et al. 2002; Jung et al. 2004). Association mapping in maize has focused on candidate gene markers from known pathways and genes. This has led to the identification of SNP markers for genes affecting starch (Wilson et al. 2004), carotenoid (Palaisa et al. 2003), and maysin contents (Szalma et al. 2005), as well as aluminum tolerance (Krill et al. 2006).

With the availability of dense genomewide genotyping in maize, a novel integrated association mapping strategy has been developed for both qualitative and quantitative traits. This has been named nested association mapping (NAM), and has been developed based on 25 maize populations, each of which comprises 200 RILs derived by crossing 25 diverse inbred lines to a common inbred line, B73. With a dense coverage (2.6 cM) of common-parent-specific (CPS) markers, the genome information for the 5000 RILs can be inferred based on the parental genome information leading to genome-wide high-resolution mapping. The power of NAM with 5000 RIL allowed 30% to 79% of the simulated QTL to be precisely identified (Stich et al. 2007; J. Yu et al. Cornell, personal communication). With the ongoing genome sequencing projects, NAM will greatly facilitate the dissection of complex traits in many species in which a similar strategy can be readily applied.

Both linkage analyses and LD mapping have limitations when used alone. Association mapping will not replace linkage mapping for determining marker-gene associations, as it suffers from false positive results (NCI-NHGRI 2007), but it will provide a valuable first step in many cases and a method to validate associations found through independent analyses or germplasm. In addition, a joint linkage and

LD mapping strategy has been devised for genetic mapping (Wu and Zeng 2001; Wu et al. 2002). This strategy has power to simultaneously capture the information about the linkage of the markers (as measured by recombination fraction) and the degree of LD created during historic time. The NAM populations developed by the Molecular and Functional Diversity of the Maize Genome Project (<http://www.panzea.org/>) provide an opportunity for a joint linkage and LD mapping.

#### ***14.3.4 Marker Validation***

Marker-trait associations need to be validated before entering into large-scale marker-assisted selection (MAS) applications, whatever methodology was used to identify the association (Nicholas 2006). Several factors contribute to the inconsistency of QTL mapping results including population structure and size, genetic background and epistasis effects, QTL-by-environment interaction, and level of LOD threshold (Beavis 1998; Moreau et al. 1998). Additionally, inaccurate phenotyping of the mapping populations further reduces the power and precision of QTL detection. Cross validation of QTL in independent populations, different genetic backgrounds and environments is necessary to obtain unbiased estimates of QTL position and effects.

The availability of thousands of SNP markers, rather than several hundred SSR markers, makes it practical to validate marker-trait association through high-precision genotyping using the same set of markers to screen different parental lines and breeding populations. Alternatively, marker validation can also be carried out at the same time by mapping multiple independent populations, selective genotyping and pooled DNA analysis, and development of gene-based, closely linked markers. Validation requirements can be minimized by focusing on large-effect QTL (Price 2006); precision phenotyping; identification of context independent QTL; mapping as you go approaches (Podlich et al. 2004); association mapping using large numbers of inbreds; genomewide association scans (as shown in human genomics, e.g., Meaburn et al. 2006); using breeding materials for mapping; and utilization of haplotype-based selection rather than single-marker based selection. Another useful strategy used for confirmation of candidate QTL or fine mapping is the application of NIL (near-isogenic lines). This approach reduces much of the “noise” caused by genetic background effects, thus mapping with NIL offers more accurate QTL effect estimates than RILs if multiple QTL are segregating in the populations, although power to detect a single QTL may be greater in RILs than NILs (Kaepler 1997; Szalma et al. 2007).

### **14.4 Physical Mapping and Genome Sequencing**

Genetic markers, genes, and other genetic elements in the maize genome can be characterized by their physical positions and biochemical composition. The finest physical map is the nucleotide sequence from which it is possible to determine

gene structure and function. Physical mapping and genome sequencing are essential prerequisites for gene isolation and functional characterization of genes. Based on technical advances in the related fields, physical mapping and genome sequencing has become much easier in recent years for large-genome plants including maize.

#### ***14.4.1 Development of BAC Libraries***

The construction of a physical framework for the maize genome has been based on a large insert genomic library generated from a temperate maize genotype. Assuming an average insert size of 100 kb, almost 300,000 colonies would be required for an 11-fold representation of the haploid maize genome. In order to develop a comprehensive physical framework for maize using fingerprinting and BAC end sequencing technologies, two deep coverage BAC libraries were developed from the maize inbred lines LH132 Dekalb and B73. The LH132 library consists of 427,392 clones stored in over one thousand 384-well microtiter plates. Based on a haploid genome size of 2,500 Mb, the coverage of the library is about 20 maize genome equivalents (Tomkins et al. 2000a). The coverage of the B73 library is about 13.5 maize genome equivalents (Tomkins et al. 2000b). The two libraries are well suited to construct a comprehensive physical framework of the maize genome due to their high overlap and large average insert size. A third BAC library was constructed using CHORI-201 (<http://bacpac.chori.org/maize201.htm>). It is this library that is being used for physical mapping and sequencing of the maize genome. The BAC clones have been arrayed into nearly three hundred 384-well microtiter plates and gridded onto nylon high-density filters for screening by probe hybridization. The Maize Mapping Project (MMP, <http://www.maize-map.org/>) has also constructed two BAC libraries from the inbred B73 (*HindIII* + *EcoRI*). The BAC libraries from both sources consist of nearly half a million clones which cover the B73 genome of 2,365 Mb nearly 30 times. The MMP is also generating a physical map by DNA fingerprinting of the BAC libraries and linking them to the IBM map.

#### ***14.4.2 BAC Physical Mapping***

Several genome-wide physical maps are reported in maize (Yim et al. 2002; Coe et al. 2002; Cone et al. 2002) that are useful in genome sequencing, targeted marker development, efficient positional cloning, and high throughput EST mapping. The IBM markers have been hybridized to fingerprinted BACs and the BAC-marker associations have been used to create fingerprint contigs and thereby integrate genetic and physical maps (Coe et al. 2002).

Anchoring the physical and genetic maps requires many high-quality single-locus markers placed on both maps, and the unavailability of such data is one of the significant bottlenecks of the map integration process. In addition to classical RFLP- or SNP-based projects, numerous other approaches have been used to

generate denser maps, including ESTs (Wen et al. 2002), radiation hybrid panels (Okagaki et al. 2001, Davis et al. 2000) and physically sheared DNA (Dear and Cook 1993).

Oat-maize addition (OMA) and radiation hybrid lines have been developed for physical and genetic mapping (Okagaki et al. 2006b). OMA lines are available for maize chromosomes 1–10, and also where a maize B chromosome has been individually added to the oat genome by wide crossing. Previously, maize chromosomes 1–10 from Seneca 60 had been recovered individually in OMA lines. Gamma irradiation of the OMA lines has yielded several hundred Radiation Hybrid (RH) lines each having just a fragment of a maize chromosome either after partial deletion of the rest of the maize chromosome or after translocation of a maize chromosome fragment into an oat chromosome.

These genetic resources, BAC libraries, and physical maps in temperate maize provide an important foundation for genomics of tropical maize. However, DNA libraries from tropical maize lines will now be important in order to exploit the genetic diversity and novel alleles that do not exist in temperate maize germplasm.

### ***14.4.3 BAC DNA Sequencing***

Sequencing the maize genome will greatly improve our potential to understand the molecular basis of important agronomic traits, gene regulation, genome evolution, plant development and biology. However, the large size of the maize genome and the high frequency of repetitive elements have prompted the examination of sequencing technologies expected to target gene rich regions as an alternative to whole genome sequencing. Based on the one-eighth of the maize genome already sequenced, (307 Mb) repeat sequences represent 58–66% while the predicted 42–59,000 genes represent just 7.5% of the genome (Messing et al. 2004; Haberer et al. 2005).

A large-scale effort to sequence the maize genome was commenced in 2006 through the NSF-funded Maize Genome Project (a collaboration between the Washington University Genome Sequencing Center, the Arizona Genomics Institute, Iowa State University, and Cold Spring Harbor Laboratory), aiming to sequence the maize genespace of a maize inbred B73 using a BAC-based approach (Wilson et al. 2006). This effort will utilize a minimal tiling path of approximately 19,000 mapped BAC clones, and will focus on producing high-quality sequence coverage of all identifiable gene-containing regions of the maize genome. These regions will be ordered, oriented, and, along with all of the intergenic sequences, anchored to the extant physical and genetic maps of the maize genome.

To further prepare for sequencing the entire genome, the Sequencing the Maize Genome Project (STMG, PI, J. Messing, Rutgers University) sought to improve on the B73 physical map by high information content fingerprinting (HICF) and by BAC end sequencing (BES). The Consortium for Maize Genomics (CMG), consisting of The Donald Danforth Plant Science Center, The Institute for Genomic Research (TIGR), Purdue University, and Orion Genomics, sought to test fraction-

ation techniques of genomic regions containing only genes and not repetitive DNA elements. Two strategies, methyl-filtration and high-  $C_0t$  selection, have been proposed to enrich for gene rich regions. Both STMG and CMG are collaborating to sequence two 5 Mb homoeologous regions of the maize genome and to analyze the maize genome structure and shotgun sequence assemblies of a larger interval. Using a methylation filtration strategy, Bedell et al. (2005) sequenced 96% of the genes with an average coverage of 65% across their length in sorghum. This strategy filtered away a high proportion of repetitive elements when sequencing the genome of sorghum that reduced the amount of sorghum DNA to be sequenced by two thirds, from 735 Mb to approximately 250 Mb. Both methylation filtration and high  $C_0t$  have already been used for efficient characterization of the maize gene space (Palmer et al. 2003; Whitlaw et al. 2003). These methods are used for highly repetitive genomes such as maize and sorghum (see above).

A recent report (July 2007) from the Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV), Mexico, indicates that a major sequencing project is being carried out using bulked plants from a landrace Palomero accession (Mexican popcorn maize). This maize accession has 30% less DNA and is phylogenetically closer to teosinte than to B73. The project focuses on important gene rich regions. The sequence generated so far has about 3–7 fold coverage of the popcorn genome. Assembly and annotation are still on-going (<http://www.niherst.gov.tt/s-and-t/s-and-t-news/>; Dr. Alfredo Herrera Estrella, CINVESTAV, personal communications).

