Indian J. Genet., 70(4): 315-327 (2010)

Review paper

Phenotypic and molecular diversity of maize landraces: characterization and utilization

B. M. Prasanna*

International Maize and Wheat Improvement Center (CIMMYT), ICRAF House, United Nations Avenue, Gigiri, Nairobi, Kenya

(Received: September 2010; Revised: October 2010; Accepted: November 2010)

Abstract

A well-characterized and well-evaluated germplasm collection would have greater chances of contributing to the development of new varieties, and consequently, greater realization of benefits for the resource-poor farmers. Landraces, the germplasm maintained by the farmers over the decades or centuries, were evolved and selected to thrive under particular environmental conditions and to meet local food preferences. Although maize originated in Mexico, landraces of this crop of worldwide importance are widely spread across the continents. India too forms a rich hub of diversity for maize landraces, particularly in the North-Eastern Himalayan (NEH) region. Maize landraces of Americas and Europe, and more recently of Asia, have been subjected to intensive molecular analyses, leading to significant insights regarding their diversity and population genetic structure. Comprehensive analysis of phenotypic and molecular diversity of the landraces is critical for their effective use in breeding programmes. Utilizing the rapidly advancing genome sequencing and genotyping technologies, along with phenotypic characterization through a global phenotyping network, are required for identifying trait-specific donors as well as favorable genes/alleles that can be channelized in breeding strategies.

Key words: Landraces, diversity, phenotypes, molecular markers, breeding, *Zea mays* L.

Introduction

From their region of domestication, many crop species have spread across the globe into new countries and environments to serve the ever growing human population as sources of food, feed, fiber and medicine. In their new environments, these crops have responded to natural as well as human selection pressures and differentiated into a range of adapted variants [1]. Parallel to creating such a vast and unprecedented diversity of crops, domestication and selection have also resulted in dwindling resources of genetic diversity. One such major factor and outcome is the 'bottleneck effect' which has predominantly been seen in many crop species [2, 3]. Genetic erosion and habitat destruction by modern agriculture has increased the importance of germplasm collection and characterization, as genetic variability is essential for present and future plant breeding programmes.

Matsuoka *et al*. [4] showed that the domestication of maize is based on a unique event, and that maize accessions from the highlands of Oaxaca in Mexico are genetically the closest to the wild ancestor of maize (*Zea mays* ssp. *parviglumis*). As the center of maize domestication and diversity, Mexico can be considered as one of the largest reservoirs of maize genetic resources. The spread of maize from its center of origin to various parts of the world has been as remarkable as its evolution into a cultivated and productive food plant. During the past century, the existing landraces were the bases for developing modern open pollinated varieties (OPVs), which have in turn begun to replace landraces in the developing world. Although worldwide about half of the non-temperate maize-growing area is still sown with landraces, this seems to be on a decreasing trend [5, 6]. While most of the genetic

---

*Corresponding author's e-mail: b.m.prasanna@cgiar.org

Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

variability is represented within and between landraces maintained by the traditional family farming systems, the main reason for accelerated genetic erosion was the steady substitution of landraces by a small number of improved varieties.

Is it possible to provide an accurate definition of a 'landrace'? Harlan [7] considered this as very difficult task due to the complexity. Zeven [8] defined 'landrace' as follows: "An autochthonous landrace is a variety with a high capacity to tolerate biotic and abiotic stresses, resulting in high yield stability and an intermediate yield level under a low input agricultural system". Irrespective of the definition, there are certain core principles that can define a landrace. Landraces usually consist of mixture of genotypes, all of which are reasonably well adapted to the region in which they evolve but which differ in detail as to specific adaptations to the particular conditions within the environment. They have evolved under subsistence agriculture and are still cultivated by farmers in regions of crop domestication and diversity. They may differ in reaction to insect-pests and diseases, adaptation to low soil fertility, and tend to be rather low yielding but dependable. They are more often adapted to crude land preparation, seeding, weeding and harvesting procedures of traditional agriculture. They are recognizable morphologically; farmers have named them usually by the region where the particular landrace is predominantly cultivated and importantly they are genetically diverse.