## 14.5 Functional Genomics

Functional genomics can be defined as a field of molecular biology that makes use of the vast wealth of data and information produced in genomics to define gene (and protein) functions and interactions. It includes function-related aspects of the genome itself such as mutation and polymorphism analysis, as well as measurement of molecular activities and characterization of the phenotype. The latter comprise a number of “-omics” such as transcriptomics (gene expression), proteomics (protein expression), phosphoproteomics and metabolomics by quantifying the various biological processes to drive increased understanding of gene and protein functions and interactions.

### 14.5.1 Insertional Mutation

The insertion of a T-DNA element into a gene can lead to the loss or gain of a function. The use of this phenomenon has led to the identification of many genes and regulatory elements in *Arabidopsis* and many other plant species including maize (Cowperthwaite et al. 2002; Singh et al. 2003; Ma and Dooner 2004; Kolkman et al. 2005); for a review on maize insertional mutations, see Lisch (2002). With

classical genetic techniques and non-transgenic materials, Ahern et al. (2006) are generating a collection of 10,000 families, each harboring a unique *Ds* insertion distributed throughout the genome. DNA sequences flanking the *Ds* elements are cloned and sequenced providing a precise physical location for each insertion in the maize genome (Liu et al. 2006). Importantly, each *Ds* insertion is stable in the absence of *Ac*, but can be remobilized using a stabilized transposase source. As *Ds* tends to move to closely linked, gene-rich regions of the genome, each insertion will also serve as a platform for additional rounds of mutagenesis targeting linked genes. In addition, *Ac/Ds* transposons can be used for generation of an allelic series within a single gene (Bai et al. 2007).

Another type of DNA transposon, the *Mu* elements, accumulates to high-copy numbers within maize lines, which allows a relatively small population of ~40,000 plants to have a high chance of mutating most genes within the genome. Consequently, multiple groups have developed *Mu* transposon-tagging populations that can be used for both forward and reverse genetics (as reviewed by Settles 2005).

Gene knockouts are an essential resource for functional genomics. Many groups have developed reverse genetics populations in order to identify knockouts in any gene within the maize genome. These include: the Trait Utility System for Corn (Pioneer Hi-bred International; <http://www.pioneer.com>), the *RescueMu* population (Maize Gene Discovery Project; <http://www.maizegdb.org/rescuemu-phenotype.php>), and the *Mu*AFLP-based resources (BBSRC Gene Function Initiative). In addition, flanking sequence tags (FSTs) have been generated using DNA transposons to anchor each mutant to a specific locus within the genome. Settles et al. (2006) have shown that FSTs from the UniformMu population create easy to use knockout resources. The genetic markers in the UniformMu population allow for the selection of stable transposition events.

### 14.5.2 EST Development

Expressed sequence tags (ESTs) are currently the most abundant group of sequence resources. ESTs provide a robust sequence resource that can be exploited for gene discovery, genome annotation and comparative genomics. However, a large proportion of ESTs in public databases are unedited, automatically processed, single read sequences produced from cDNAs that provide only a very preliminary indication of nature and potential function of candidate genes. There are over a million maize ESTs in Genebank ([http://www.ncbi.nlm.nih.gov/dbEST\\_summary.html](http://www.ncbi.nlm.nih.gov/dbEST_summary.html)) including a large number from tissue and growth stage specific libraries or even limited populations of plant cells (e.g. Fernandes et al. 2002; Lê et al. 2005).

A genomics initiative to sequence full length cDNA's for 30,000 genes is underway through the Arizona Genomics Institute (<http://www.maizecdna.org/>). This effort is based on EST alignment assemblies followed by primer walking. A full length cDNA library from maize seedlings contributed 2073 full length cDNA sequences, of which over 80% represented new genes in the databases (Jia et al. 2006).

Such cDNAs form an excellent research resource as well as aiding efforts to annotate the maize genome.

Recently, a new sequence technology (454 Life Sciences; Emrich et al. 2007) was used to generate more than 261,000 ESTs from laser capture microdissection (LCM) of the shoot apical meristem from a single sequencing run. From this data > 25,000 maize genomic sequences were annotated, and several novel EST sequences were discovered (Emrich et al. 2007).

One of major uses of ESTs is to develop gene-based markers that are perfectly associated with the trait of interest, more conserved among related species, and may lead to elucidation of the function of genes influencing the target trait. ESTs have been localized on high resolution maps using different methods such as conventional mapping of the IBM RILs (Chen et al. 2007; <http://maize-mapping.plantgenomics.iastate.edu/>) and maize single feature polymorphism Genechips (Zhu et al. 2006). Integration of these gene markers with other types of genetic and physical markers will provide an important resource for the identification and marker-assisted selection of genes that control complex traits. The best functional and positional candidates should first be subjected to a functional validation process, such as reverse genetics mutant collection screening, overexpression or knockout transgenic approaches, or association studies using germplasm with allelic polymorphism of the gene and phenotypic data.

### 14.5.3 Gene cloning

Progress in maize genetics and gene discovery is confounded by the following: (1) the maize genome is big (thus not amendable to whole genome sequencing as has been successful in Arabidopsis and rice); (2) subspecies genome size and even gene order varies greatly; (3) the maize genomes contain multiple copies of most genes; and (4) jumping genes or transposons make up a large portion of the genome.

Traditionally, gene discovery looks for genes in two complementary ways. One method searches for ESTs. These represent genes that are turned “on” in a specific tissue, at a specific development stage, or in response to a specific biotic or abiotic stress. The second method, takes advantage of transposons that insert copies of themselves inside maize genes. Researchers evaluate the phenotype of maize plants that contained a specific, engineered transposon tag called *RescueMu*. The transposon, whose sequence is known and easily traceable, inserts itself in new chromosome locations, but always within a gene. The researchers then find genes by sequencing the DNA on both sides of *RescueMu*. The latter approach has the added advantage of being able to directly compare the sequence of the interfered gene with changes in phenotype.

Positional cloning in maize has been considered near impossible because of the vast amounts of repetitive DNA. However, conservation of synteny across the cereal genomes, in combination with new maize resources, has made chromosomal walk-

ing much faster than the more traditional methods of gene isolation (for a review, see Bortiri et al. 2006a). The first gene isolated through positional cloning was the *teosinte glume architecture (tga1)* locus, which encodes a transcriptional regulator (Wang et al. 2005). A recent report on cloning of *indeterminate gametophyte1* used a combination of positional cloning and transposon insertion to test candidate genes (Evans 2007). Buckler et al. (2006) have recently reviewed the advent of positional cloning and association approaches that allow for the dissection of complex trait down to the gene and nucleotide level. These are clearly highly relevant to both temperate and tropical maize.

**Reproductive Biology:** It is well established that small gene sequence changes can have dramatic effects on flowering time (Thornsberry et al. 2001). Recently, several genes that regulate inflorescence architecture in maize have been cloned. The gene responsible for the mutated phenotype of a highly branched tassel and a branched ear, *ra1*, was cloned by transposon tagging rather than by using synteny with rice (Vollbrecht et al. 2005). Two other maize inflorescence genes have been cloned using the map position of the mutation in combination with synteny with a candidate gene in rice. Mutants at the *barren stalk 1 (ba1)* locus lack tassel branches, spikelets, and ears. The positional cloning of *lax panicle* in rice (Komatsu et al. 2003) provided a candidate gene for *ba1*. In the second case, a maize *clavatal 1 (clv 1)* ortholog was mapped to chromosome 5 in the same region as *thick tassel dwarf 1 (td1)*. The phenotype of *td1* mimics that of *Arabidopsis clv* mutants, which have larger inflorescence meristems and more floral organs. Proof that *td1* was the *clv1* ortholog came from analysis of a large number of *Mu*-induced alleles (Bommert et al. 2005). More recently, *ra2* (Bortiri et al. 2006b), *ra3*, and *tasselseed4 (ts4)* have also been isolated through positional cloning.

**Forage Quality:** Silage maize is a major source of forage for dairy cattle due to its high energy content and good digestibility. Lignin structure and cross-linking between cell wall components influence digestibility (Barrière et al. 2003). Analysis of allelic diversity in relation to cell wall digestibility revealed *ZmPox3* peroxidase as a candidate gene for improvement of silage maize digestibility (Guillet-Claude et al. 2004) as it is co-localized with a cell wall digestibility and lignification QTL (Barrière et al. 2003). Brown midrib (*bm*) mutants in maize have an increased digestibility but inferior agronomic performance (Barrière and Argillier 1993). Two of the four *bm* genes (*bm1* and *bm3*) have been shown to be involved in monolignol biosynthesis (Barrière et al. 2003). These and other lignin biosynthesis genes have been isolated based on sequence homology. Candidate genes, putatively affecting forage quality, have been identified in a collection of maize inbred lines by expression profiling using isogenic *bm* lines, leading to the detection of association between a polymorphism at the caffeic acid *O*-methyl transferase locus and the digestible neutral detergent fiber locus (Lübberstedt et al. 2005).

**Pest and Disease Resistance:** Conserved domains or motifs shared amongst known resistance genes have been extensively exploited to identify resistance gene analogs (RGAs). In an attempt to isolate and map all potential RGAs from the maize

genome, three approaches were adopted by Xiao et al. (2006), including modified AFLP, modified rapid amplification of cDNA ends (RACE), and data-mining.

In response to attack by herbivorous insects, plants synthesize and release volatile chemical signals that attracts the natural enemies of the herbivore. Lin et al. (2006) reported the isolation and characterization of the maize *sesquiterpene cyclase2* gene (*stc2*) that is an ortholog of *stc1*, a gene induced in response to attack by beet army-worm larvae.

#### 14.5.4 Transcription Profiling

Comprehensive, low-cost, public sector long-oligonucleotide (70mer) microarrays have been developed for gene expression analysis in maize based on single and assembled ESTs plus some non-redundant repeat elements, organelle genes, and other community favorites (Iniguez et al. 2006). Transcriptional profiling has already been applied to the study of a range of traits in temperate maize and most of these are highly relevant to tropical production systems.

*eQTL Mapping:* Transcript abundance levels that differ between the parents of a mapping populations and segregate amongst the progeny can be mapped and characterized as quantitative traits (Cheung and Spielman 2002). Using microarrays the expression levels of large numbers of genes can be determined and compared with variation in the phenotype of target traits. The genomic regions of these gene expression QTLs (eQTL) can then be determined using statistical tools developed for conventional QTL analysis (Jansen and Nap 2001).

*Heterosis:* Recent data suggest that regulation of gene expression might play an important role in determining hybrid vigor in maize. Between 800 to 1000 genes were identified as being significantly over expressed in the F<sub>1</sub> hybrid as compared to the parental genotypes (Swanson-Wagner et al. 2006; Scheuring et al. 2006). A parallel study concluded that cis-transcriptional variation between genes from the different parents led to additive expression patterns in the F<sub>1</sub> hybrid (Stupar and Springer 2006). A further study found both dominance and over-dominance components were involved in non-additive gene expression variation encompassing a wide variety of biological processes (Pea et al. 2006).

*Grain Quality:* Transcript profiling has also been carried out on maize material associated with the longest continuous genetic selection experiment in higher plants (Moose et al. 2006). Microarray comparisons of developing seeds revealed significant expression differences in many genes, with the seed storage protein genes exhibiting the most dramatic changes.

*Abiotic Stress Tolerance:* Gene expression profiling has been widely used for studying abiotic stress tolerance. Flowering is the developmental stage that is most vulnerable to abiotic stress leading to significant yield loss associated with the resultant aberrant floral development, and impaired ear and kernel growth. Genes within the starch biosynthetic pathway are collectively down-regulated during drought

stress, resulting in reduced starch content. Many other genes are consistently up-regulated or down-regulated by drought stress (Zinselmeier et al. 2002; Yu and Setter 2003). There are similar reports for cold stress during germination and desiccation tolerance (Kollipara et al. 2002). Finally, transcriptome analysis of the low-phosphorus responses in roots and shoots of a phosphorus-efficient *Zea mays* line identified alterations of several metabolic and physiological processes (Calderon-Vazquez et al. 2006).

### 14.5.5 Transformation

Transformation is an important tool for maize genetic research and germplasm improvement, in addition to its extensive use in the development of pest- and herbicide-resistant new varieties. *Agrobacterium tumefaciens*-mediated transformation is the preferred method for genetic transformation because it generates a high proportion of independent events with single, or low, transgene copy numbers, which is considered to favor consistent transgene expression in progeny generations (Meyer and Saedler 1996).

Maize varieties resistant to glufosinate (Liberty) and Roundup herbicides have been produced in the USA. Maize varieties have also been transformed to express the *Bt* toxin by inserting a gene from the soil-dwelling bacteria *Bacillus thuringiensis*. This gene codes for a toxin that will crystallize in the digestive tract of insect larvae, leading to its starvation. This has been particularly effective against the European corn borer *Ostrinia nubilalis* that destroys corn crops by burrowing into the stem, causing the plant to lodge. It can be expected that transgenic maize will have a significant influence on tropical maize production once the public concern issues related to genetically modified organisms have been resolved.

Biswas et al. (2006) demonstrated the feasibility of producing a bacterial cellulose within maize biomass for possible biomass conversion into fermentable sugars by introducing the catalytic domain of an endo-1,4-p-D-glucanase gene from the eubacterium, *Acidothermus cellulolyticus*. For the food industry, the availability of foods that are low in sugar content, yet high in flavor, is critically important to millions of individuals conscious of carbohydrate intake in relation to diabetic or dietetic concerns.

The reported discovery of transgenes in maize landraces of small-scale Mexican farmers (Quist and Chapela 2001) raised questions about whether the commercial introduction of transgenic maize varieties might have a deleterious effect on the diversity of maize landraces and on traditional small-scale agricultural systems. An important concern in assessing the risk of growing a genetically modified crop in its center of domestication is gene flow between the transgenic crop and its landraces and wild relatives. However, a more recent study suggests that it is unlikely that the presence of transgenes per se will automatically reduce the diversity of alleles in local maize populations or the level of diversity of morphological variants managed by small-scale farmers (Bellon and Berthaud 2004).

### 14.5.6 TILLING

A non-transgenic method for reverse genetics called Targeting Induced Local Lesions In Genomes (TILLING) has been developed as a method for inducing and identifying novel genetic variation. TILLING is a targeted version of conventional mutation breeding with the added advantage of mutation detection in the gene of interest. TILLING employs a mismatch-specific endonuclease to detect single-base-pair (bp) allelic variation in a target gene using a high-throughput assay. Its advantages over other reverse genetic techniques include its applicability to virtually any organism, its high throughput nature, and its independence of genome size, reproductive system or generation time (Gilchrist and Haughn 2005). A public TILLING service has been established through the Maize TILLING Project (MTP) at Purdue University (<http://genome.purdue.edu/maizetilling>) (Till et al. 2004). The current TILLING population contains ~2,900 mutant lines with ~165,000 mutations in exons (Monde et al. 2006). In addition, the mtmDB web site (<http://mtm.cshl.org>) contains a knockout resource of a population of 43,776 plants containing stabilized *Mu* insertions that are available to the global scientific community (May et al. 2003).

## 14.6 Genomic databases and tools

As more and more information and data have been generated in various fields of genomics, databases and bioinformatic tools are needed to store, integrate, and manage the data plus extract and analyze useful information for use in genetic improvement. One of the most important genomic databases and tools for maize is MaizeGDB (<http://www.maizegdb.org>). The site features a wealth of data and resources facilitating the scientific study of maize:

- Sequence databases including integration with various contig assemblies
- Detailed genetic, physical, and cytogenetic maps
- Molecular marker primer databases
- Integrated tools for map comparisons, sequence similarity searches, and comparisons with and links to other databases, such as Gramene (<http://www.gramene.org/>) and NCBI (<http://www.ncbi.nlm.nih.gov/>)
- Web-based community curation tools that enable researchers to edit and annotate their own data and to enter new data into MaizeGDB directly
- Informatics support for maize community initiatives such as the annual Maize Genetics Conference and community-wide workshops, and maintains data for maize community research projects
- QTL information in the literature from the mid-1990's coupled to other information about germplasm, nearby loci and sequence information

To permit this work to continue at MaizeGDB, a new, web accessible curation interface has been designed and implemented. The new design accommodates a legacy trait hierarchy developed at MaizeGDB and recently harmonized with the

rice Trait Ontology at Gramene, and trait descriptors used by GRIN (the Germplasm Resources Information Network).

The Maize Assembled Genomic Island (MAGI, <http://magi.Plant.genomics.iastate.edu>) is a resource for maize genome assembly, annotation and mapping. ~3,100,000 maize genomic sequences primarily composed of gene-enriched GSSs, random whole genome shotgun (WGS) sequences, and BAC shotgun reads were assembled into MAGI (Emrich et al. 2004). Similarly ~550,000 methyl filtered (MF) sequence reads from *Sorghum bicolor* (BT × 623) were assembled into Sorghum Assembled genoMic Islands (SAMIs). To identify genomic contigs associated with particular genes, MAGIs and SAMIs can be searched using the BLAST tool. GBrowse, a component of GMOD, is used to display annotated assemblies. Segregation data in the IBM RIs have been generated for ~5,000 MAGIs and ESTs. A new genetic map based on these data and generated using MultiMap, including linkages to AGI's physical map, can be viewed via CMap. The MAGI website serves as a community resource for map-based cloning projects as well as for analyses of genome structure and comparative genomics.

There are many computer software and decision support tools developed by the plant genomics community, including those for germplasm evaluation, breeding population management, genetic map construction, marker-trait association analysis, marker-assisted selection, genotype-by-environment interaction analysis, breeding design and simulation, and information management. Since these programs have been reviewed else-where (Dwivedi et al. 2007), only two software packages that are more specific to maize will be discussed here. The first is TE Nest that was developed to facilitate the annotation of the 1.5 Mb chromosome 3 centromeric *rfl*-spanning sequence constructed from 19 contiguous BAC clones (Kronmiller et al. 2006). Considering that 85% of the maize genome consists of transposable elements (TEs), with more than 70% of TEs found nested within one another, an accurate nested TE identification tool for complete annotation of the maize genome was needed. TE Nest contains an up-to-date database of maize canonical TEs and their associated long terminal repeats (LTRs), if applicable. The second software is an integrated program developed by Schroeder et al. (2006) to aid researchers in the SNP discovery process across several maize, teosinte, and *Tripsacum* lines. An integrated set of tools consisting of a relational database and applications for data loading, editing and reporting has been developed. All stages of SNP discovery from tracking sequences, generating alignments, editing alignments, and reporting are covered. Central to this system is an intuitive, quality score based alignment editing tool designed to simplify manual editing of the highly polymorphic and complex *Zea* alignments.

## 14.7 Genomics-assisted breeding of tropical maize

One of the most important uses of plant genomics is the application of molecular biology information and tools to improve the efficiency and scope of plant breeding. Genomics-assisted plant breeding includes using molecular markers associated

with traits of agronomic importance to help improve selection efficiency, or using genetic transformation with functionally characterized genes. Here we are focusing on the use of markers to improve the identification, introgression, and manipulation of genetic variation. Molecular markers can increase the accuracy and speed of all three of these aspects of the breeding process compared to conventional phenotypic selection. Contrary to the situation in developed countries, where almost all maize grown by farmers are hybrid varieties, developing countries (almost all of which are in the tropics) are growing various types of maize including hybrid, synthetic and open-pollinated varieties, and landraces. Irrespective of the nature of the target breeding product, breeding objectives must focus on traits required for tropical environments, which are very different from those in temperate cropping regions, particularly regarding abiotic and biotic stresses.

Large multi-national seed companies are now routinely using applied genomic tools to (i) dissect the genetic structure of their germplasm to understand gene pools and germplasm (heterotic) groups, (ii) provide insights into allelic content of potential germplasm for use in breeding, (iii) screen early generation breeding populations to select segregants with desired combinations of marker alleles associated with beneficial traits (in order to avoid costly phenotypic evaluations), and (iv) establish genetic identity (fingerprinting) of their products (Fu and Dooner 2002; Xu 2003; Niebur et al. 2004; Cooper et al. 2004; Crosbie et al. 2006). MAS has been successfully applied in the private sector for maize variety development for recovering an ideal genotype, defined as a mosaic of favorable chromosomal segments from the parental genotypes. More specifically MAS has been used to simultaneously select for multiple traits (selection based on marker information only) such as yield, biotic and abiotic stress resistance, and quality attributes (Ragot et al. 2000; Eathington 2005), several of which are polygenic in nature. Using these approaches, commercial breeding programs have reported twice the rate of genetic gain over phenotypic selection in maize (Eathington 2005; Crosbie et al. 2006). The first commercial products of holistic molecular breeding (rather than unilateral MAS interventions) are expected from all the multinational breeding companies very soon. The first molecular breeding hybrids developed by Monsanto entered the U.S.A. commercial portfolio in the 2006 cropping season, and it is estimated that by 2010 over 12% of the commercial crop in the U.S.A. will be derived from molecular breeding (Fraley 2006).