Geographical and racial diversity of maize

Wellhausen *et al*. [9] classified the Mexican maize collection, based on tassel type, ear and kernel characteristics, as proposed by Anderson and Cutler [10] into four major groups (i) Ancient Indigenous-consisting of Palomero Toluqueno, Nal-Tel, Chaplote and Arrocillo Amerillo, (ii) Pre-Columbian Exotic-comprising Cacahuacintle, Harinoso de Ocho, Oloton and Maiz Dulce (iii) Mestizos consisting of 13 new races arising out of the pre-Columbian exotic races with ancient indigenous or teosinte or perhaps *Tripsacum* also, and (iv) Modern Incipient consisting of four races that came into existence within historical times. Later a number of studies were conducted by various researches to describe races of maize from Central America to South America. The basis of classification were more or less similar, that is, they included photographs and means of many plants, tassel, ear and kernel characteristics and sometimes physiological characters as well as chromosome morphology and chromosome knob data.

The pre-Columbian farmers bred most of the major varieties of maize that exist today, including red corn, blue corn, yellow corn, flint corn, dent corn, sweet corn, popcorn, pod corn and flour corn. It also appears that at the earliest level of domestication, kernel size was small and was, thus, believed to be popcorn. Later, kernels of varying endosperm constitution and sizes have evolved. The largest kernels encountered are of Cuzco Gigante, which are about 2 cm in length and width and about 1 cm in thickness. All kinds of coloration are found in the endosperm (white, yellow and orange) and in the aleurone (colorless and various pinks, reds, yellows, browns and purples). Further, aleurone and/or pericarp may be patterned (stippled, marbled, speckled, dotted, striped, streaked etc) (Fig. 1). Most modern maize is either yellow or white, flint or dent, but American Indians still maintain maize types that are highly colored and floury in texture.

It may also be of interest to note that about 50% of the races are adapted to low altitudes (0–1000 m), almost 40% grow at elevations higher then 2000m, and slightly more than 10% are from intermediate elevations (1000–2000 m). Regarding endosperm types, about 40, 30, 20, 10, and 3 per cent are floury, flints, dents, popcorns and sweet corns, respectively [11]. The adaptation to different altitudes may be mainly the result of natural selection, whereas the distribution of different endosperm types may be primarily due to human preference.

To describe maize descendents from a common progenitor, Manglesdorf [12] assigned races to 'lineages'. The variability in various Latin American corn races was classified into six lineages (a) Palomero Toluqueno, a Mexican pointed seed popcorn, (b) Chaplote-Naltel comples of Mexico, (c) Pira Niranja of Columbia, the progenitor of tropical flint corn with orange endosperm, (d) Confit Morocho of Peru, the progenitor of all eight rowed corn, (e) Chullupi of Peru, the progenitor of all sweet corn and related starchy seeded form with globular ear, and (f) Kculli, the Peruvian dye corn, the progenitor of all races with complex of pericarp and aleurone colors. Goodman and Brown [13] reviewed the previous work done and established inter-racial relationships in Latin America, Mexico, Central America, West Indies, South America, lowland Northern South America, Amazon Basin and surrounding lowlands, lowlands Southern South America and the Andean region and USA. Nearly 300 maize races from various countries were described and well-documented by the National Academy of Sciences and National Research

Council, USA. These races have varied geographical distribution, adaptation as well as human preference characteristics. Some of the original maize races are still grown in America. At present, the CIMMYT Maize Gene Bank conserves more than 21,000 landrace accessions collected worldwide, especially from Latin America [14].

Diversity of maize landraces in India

The diversity of maize in India is interesting from an evolutionary viewpoint because of its distance from the center of diversity, and once introduced to India, they were subjected to local cultivation in small isolated pockets of the country. Although the racial diversity of maize in India is small when compared to the Americas but the selection for genetic uniformity and local adaptation indicate a potential for unique germplasm for the maize geneticists and breeders.