One of the most important applications of MAS is for gene pyramiding to maximize utilization of existing gene resources. Genes controlling resistance to different races or biotypes of pests and pathogens can be pyramided together with agronomic and/or seed quality traits to ensure simultaneous introgression of several traits into an improved genetic background. Traditionally, wild relatives are considered as good sources of resistance to many pests and diseases not found in cultivated species, thus making them a valuable resource for genes to transfer to cultivated species. Both conventional crossing and selection, and molecular biology techniques (MAS and transgenic approaches) have been used to transfer pest- and disease-resistance from wild relatives to cultivated crop species. Resistant gene(s) from wild relatives have enabled large-scale cultivation of crops in disease/pest endemic regions of the world. Many major genes (recessive or dominant) and/or

QTL conferring resistance to pests and diseases have been reported in maize. Using MAS coupled with field evaluation, researchers have been able to combine multiple resistances to these pests and diseases (Widstrom et al. 2003; Quint et al. 2002). More recently, modeling and simulation analysis has been able to define the most efficient breeding strategies for generating such marker-assisted pyramided products (Wang et al. 2007).

Development of exotic genetic libraries, also known as chromosome segment substitution line (CSSL), introgression lines (IL), and contig lines is another approach to enhance utilization of wild relatives to expand crop gene pools. These genetic stocks consist of marker-defined genomic regions taken from wild species and introgressed into the background of elite crop lines, thus providing a potential resource for overcoming the yield barriers through pyramiding of beneficial loci and fixing positive heterosis.

### ***14.7.1 Yield and Heterosis***

Dominance, overdominance and epistasis have all been proposed to have a role in the genetic control of superior hybrid performance. The dominance model attributes increased vigor to the action of favorable dominant alleles from both parents combined in the hybrid, whereas the overdominance model postulates the existence of loci at which the heterozygous state is superior to either homozygote (Xiao et al. 1995; Yu et al. 1997; reviewed by Xu 2003). Evidence for the role of epistasis (interaction of the favorable alleles at different loci contributed by the two parents) in hybrid vigor has also been reported (Stuber et al. 1992; Li et al. 2001; Luo et al. 2001; reviewed by Xu 2003). Further, detailed description of the genetic basis of heterosis, heterotic groups, hybrid prediction and hybrid performance, relationships between heterozygosity and genetic distance with hybrid performance and heterosis, and use of MAS in hybrid breeding has been discussed elsewhere (Xu 2003).

The establishment of heterotic groups and heterotic patterns is an empirical task in hybrid maize breeding that has, in temperate maize germplasm, contributed to large increases in yield. Reciprocal recurrent selection (RRS) programs have proven to be effective in the improvement of heterotic groups through maximizing selection gains within a heterotic group and differences between heterotic groups. In temperate maize, such as the U.S. Corn Belt germplasm, the Reid Yellow Dent  $\times$  Lancaster Sure Crop, a heterotic pattern was recognized by Sprague over 60 years ago (from Iowa State Corn Breeding Annual Report 1939, 1940) (referred to in Troyer and Rocheford 2002). However, the first mention of the term “heterotic pattern” (or heterotic group) was in 1972 by B. Tsotsis (1972); the concept was further developed through the 1970s (Tracy and Chandler 2006). As an example, inbred lines such as B73 and Mo17, which are from two different heterotic groups, were chosen as testers for the selection of new maize inbreds.

Following successful deployment of hundreds of OPVs in the 1970s and early 1980s, the International Maize and Wheat Improvement Center (CIMMYT) maize program began the development of hybrid maize to meet the needs of hybrid-oriented farms and markets in the developing world. In the 1990s, 10 pairs of subtropical, midaltitude, and highland populations were developed as heterotic partners. These were subsequently used in the RRS programs at CIMMYT to enlarge genetic distance between partner groups and maximize the heterosis between inbred lines selected from complementary populations. The heterotic patterns include: Pop33 x Pop45, Pop42 x Pop44, Pop501 x Pop502, Pop401 x Pop402, Pop445 x Pop446, INT-A x INT-B, LAT-A x LAT-B, DR-A x DR-B, Z97EWA x Z97EWB, and Pop902 x Pop903. More recently, testers from each population have been used to test the hybrid performance of inbreds from the partner populations and to help assign new inbred lines to an appropriate heterotic group (Xia et al. 2005).

Molecular markers are a powerful complement to help define heterotic groups and to examine the relationships among inbred lines at the DNA level. Various molecular marker types have been used to investigate relationships among inbred maize lines from different heterotic groups (for example in tropical maize, see Xia et al. 2004). Markers can also be used to assign lines to new or currently existing heterotic groups (Dubreuil et al. 1996; Smith et al. 1997; Yuan et al. 2001).

### 14.7.2 Quality

Micronutrient deficiencies affect millions of people worldwide, particularly in tropical countries. Although maize can supply the minimum daily caloric requirement for humans, it is a poor source of the essential amino acids lysine and tryptophan. A diet in which maize predominates can lead to serious deficiency disorders such as pellagra and kwashiorkor. Mertz et al. (1964) discovered the mutant *opaque 2* (*o2*) that increases the lysine content in maize endosperm. Unfortunately, this gene is associated with inferior agronomic traits, including brittleness and insect susceptibility. However, with the discovery of “modifier genes” (*mo2*) that alter the soft, starchy texture of the endosperm, maize breeders developed hard endosperm *o2* mutants designated as “Quality Protein Maize” (Prasanna et al. 2001). These have the phenotypes and yield potential of normal maize while maintaining the increased lysine content of *o2*. As summarized by Bjarnason and Vasal (1992) and Krivanek et al. (2007), breeding of QPM varieties requires manipulation of three genetic systems: 1) the *opaque-2* (*o2*) gene must be in its homozygous recessive form, thereby reducing the rate of transcription of genes encoding zein proteins, which contain very small quantities of lysine and tryptophan; 2) modifier genes of the *o2* gene must be selected, to modify the undesirable soft and chalky (opaque) kernel features that are typical of *opaque-2* maize; and 3) additional (non-*o2*) genes affecting lysine and tryptophan concentration in grain must be selected to ensure that concentrations of these amino acids are within the high range of variation observed for maize.

Using SSRs and backcross breeding, Babu et al. (2004) developed maize lines that had twice the amount of lysine and tryptophan than the native lines and recovered up to 95% of the recurrent parent genome. Yang et al. (2005) reported a new lysine mutant, *o16*, which contained a similar level of lysine content to *o2*, but was located on a different chromosome. The genetic effect of *o16* needs to be confirmed under different genetic backgrounds. Then MAS for combining both *o2* and *o16* alleles will help develop new high lysine maize varieties.

### **14.7.3 Abiotic stresses**

The most important abiotic stress in many tropical countries, particularly in Africa, is drought, which affects agricultural production in about 60% of the land area in the tropics. Other abiotic stresses in the tropics include low soil fertility stress, soil acidity and high aluminum saturation, extreme temperatures, waterlogging, and salinity. Maintenance of root elongation is an important adaptive response to drought conditions. In addition, abscisic acid accumulation is required for root growth maintenance under water deficits (Leach et al. 2006). Short anthesis-silking interval (ASI) has been used as an important criterion for drought tolerance in maize. Thus, CIMMYT initiated a major marker-assisted breeding program to transfer five genomic regions involved in the expression of short ASI from Ac7643, a drought tolerant line, to CML247, an elite tropical breeding line (Ribaut et al. 1996, 1997). As a result, the best five marker assisted backcrossing-derived hybrids yielded, on average, at least 50% more than the control hybrids under water stress conditions (Ribaut et al. 2002; Ribaut and Ragot 2007). However, drought tolerance is genetically so complex that success stories from public sector MAS program are limited, or have so far been negligible in maize (Tuberosa and Salvi 2006). With all the recent technological breakthroughs (identifying and pyramiding QTL of minor effects from diverse germplasm resources) it is likely that genomics-assisted breeding will soon lead to the release of cultivars improved for quantitative traits.

### **14.7.4 Biotic stresses**

Diseases that are of a global nature and occur in most maize growing environments include leaf blights, leaf rusts, leaf spots, stalk rots and ear rots. Diseases that are of regional economic importance in the tropics include:

- Asia - downy mildews, which are also spreading to some parts of Africa and the Americas
- Africa - maize streak virus and the parasitic weed *Striga*
- Latin America - maize stunt and tar spot

There are several MAS reports regarding various biotic stresses but we will focus here on just three. The first example is MAS for European corn borer (ECB)

(*Ostrinia nubilalis* Hubner). Two separate experiments were conducted to assess the efficiency of both phenotype-based selection and MAS for second generation ECB tolerance and for stalk strength (Flint-Garcia et al. 2003b). In some populations MAS was more effective than phenotypic selection, although in some other populations the opposite was true. In some cases MAS was effective for selection of resistance and susceptibility, whereas in others MAS was only effective for selection of susceptibility. Finally, MAS for QTL from some sources was much more effective than MAS for QTL from other sources. In a similar study, no significant difference was observed between the products of MAS and the products of phenotypic selection (Willcox et al. 2002). In a third study, selection using MAS data only was less efficient than phenotypic selection, except when combining marker and phenotypic data which increased the relative efficiency, but only by 4% (Bohn et al. 2001).

In summary, MAS has been widely used in large breeding companies for both major gene and QTL controlled traits, while in the public sector MAS applications have generally focused on simply inherited disease and insect resistances. In tropical maize, major efforts have been devoted to genetic mapping for drought tolerance, although as yet without any resultant MAS successes. From several reports comparing MAS and phenotypic selection, MAS does not always provide a better selection response than conventional phenotypic selection. For example, Moreau et al. (2004) characterized 300  $F_{3;4}$  families derived from inbred an early European flint inbred and an early dent inbred from USA using 93 markers and phenotypic evaluation in multiple environments. Three methods of selection were applied – (i) two cycles of conventional phenotypic selection, (ii) two cycles of MAS based on an index combining phenotypic values and QTL genetic values, and (iii) one cycle of combined MAS followed by two cycles of selection based only on the QTL effects estimated in the first generation. In this study, the allele frequencies showed that selection using markers was very efficient only for fixing favorable QTL in the initial population. Genetic gain was significant for each method of selection. However, the differences between phenotypic selection and combined MAS were not significant. Two additional cycles of MAS using only marker data did not improve significantly the genetic value of the population, indicating that QTL effects estimated in the initial population were not stable due to epistasis and/or QTL  $\times$  E interactions. In many cases, however, MAS does not need to be superior to phenotypic selection to still have a significant impact on the overall breeding efficiency, especially when the cumulative effects of multiple traits are considered.

## 14.8 Future Perspectives

Despite the abundance of recent technological breakthroughs, the overall contribution of genomics-assisted breeding to the release of maize cultivars improved for quantitative traits such as drought tolerance has so far been negligible (Tuberosa and Salvi 2006). This may be because the majority of achievements in maize genomics have been based on temperate maize germplasm where drought tolerance is

less important than it is in tropical maize germplasm. However, markers and gene sequences can still be valuable for tropical maize, although clearly they must first be carefully validated. There are significant differences in applied genomics between temperate and tropical maize. Technology transfer from temperate maize to tropical maize and capacity building in tropical countries are needed for improvement of tropical maize. Comparative genomics across tropical maize germplasm and temperate maize will help identify novel genes and alleles required for improvement of both temperate and tropical maize. Introgression of genes between temperate and tropical maize should be emphasized in order to further improve maize in both regions. North-south collaborations in maize genomics should be strengthened through scientists in both theoretical and applied genomics.

As for the future of genetics and genomics studies in maize, we would like to add our support to the 15 priority areas identified by the 48th Maize Genetics Conference:

1. Turning the analysis of phenotypes and traits into a high-throughput endeavor without sacrificing agronomic relevance
2. Surveying diverse maize (teosintes and landraces) for novel genes, genetic polymorphisms and phenotypic variation
3. Simultaneously manipulating multiple alleles for scores of genes (QTL) across diverse genetic backgrounds
4. Using proteomics/mass spectrometry as a tool for the analysis of maize mutants/QTL
5. Approaches for mapping all the mutants to the gene space so one can quickly move from phenotype to gene candidate
6. A complete collection of all ESTs from all tissues and developmental stages to make more complete microarrays (for example, meiotic genes are greatly under-represented in GSS and EST collections)
7. Sequence-indexed collections for all the tools related to gene discovery including TILLING, MTMdB, PML, Rescue*Mu*, Uniform*Mu*, and *Ac/Ds*
8. More reverse genetics resources, i.e., more mutants in more genes (TUSC, Tilling etc)-targeted gene disruption strategies (Zn finger nucleases, etc)
9. Additional expression profiling tools or informational resources (e.g., detailed analysis of antisense expression, active promoter mapping, and alternative splicing)
10. Sequencing-based expression profiling platforms and their advantages and disadvantages over current hybridization based approaches
11. The B73 genome sequence and its annotation (and even ESTs) for proteomics/mass spectrometry analysis of other inbred lines and (distant) landraces (e.g. tropical highland varieties)
12. Better events (single or low transgene copy, site specific insertion to minimize the transgene expression variation due to transgene random integration on the chromosome)
13. A few transformable inbred lines amendable for *Agrobacterium*-mediated transformation

14. Cost effective plant transformation: introduction of GFP, tap-tagged fusion proteins of choice under native or conditional promoters
15. Strategies, tools, and policies that can be developed to increase data submission into the various public databases

We can expect that the rapid developments across maize genetics and genomics, although currently based mainly on temperate maize germplasm, will be transferable and increasingly valuable for tropical maize improvement. Applied maize genomics in tropical countries should focus on the areas specific to tropical maize including: (1) fingerprinting of tropical maize germplasm including adapted landraces; (2) establishing distinct “haplotypes” for tropical maize germplasm; (3) allele mining and gene discovery from tropical germplasm; (4) understanding of genotype-by-environment interactions that are specific to the tropics; (5) developing decision support tools that are more suitable for developing countries and institutions in the tropics; (6) developing information and data management systems that facilitate North-South collaborations; and (7) establishing networks and supporting systems that promote applications of genomics in maize breeding. With the resolution of many practical, logistical and genetical bottlenecks in MAS (review by Xu and Crouch 2008) and the ongoing development of powerful decision support tools for molecular plant breeding (Wang et al. 2007), it can be expected that genomics-assisted breeding will increasingly become a routine component of breeding programs focused on the development of tropical maize varieties.

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## References

- Ahern K, Deewatthanawong P, Conrad L, Schnable J, Dong Q, et al. (2006) A two component *Activator/Dissociation* platform for reverse and forward genetic analysis in maize. *Maize Genet Conf* (abstr) 48, P155
- Babu ER, Man VP, Gupta HS (2004) Combining high quality protein and hard endosperm traits through phenotypic and marker assisted selection. In: Fisher T (ed) *New Directions for a Diverse Planet*. Proc 4th Intl Crop Sci Congress, Published on CDROM. Website [www.cropscience.org.au](http://www.cropscience.org.au).
- Bai L, Singh M, Lauren Pitt L, Sweeney M, Brutnell TP (2007) Generating novel allelic variation through activator (Ac) insertional mutagenesis in maize. *Genetics* 175:981–992
- Barrière Y, Argillier O (1993) Brown-midrib genes of maize: A review. *Agronomie* 13:865–876
- Barrière Y, Guillet C, Goffner D, Pichon M. (2003) Genetic variation and breeding strategies for improved cell digestibility in annual forage crops: a review. *Animal Res* 52:193–228
- Batley J, Barker G, O’Sullivan H, Edwards KJ, Edwards D (2003) Mining for single nucleotide polymorphisms and insertions/deletions in maize expressed sequence tag data. *Plant Physiol* 132:84–91
- Beavis WD (1998) QTL analysis: power, precision and accuracy. pp 145–162. In: Paterson AH (ed) *Molecular Dissection of Complex Traits*. CRC Press, Boca Raton, FL

- Beavis WD, Grant D (1991) A linkage map based on information from 4 F<sub>2</sub> populations of Maize (*Zea mays* L.). *Theor Appl Genet* 82:636–644
- Bedell JA, Budiman MA, Nunberg A, Citek RW, Robbins D, et al. (2005) Sorghum genome sequencing by methylation filtration. *PLoS Biol* 3:0103–0115
- Bellon MR, Berthaud J (2004) Transgenic maize and the evolution of landrace diversity in Mexico. The importance of farmers' behavior. *Plant Physiol* 134:883–888
- Bernier, J., Kumar, A., Venuprasad, R., Spaner, D., and Atlin, G. (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci* 47, 505–517.
- Betrán FJ, Ribaut JM, Beck D, Gonzalez de León D (2003) Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. *Crop Sci* 43:797–806
- Biswas GCG, Ransom C, Sticklen M (2006) Expression of biologically active *Acidothermus* cellulolytic endoglucanase in transgenic maize plants. *Plant Sci* 171:617–623
- Bjarnason M, Vasal SK (1992) Breeding of quality protein maize (QPM). *Plant Breed Rev* 9:181–216
- Bohn M, Khairallah MM, González-de-León D, Hoisington DA, Utz HF, et al. (1996) QTLs mapping in tropical maize: I. Genomic regions affecting leaf feeding resistance to sugarcane borer and other traits. *Crop Sci* 36:1352–1361
- Bohn M, Khairallah MM, Jiang C, González-de-León D, Hoisington DA, et al. (1997) QTL mapping in tropical maize: II. Comparison of genomic regions for resistance to *Diatraea* spp. *Crop Sci* 37:1892–1902
- Bohn M, Groh S, Khairallah MM, Hoisington DA, Utz HF, et al. (2001) Re-evaluation of the prospects of marker-assisted selection for improving insect resistance against *Diatraea* spp. in tropical maize by cross validation and independent validation. *Theor Appl Genet* 103:1059–1067
- Bommert PB, Lunde C, Nardmann J, Vollbrecht E, Running PM, et al. (2005) *thick tassel dwarf1* encodes a putative maize orthologue of the *Arabidopsis* CLAVATA1 leucine-rich receptor-like kinase. *Development* 132:1235–1245
- Bortiri E, Jackson D, Hake S (2006a) Advances in maize genomics: the emergence of positional cloning. *Curr Opin Plant Biol* 9:164–171
- Bortiri E, Chuck G, Vollbrecht E, Rochefort TF, Martienssen R, et al. (2006b) *ramosa2* encodes a LOB domain protein that determines the fate of stem cells in branch meristems of maize. *Plant Cell* 18:574–585
- Briggs WH, McMullen M, Gaut BS, Doebley J (2006) QTL analysis of morphological traits in a large maize-teosinte backcross population. *Maize Genet Conf (abstr)* 48:T24
- Buckler ES, Gaut BS, McMullen MD (2006) Molecular and functional diversity of maize. *Curr Opin. Plant Biol* 9:172–176
- Burr B, Burr F, Thompson KH, Albersten M, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. *Genetics* 118:519–526
- Calderon-Vazquez C, Ibarra-Laclette E, Caballero-Perez J, Herrera-Estrella A, Martinez de la Vega O, et al. (2006) Transcriptome analysis of the low-phosphorus responses in roots and shoots of a phosphorus-efficient *Zea mays* line identifies alterations of several metabolic and physiological processes. *Maize Genet Conf (abstr)* 48:P203
- Causse M, Santoni S, Damerval C, Maurice A, Charcosset A, et al. (1996) A composite map of expressed sequences in maize. *Genome* 39:418–432
- Chen HD, Guo L, Fu Y, Enrich SJ, Ronin YI, et al. (2007) High-density genetic map of maize genes. *Maize Genet Conf (abstr)* 49, P 141
- Cheung VG, Spielman RS (2002) The genetics of variation in gene expression. *Nat Genet* 32 (suppl.):522–525
- Ching A, Caldwell KS, Jung M, Dolan M, Smith OS, et al. (2002) SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. *BMC Genet* 3:19
- Cho RJ, Mindrinos M, Richards DR, Sapolsky RJ, Anderson M, et al. (1999) Genome-wide mapping with biallelic markers in *Arabidopsis thaliana*. *Nat Genet* 23:203–207

- Coe EH, Hoisington DA, Neuffer MG (1987) Linkage map of corn (maize) (*Zea mays* L.). *Maize Genet Coop Newsl* 61:116–147
- Coe E, Cone K, McMullen M, Chen S-S, Davis G, et al. (2002) Access to the maize genome: An integrated physical and genetic map. *Plant Physiol* 128:9–12
- Comai L, Young K, Till BJ, Reynolds SH, Greene EA, et al. (2004) Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. *Plant J* 37:778–786
- Cone KC, McMullen MD, Bi IV, Davis GL, Yim Y-S, et al. (2002) Genetic, physical, and informatics resources for maize. On the road to integrated map. *Plant Physiol* 130:1598–1605
- Cooper M, Smith OS, Graham G, Arthur L, Feng L, et al. (2004) Genomics, genetics, and plant breeding: A private sector perspective. *Crop Sci* 44:1907–1913
- Cowperthwaite M, Park W, Xu Z, Yan X, Maurais SC, et al. (2002) Use of the transposon *Ac* as a gene-searching engine in the maize genome. *Plant Cell* 14:713–726
- Crosbie TM, Eathington SR, Johnson GR, Edwards M, Reiter R, et al. (2006) Plant breeding: past, present, and future. pp. 3–50. In: Lamkey KR, Lee M (eds) *Plant Breeding: The Arnel R. Hallauer International Symposium*. Blackwell Publishing, Ames, Iowa
- Crossa J, Vargas M, Van Eeuwijk FA, Jiang C, Edmeades GO, et al. (1999) Interpreting genotype  $\times$  environment interaction in tropical maize using linked molecular markers and environmental covariables. *Theor Appl Genet* 99:611–625
- Danilova T, Lamb J, Bauer M, Meyer J, Birchler J (2006) Development of PCR based FISH probes for identification of maize mitotic chromosomes. *Maize Genet Conf (abstr)* 48:P74
- Davis DW, Cone KC, Chomet P, Cox D, Brady S, et al. (2000) Maize whole-genome radiation hybrids: a progress report. *Plant Animal Genome Conf* 8:P255
- Davis GL, McMullen MD, Baysdorfer C, Musket T, Grant D, et al. (1999) A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. *Genetics* 152:1137–1172
- Dear PH, Cook PR (1993) Happy mapping: linkage mapping using a physical analogue of meiosis. *Nucleic Acids Res* 21:13–20
- Doebley J, Stec A, Gustus C (1995) *Teosinte branched 1* and the origin of maize: Evidence for epistasis and the evolution of dominance. *Genetics* 141:333–346
- Doebley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. *Nature* 386:485–488
- Dorweiler J, Stec A, Kernicle J, Doebley J (1993) *Teosinte glume architecture 1*: a genetic locus controlling a key step in maize evolution. *Science* 262:233–235
- Dubreuil P, Dufour P, Drejci E, Causse M, de Vienne D, et al. (1996) Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. *Crop Sci* 36:790–799
- Dvorak J, Luo MC, Yang ZL (1998) Restriction fragment length polymorphism and divergence in the genomic regions of high and low recombination in self-fertilizing and cross-fertilizing *Aegilops* species. *Genetics* 148:423–434.
- Dwivedi SL, Crouch JH, Mackill DJ, Xu Y, Blair MW, et al. (2007) The molecularization of public sector crop breeding: progress, problems and prospects. *Adv Agron* 95:163–318.
- Eathington SR (2005) Practical applications of molecular technology in the development of commercial maize hybrids. In: *Proc 60th Ann Corn and Sorghum Seed Res Conf*. American Seed Trade Association, Washington, D.C.
- Edwards MD, Helentjaris T, Wright S, Stuber CW (1992) Molecular-marker-facilitated investigations of quantitative trait loci in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. *Theor Appl Genet* 83 :765–774
- Emrich SJ, Aluru S, Fu Y, Wen TJ, Narayanan M, et al. (2004) A strategy for assembling the maize (*Zea mays* L.) genome. *Bioinformatics* 20:140–147
- Emrich SJ, Barbazuk WB, Li L, Schnable PS (2007) Gene discovery and annotation using LCM-454 transcriptome sequencing. *Genome Res* 17:69–73
- Evans MMS (2007) The *indeterminate gametophyte1* gene of maize encodes a LOB domain protein required for embryo sac and leaf development. *Plant Cell* 19:46–62

- Falque M, Décousset L, Dervins D, Jacob AM, Joets J, et al. (2005) Linkage mapping of 1454 new maize candidate gene loci. *Genetics* 170:1957–1966
- Fernandes J, Brendel V, Gai X, Lal S, Chandler VL, et al. (2002) Comparison of RNA profiles based on maize expressed sequence tag frequency analysis and micro-array hybridization. *Plant Physiol* 128:896–910
- Figueroa D, Amarillo I, Ring B, Strobel C, Lawrence C, et al. (2006) Constructing a cytogenetic map of maize core bin markers in oat addition lines using sorghum BACs as FISH probes. *Maize Genet Conf* (abstr) 48: P71
- Flint-Garcia SA, Thornsberry JM, Buckler IV ES (2003a) Structure of linkage disequilibrium in plants. *Ann Rev Plant Biol* 54:357–374
- Flint-Garcia SA, Darrah LL, McMullen MD, Hibbard BE (2003b) Phenotypic versus marker-assisted selection for stalk strength and second-generation European corn borer resistance in maize. *Theor Appl Genet* 107:1331–1336
- Fraley R (2006) Presentation at Monsanto European Investor Day, 10 November 2006. [www.monsanto.com](http://www.monsanto.com)
- Fu H, Dooner HK (2002) Intraspecific violation of genetic colinearity and its implications in maize. *Proc Natl Acad Sci USA* 99:9573–9578
- Gardiner JM, Coe EH, Melia-Hancock S, Hoisington DA, Chao S (1993) Development of a core RFLP map in maize using an immortalized F2 population. *Genetics* 134:917–930
- Gaut BS, Le Thierry I EM, Peek AS, Saukins MC (2000) Maize as a model for the evolution of plant nuclear genomes. *Proc Natl Acad Sci USA* 97:7008–7015
- Gilchrist EJ, Haughn GW (2005) TILLING without a plough: a new method with applications for reverse genetics. *Curr Opin Plant Biol* 8:1–5
- Grohn S, González-de-León D, Khairallah MM, Jiang C, Bergvinson M, et al. (1998) QTL mapping in tropical maize: III. Genomic regions for resistance to *Diatraea* spp. and associated traits in two RIL populations. *Crop Sci* 38:1062–1072
- Guillet-Claude C, Birolleau-Touchard C, Manicacci D, Rogowsky PM, Rigau J, et al. (2004) Nucleotide diversity of the *ZmPox3* maize peroxidase gene: Relationships between a MITE insertion in exon 2 and variation in forage maize digestibility. *BMC Genet* 5:19
- Haberer G, Young S, Bharati AK, Gundlach H, Raymond C, et al. (2005) Structure and architecture of the maize genome. *Plant Physiol* 139:1612–1624
- Hamblin MT, Aquadro CF (1999) DNA sequence variation and the recombinational landscape in *Drosophila pseudoobscura*: a study of the second chromosome. *Genetics* 153:859–869
- Iniguez AL, Gardiner J, Hogan M, Smith A, Buell R, et al. (2006) Antisense expression analysis in the maize transcriptome and microarray crossplatform comparisons. *Maize Genet Conf* (abstr) 48:P2
- Jansen RC, Nap J-P (2001) Genetical genomics: the added value from segregation. *Trends Genetics* 17:388–391
- Jia J, Fu J, Zheng J, Zhou X, Huai J, et al. (2006) Annotation and expression profile analysis of 2073 full-length cDNAs from stress-induced maize (*Zea mays* L.) seedlings. *Plant J* 48:710–727
- Jung M, Ching A, Bhattaramakki D, Dolan M, Tingey S, et al. (2004) Linkage disequilibrium and sequence diversity in a 500-kbp region around the *adh1* locus in elite maize germplasm. *Theor Appl Genet* 109:681–689
- Kaeppeler SM (1997) Quantitative trait locus mapping using sets of near-isogenic lines: relative power comparisons and technical considerations. *Theor Appl Genet* 95:384–192
- Khairallah MM, Bohn M, Jiang C, Deutsch JA, Jewell DC, et al. (1998) Molecular mapping of QTL for southwestern corn borer resistance, plant height and flowering in tropical maize. *Plant Breed* 117:309–318
- Kolkman JM, Conrad LJ, Farmer PR, Hardeman K, Ahern KR, et al. (2005) Distribution of *Activator* (*Ac*) throughout the maize genome for use in regional mutagenesis. *Genetics* 169:981–995
- Kollipara KP, Saab IN, Wych RD, Lauer MJ, Singletary GW (2002) Expression profiling of reciprocal maize hybrids divergent for cold germination and desiccation tolerance. *Plant Physiol* 129:974–992

- Komatsu K, Maekawa M, Ujiie S, Satake Y, Furutani I, et al. (2003) LAX and SPA: major regulations of shoot branching in rice. *Proc Natl Acad Sci USA* 100:11765–11770
- Koumbaris G, Bass HW (2003) A new single-locus cytogenetic mapping system for maize (*Zea mays* L.): overcoming FISH detection limits with marker-selected sorghum (*S. propinquum* L.) BAC clones. *Plant J* 35:647–659
- Krill A, Hoenkenga O, Kirst M, Kochian L, Buckler E (2006) Association analysis of candidate genes for aluminum tolerance in maize. *Maize Genet Conf (abstr)* 48:P210
- Krivanek AF, De Groote H, Gunaratna NS, Diallo AO, Friesen D (2007) Breeding and disseminating quality protein maize (QPM) for Africa. *African J Biotechnol* 6:312–324
- Kronmiller B, Werner K, Wise R (2006) TE Nest: Automated chronological annotation and visualization of maize nested transposable elements. *Maize Genet Conf (abstr)* 48:P58
- Lanza LLB, Souza Jr CL, Ottoboni LLM, Vieira MLC, Souza AP (1997). Genetic distance of inbred lines and prediction of maize single cross performance using RAPD markers. *Theor Appl Genet* 94:1023–1030
- Laurie CC, Chasalow SD, LeDeaux JR, McCarroll R, Rush D, et al. (2004) The genetic architecture of response to long-term artificial selection for oil concentration in the maize kernel. *Genetics* 168:2141–2155
- Lê Q, Gutiérrez-Marcos JF, Costa LM, Meyer S, Dickinson HG, et al. (2005) Construction and screening of subtracted cDNA libraries from limited populations of plant cells: a comparative analysis of gene expression between maize egg cells and central cells. *Plant J* 44:167–178
- Leach K, Davis D, Maltman R, Hejlek L, Nguyen H, et al. (2006) Genetic diversity of maize primary root growth and abscisic acid content to water stress. *Maize Genet Conf (abstr)* 48:P219
- Lee M, Sharopova N, Beavis WD, Grant D, Katt M, et al. (2002) Expanding the genetic map of maize with the intermated B73 x Mo17 (IBM) population. *Plant Mol Biol* 48:453–461
- Li ZK, Luo LJ, Mei HW, Wang DL, Shu QY, et al. (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. *Genetics* 158:1737–1753
- Lima MLA, de Souza CL, Bento DAV, de Souza AP, Carlini-Garcia LA (2006) Mapping QTL for grain yield and plant traits in a tropical maize population. *Mol Breed* 17:227–239
- Lin C, Shen B, Xu Z, Dooner H (2006) Isolation and characterization of maize sesquiterpene cyclase2 (*stc2*) gene involved in insect resistance. *Maize Genet Conf (abstr)* 48:P21
- Lisch D (2002) Mutator transposons. *Trends Plant Sci* 7:498–504
- Liu K, Goodman M, Muse S, Smith JS, Buckler ED, et al. (2003) Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. *Genetics* 165:2117–2128
- Liu W, Gao Y, Teng F, Shi Q, and Zheng Y (2006) Construction and genetic analysis of mutator insertion mutant population in maize Construction and Genetic analysis of the maize mutator-transposon insertional mutant pool. *Chinese Sci Bull* 51:2604–2610
- Lizarraga Guerra R, Gibbon B, Larkins B (2006) Genetic analysis of opaque2 modifier genes. *Maize Genet Conf (abstr)* 48:P15
- Lübberstedt TL, Zein I, Andersen JR, Wenzel G, Krützfeldt B, et al. (2005) Development and application of functional markers in maize. *Euphytica* 146:101–108
- Luo LJ, Li ZK, Mei HW, Shu QY, Tabein R, et al. (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. *Genetics* 158:1755–1771
- Ma Z, Dooner HK (2004) A mutation in the nuclear-encoded plastid ribosomal protein S9 leads to early embryo lethality in maize. *Plant J* 37:92–103
- Mangolin CA, Souza Jr CL, Garcia AAF, Garcia AF, Sibov ST, et al. (2004) Mapping QTLs for kernel oil content in a tropical maize population. *Euphytica* 137:251–259
- May BP, Liu H, Vollbrecht E, Senior L, Rabinowicz PD, et al. (2003) Maize-targeted mutagenesis: A knockout resource for maize. *Proc Natl Acad Sci USA* 100:11541–11546
- Meaburn E, Butcher LM, Schalkwyk LC, Plomin R (2006) Genotyping pooled DNA using 100K SNP microarrays: a step towards genomewide association scans. *Nucleic Acids Res* 34:No.4, e28

- Mertz ET, Bates LS, Nelson OE (1964) Mutant gene that changes protein composition and increases lysine content of maize endosperm. *Science* 145:279–280
- Messing J, Bharti AK, Karlowski WM, Gundlach H, Kim HR, et al. (2004) Sequence composition and genome organization of maize. *Proc Natl Acad Sci USA* 101:14349–14354
- Meyer P, Saedler H (1996) Homology-dependent gene silencing in plants. *Ann Rev. Plant Physiol Plant Mol Biol* 47:23–48
- Monde R-A, Till BJ, Sahn H, Laport R, Haywood N, et al. (2006) The Maize TILLING Project: progress report for year 3. *Maize Genet Conf* (abstr) 48:P31
- Moose S, Schneerman M, Zhang M, Zhang K, Schneeberger R, et al. (2006) Transcript profiling of the Illinois protein strains and derived germplasm. *Maize Genet Conf* (abstr) 48:P201
- Moreau L, Charcosset A, Hospital F, Gallais A (1998) Marker-assisted selection efficiency in populations of finite size. *Genetics* 148:1353–1365
- Moreau L, Charcosset A, Gallais A (2004) Experimental evaluation of several cycles of marker-assisted selection in maize. *Euphytica* 137:111–118
- NCI-NHGRI Working Group on Replication in Association Studies (2007) Replicating genotype-phenotype associations. *Nature* 447:655–660
- Nicholas FW (2006) Discovery, validation and delivery of DNA markers. *Aus J Exp Agric* 46:155–158
- Niebur WS, Rafalski JA, Smith OS, Cooper M (2004) Applications of genomics technologies to enhance rate of genetic progress for yield of maize within a commercial breeding program. In: Fhisher T (ed) *New Directions for a Diverse Planet. Proc 4<sup>th</sup> Intl Crop Sci Congr*, [www.cropscience.org.au](http://www.cropscience.org.au)
- Okagaki RJ, Kynast RG, Livingston SM, Russell CD, Rines HW, et al. (2001) Mapping maize sequences to chromosomes using oat-maize chromosome addition materials. *Plant Physiol* 125:1228–1235
- Okagaki R, Jacobs M, Schneerman M, Kynast R, Buescher E, et al. (2006a) A comparison of centromere mapping techniques. *Maize Genet Conf* (abstr) 48:P68
- Okagaki R, Kynast R, Stec A, Schmidt C, Jacobs M, et al. (2006b) Oat-maize addition and radiation hybrid lines for the physical and genetic mapping of the maize genome. *Maize Genet Conf* (abstr) 48:P149
- Ortiz R, Crouch JH, Iwanaga M, Sayre K, Warburton M, et al. (2006) Agriculture and energy in developing countries: Bio-energy and Agricultural Research-for-Development. IFPRI “2020 Focus” Policy Brief #14 ([www.ifpri.org/pubs/catalog.htm#focus](http://www.ifpri.org/pubs/catalog.htm#focus))
- Rosegrant MW, Msangi S, Sulser T, Valmonte-Santos R (2006) Biofuels and the Global food Balance. IFPRI “2020 Focus” Policy Brief #14 ([www.ifpri.org/pubs/catalog.htm#focus](http://www.ifpri.org/pubs/catalog.htm#focus))
- Palaisa KA, Morgante M, Williams M, Rafalski A (2003) Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci. *Plant Cell* 15:795–1806
- Paliwal RL (2000) Introduction to maize and its importance. pp. 1–3. In: Paliwal RL, Granados G, Lafitte HR, Violic AD, Marathe JP (eds) *Tropical Maize: Improvement and Production*. FAO, Rome
- Paliwal RL, Granados G, Lafitte HR, Violic AD, Marathe JP (2000) *Tropical Maize: Improvement and Production*. FAO, Rome. 363 pp
- Palmer LE, Rabinowicz PD, O’Shaughnessy AL, Balija VS, Nascimento LU, et al. (2003) Maize genome sequencing by methylation filtration. *Science* 302:2115–2117
- Pandey S, Gardner CO (1992) Recurrent selection for population, variety, and hybrid improvement in tropical maize. *Adv Agron* 48:1–87
- Pea G, Ferron S, Gianfranceschi L, Krajewski P, Pe ME (2006) Wide-scale survey of transcriptional heterosis in F<sub>1</sub> maize immature ear. *Maize Genet Conf* (abstr) 48:P207
- Podlich DW, Winkler CR, Cooper M (2004) Mapping as you go: an effective approach for marker-assisted selection of complex traits. *Crop Sci* 44:1560–1571
- Prasanna BM, Vasal SK, Kassahun B, Singh NN (2001) Quality protein maize. *Current Sci* 81:1308–1319

- Price AH (2006) Believe it or not, QTLs are accurate! *Trends Plant Sci* 11:213–216
- Quint M, Mihaljevic R, Dussle C, Xu ML, Melchinger A, et al. (2002) Development of RGA-CAPS markers and genetic mapping of candidate genes for sugarcane mosaic virus resistance in maize. *Theor Appl Genet* 105:355–363
- Quist D, Chapela IH (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414:41–543
- Ragot M, Gay G, Muller J-P, Durovray J (2000) Efficient selection for the adaptation to the environment through QTL mapping and manipulation in maize. pp. 128–130. In: Ribaut J-M, Poland D (eds) *Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments*. CIMMYT, México, D.F.
- Reif JC, Xia XC, Melchinger AE, Warburton ML, Hoisington DA, et al. (2004) Genetic diversity determined within and among CIMMYT maize populations of tropical, subtropical, and temperate germplasm by SSR markers. *Crop Sci* 44:326–334
- Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, et al. (2001) Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proc Natl Acad Sci USA* 98:11479–11484
- Ribaut JM, Ragot M (2007) Marker-assisted selection to improve drought adaptations in maize: the backcross approach, perspectives, limitations, and alternatives. *J Exp Bot* 58:351–360.
- Ribaut J-M, Hoisington DA, Deutsch JA, Jiang C, Gonzalez-de-Leon D (1996) Identification of quantitative trait loci under drought conditions in tropical maize 1. Flowering parameters and the anthesis-silking-interval. *Theor Appl Genet* 92:905–914
- Ribaut J-M, Jiang C, Gonzalez-de-Leon D (1997) Identification of quantitative trait loci under drought condition in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theor Appl Genet* 94:887–896
- Ribaut JM, Bänziger M, Betran J, Jiang C, Edmeades GO, et al. (2002) Use of molecular markers in plant breeding: drought tolerance improvement in tropical maize. In: Kang MS (ed) *Quantitative Genetics, Genomics, and Plant Breeding*. CABI Publishing, pp. 85–99
- Ribaut J-M, Bänziger M, Setter T, Edmeades G, Hoisington D (2004) Genetic dissection of drought tolerance in maize: a case study. pp. 571–611. In: Nguyen H, Blum A (eds) *Physiology and Biotechnology Integration for Plant Breeding*. Marcel Dekker Inc., New York
- Rosegrant MW, Msangi S, Sulser T, Valmonte-Santos R (2006) Biofuels and the Global food Balance. IFPRI “2020 Focus” Policy Brief #14 ([www.ifpri.org/pubs/catalog.htm#focus](http://www.ifpri.org/pubs/catalog.htm#focus))
- Schaeffer M, Sanchez-Villeda H, Gerau M, McMullen M, Coe E (2006) The New IBM Neighbors: genetic and physical probed sites. *Maize Genet Conf (abstr)* 48:P151
- Scheuring C, Barthelson R, Galbraith D, Betran J, Cothren JT, et al. (2006) Preliminary analysis of differential gene expression between a maize superior hybrid and its parents using the 57K maize gene-specific long-oligonucleotide microarray. *Maize Genet Conf (abstr)* 48:P193
- Schon CC, Utz HF, Groh S, Truberg B, Openshaw S, et al. (2004) Quantitative trait locus mapping based on resampling in a vast maize testcross experiment and its relevance to quantitative genetics for complex traits. *Genetics* 167:485–498
- Schroeder S, Sanchez-Villeda H, Flint-Garcia S, Houchins K, Yamasaki M, et al. (2006) Integrated software for SNP discovery in maize. *Maize Genet Conf (abstr)* 48:P50
- Settles AM (2005) Maize community resources for forward and reverse genetics. *Maydica* 50:405–414
- Settles M, Holding D, Tan B-C, Latshaw S, Suzuki M, et al. (2006) Maize sequence indexed knockouts using the UniformMu transposon-tagging population. *Maize Genet Conf (abstr)* 48:P180
- Sheen J (2001) Signal transduction in maize and Arabidopsis mesophyll protoplasts. *Plant Physiol* 127:1466–1475
- Singh M, Lewis PE, Hardeman K, Bai L, Rose JK, et al. (2003) *Activator* mutagenesis of the *pink scutellum 1/viviparous 7* locus of maize. *Plant Cell* 15:874–884
- Smith ME, Paliwal RL (1996) Contributions of genetic resources and biotechnology to sustainable productivity increases in maize. In: Watanabe K, Pebu E (eds.) *Plant Biotechnology and Plant Genetic Resources for Sustainability and Productivity*. Lande and Academic Press, Austin, TX

- Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, et al. (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): Comparisons with data from RFLPs and pedigree. *Theor Appl Genet* 95:163–173
- Stevens R, Paul C, Islam S, Wong J, Harjes C, et al. (2006) Genetic approaches to enhance provitamins A and total carotenoids in maize grain. *Maize Genet Conf (abstr)* 48:P217
- Stich B, Yu J, Melchinger AE, Piepho H, Utz HF, et al. (2007) Power to detect higher-order epistatic interactions in a metabolic pathway using a new mapping strategy. *Genetics* 176:563–570
- Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* 132:823–839
- Stupar RM, Springer NM (2006) *Cis*-transcriptional variation in maize inbred lines B73 and Mo17 leads to additive expression patterns in the F<sub>1</sub> hybrids. *Genetics* 173:2199–2210
- Swanson-Wagner R, Jia Y, Borsuk L, DeCook R, Nettleton D, Schnable P (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F<sub>1</sub> hybrid and its inbred parents. *Proc Natl Acad Sci USA* 103:6805–6810
- Szalma SJ, Buckler ES, Snook ME, McMullen MD (2005) Association analysis of candidate genes for maysin and chlorogenic acid accumulation in maize silks. *Theor Appl Genet* 110:1324–1333
- Szalma SJ, Hostert BM, LeDeaux JR, Stuber CW, Holland JB (2007) QTL mapping with near-isogenic lines in maize. *Theor Appl Genet* 114:1211–1228
- Taramino G, Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. *Genome* 39:277–287
- Tarter JA, Goodman MM, Holland JB (2004) Recovery of exotic alleles in semiexotic maize inbreds derived from crosses between Latin American accessions and a temperate line. *Theor Appl Genet* 109:609–617
- Tenaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF, et al. (2001) Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.). *Proc Natl Acad Sci USA* 98:9161–9166
- Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, et al. (2001) *Dwarf8* polymorphisms associate with variation in flowering time. *Nat Genet* 28:286–289
- Till BJ, Reynolds SH, Weil C, Springer N, Burtner C, et al. (2004) Discovery of induced point mutations in maize by TILLING. *BMC Plant Biol* 4:12
- Tomkins JP, Frisch DA, Byrum JR, Jenkins MR, Barnett LJ, et al. (2000a) Construction and characterization of a maize bacterial artificial chromosome (BAC) library for the inbred line LH132. *Maize Genet Coop Newsl* 74:18
- Tomkins JP, Frisch DA, Jenkins MR, Barnett LJ, Luo M, et al. (2000b) Construction and characterization of a maize bacterial artificial chromosome (BAC) library for the inbred line B73. *Maize Genet Coop Newsl* 74:18–19
- Tracy WF, Chandler MA (2006) The historical and biological basis of the concept of heterotic patterns in corn belt dent maize. pp. 219–233. In: Lamkey KR, Lee M (eds) *Plant Breeding: the Arnel R. Hallauer International Symposium*. Blackwell Publishing, Ames, IA
- Troyer AF, Rocheford TR (2002) Germplasm ownership: related corn inbreds. *Crop Sci* 42:3–11.
- Tsotsis B (1972) Objectives of industry breeders to make efficient and significant advances in the future. pp. 93–107. In: Wilkinson D (ed) *Proc 27<sup>th</sup> Ann Corn and Sorghum Res Conf*. American Seed Trade Association, Washington D.C.
- Tuberosa R, Salvi S (2006) Genomics-based approaches to improve drought tolerance of crops. *Trend Plant Sci* 11: 405–412
- Vargas M, van Eeuwijk FA, Crossa J, Ribaut J-M (2006) Mapping QTLs and QTL × environment interaction for CIMMYT maize drought stress program using factorial regression and partial least squares methods. *Theor Appl Genet* 112:1009–1023
- Vigouroux Y, Mitchell S, Matsuoka Y, Hamblin M, Kresovich S, et al. (2005) An analysis of genetic diversity across the maize genome using microsatellites. *Genetics* 169:1617–1630
- Vollbrecht E, Springer PS, Gol L, Buckler ES, Martienssen R (2005) Architecture of floral branch systems in maize and related grasses. *Nature* 436:1119–1125

- Wang J, Chapman SC, Bonnett DB, Rebetzke GJ and Crouch JH (2007) Application of population genetic theory and simulation models to efficiently pyramid multiple genes via marker-assisted selection. *Crop Science* 47:582–588.
- Wang H, Nussbaum-Wagler T, Li B, Zhao Q, Vigouroux Y, et al. (2005) The origin of naked grains of maize. *Nature* 436:714–719
- Wang RL, Stec A, Hey J, Lukens L, Doebley J (1999) The limits of selection during maize domestication. *Nature* 398:236–239
- Warburton ML, Xia X, Crossa J, Franco J, Melchinger AE, et al. (2002) Genetic characterization of CIMMYT inbred maize lines and open pollinated populations using large scale fingerprinting methods. *Crop Sci* 42:1832–1840
- Wen T, Qiu F, Guo L, Lee M, Russell K, et al. (2002) High-throughput mapping tools for maize genomics. *Maize Genet Conf (abstr)* 44:8
- Whitelaw CA, Barbazuk WB, Perteua G, Chan AP, Cheung F, et al. (2003) Enrichment of gene-coding sequences in maize by genome filtration. *Science* 302:2118–2120
- Widstrom NW, Butron A, Guo BZ, Wilson DM, Snook ME, et al. (2003) Control of preharvest aflatoxin contamination in maize by pyramiding QTL involved in resistance to ear-feeding insects and invasion by *Aaperigillus* spp. *Eur J Agron* 19:563–572
- Willcox MC, Khairallah MM, Bergvinson D, Crossa J, Deutsch JA, et al. (2002) Selection for resistance to Southwestern corn borer using marker-assisted and conventional backcrossing. *Crop Sci* 42:1516–1528
- Wilson LM, Whitt SR, Ibanez-Carranza AM, Goodman MM, Rocheford TR, et al. (2004) Dissection of maize kernel composition and starch production by candidate gene association. *Plant Cell* 16:2719–2733
- Wilson R, Wing R, McCombie WR, Martienssen R, Ware D, et al. (2006) Sequencing the maize genome. *Maize Genet Conf (abstr)* 48:T11
- Wright SI, Bi IV, Schroeder SG, Yamasaki M, Doebley JF, et al. (2005) The effects of artificial selection on the maize genome. *Science* 308:1310–1314
- Wu R, Zeng Z-B (2001) Joint linkage and linkage disequilibrium mapping in natural populations. *Genetics* 157:899–909
- Wu R, Ma C-S, Casella G (2002) Joint linkage and linkage disequilibrium mapping of quantitative trait loci in natural mapping populations. *Genetics* 160:779–792
- Xia XC, Reif JC, Hoisington DA, Melchinger AE, Frisch M, (2004) Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: I. Lowland tropical maize. *Crop Sci* 44:2230–2237
- Xia XC, Reif JC, Melchinger AE, Frisch M, Hoisington DA, et al. (2005) Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: II. Subtropical, tropical midaltitude, and highland maize inbred lines and their relationships with elite U.S. and European maize. *Crop Sci* 45:2573–2582
- Xiao J, Li J, Yuan L, Tanksley SD (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. *Genetics* 140:745–754
- Xiao W, Xu M, Zhao J, Wang F, Li J, Dai J (2006) Genome-wide isolation and mapping of resistance gene analogs. *Theor Appl Genet* 113:63–72
- Xu Y (2003) Developing marker-assisted selection strategies for breeding hybrid rice. *Plant Breed Rev* 23:73–174
- Xu Y, Crouch JH (2008) Marker-assisted plant breeding: from publications to practice. *Crop Sci* (in press)
- Yang W, Zheng Y, Zheng W, Feng R (2005) Molecular genetic mapping of a high-lysine mutant gene (*opaque-16*) and the double recessive effects with *opaque-2* in maize. *Mol Breed* 15:257–269
- Yim Y-S, Davis GL, Duru NA, Musket TA, Linton EW, et al. (2002). Characterization of three maize bacterial artificial chromosome libraries toward anchoring of the physical map to the genetic map using high-density bacterial artificial chromosome filter hybridization. *Plant Physiol* 130:1686–1696

- Yu L-X, Setter TL (2003) Comparative transcriptional profiling of placenta and endosperm in developing maize kernels in response to water deficit. *Plant Physiol* 131:568–582
- Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, et al. (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proc Natl Acad Sci USA* 94:9226–9231
- Yuan LX, Fu JH, Zhang SH, Liu X, Peng Z, et al. (2001) Heterotic grouping of maize inbred lines using RFLP and SSR markers. *Acta Agron Sinica* 27:149–156
- Zhang J, Lv X, Song X, Yan J, Song T, et al. (2006) Quantitative trait loci mapping for starch, protein, and oil concentrations with high-oil maize by SSR markers. *Maize Genet Conf (abstr)* 48:P233
- Zheng Y, Gao Y, Liu W, Yang W, Shi Q, et al. (2006) Construction and Genetic analysis of the maize mutator-transposon insertional mutant pool. *Maize Genet Conf (abstr)* 48:P165
- Zinselmeier C, Sun Y, Helentjaris T, Beatty M, Yang S, et al. (2002) The use of gene expression profiling to dissect the stress sensitivity of reproductive development in maize. *Field Crops Res* 73:111–121
- Zhu T, Xia Y, Chilcott C, Dunn M, Dace G, et al. (2006) Maize ultra high-density gene map for genome-assisted breeding. *Maize Genet Conf (abstr)* 48:P181