Extensive variability in plant, ear, and tassel characteristics is observed in Northeastern and Northwestern highlands of India. The social and cultural values of the tribes inhabiting the North-Eastern Himalayan (NEH) region played an important role in the conservation of maize landraces, offering significant diversity with respect to plant type, ear characters, quality, biotic and abiotic stress resistance etc. [15].

Although early reports suggest that the local varieties in the plains of India are fairly uniform and lack adequate variability [16, 17], there exist several maize landraces with special features in these regions, such as the ‘Sathi local’ (Punjab), ‘Basi local’, ‘Maalan local’ and ‘Dausa local’ (Rajasthan), ‘Jaunpur local’ (Uttar Pradesh) etc. [15]. Landraces of North-western Himalayan region also exhibit rich diversity in kernel type, colour and maturity. Maize landraces are also grown in the foothills of South India, around 3000 to 5000 feet in elevation. These were considered to be modified New England Flints which are very rapid in their maturity. These are particularly popular among tribals in Andhra Pradesh and Orissa and are called “hungry children food” because the crop is ready and eaten green (like a sweet corn) before the rice crop is ready for harvest [18].

The North Eastern Himalayan (NEH) region of India, comprising of Arunachal Pradesh, Assam, Meghalaya, Mizoram, Manipur, Nagaland, Tripura, Sikkim and some areas in the Northern region of West Bengal, is endowed with considerable genetic diversity of maize. Anderson [19] and Stonor and Anderson [20] carried out extensive studies on the variability of maize in these North-Eastern states. With the amount of maize diversity present in the region, they considered maize to have an Asiatic origin. Singh [21] identified as many as 15 distinct races of maize, each with a characteristic complex of traits associated with it. In a crop as highly cross-pollinated as maize, the conservation of these complex traits in a relatively pure form should normally be difficult to explain. However, owing to the geography of the region, its remoteness and the poor communication facilities, it is not surprising that different tribes have been able to maintain their own maize types over the centuries.

Singh [21] grouped Indian races into four categories for convenience: (i) primitive (ii) advanced or derived (iii) recent introductions, and (iv) hybrid races. The primitive group comprised of several races of popcorn, which had differentiated at various altitudes and under diverse conditions, was distributed throughout the eastern Himalayan region. This group included *Poorvi Botapa*, *Murli* sub-race of *Poorvi Botapa*, *Tirap Nag-Sahypung*, *Arun Tepi* and *Alok Sapa*.

Studies by a number of workers, including Stonor and Anderson [20] and Suto and Yoshida [22], showed that landraces with primitive characteristics (popcorn characters and high prolificacy) exist in the eastern Himalayan region (Sikkim in India and Bhutan). Dhawan [23] christened these unique landraces with high prolificacy as “Sikkim Primitives” (SP), whose New World progenitors seem to have disappeared. Often referred locally as ‘Murli makai’, Sikkim Primitives represent a totally distinct and little grown popcorn of Sikkim and adjoining areas, occuring at mid-elevation (6000-8000 feet) and is adapted to the moist tropical forest region. These local maize populations are often used as offerings in ceremonial use among the Buddhistic peoples of the NEH region [15]. They fail to flower when grown under long summer days. But when the day length is short the plant reaches the reproductive phase from vegetative growth phase [24].

Dhawan [23] studied only two collections of primitive maize from Sikkim (SP1 and SP2), but landraces with characteristics of SP are distributed throughout the NEH, including Bhutan and Nepal. Sachan and Sarkar [26] identified 13 different strains of the primitive maize from NEH region and designated them as ‘Sikkim Primitive strains’, although these came from Nagaland, Meghalaya, Tripura and Sikkim. Mangelsdorf [12] assigned Sikkim Primitives to the lineage of Palomero Toluqueno, an ancient indigenous race of Mexico. Based on information obtained from studies on botanical, C- and Q-banding, pachytene analysis, ethnobotany, and interpretation of the archaeological findings, Sachan et al. [25] gave the hypothesis that the pre-historic wild corn, which evolved in the extreme desert environment in the Tehuacan valley of Mexico, is well preserved in the form of Sikkim Primitives.

The most important physiological attributes of SP maize are a complete lack of apical dominance, prolificacy (5-9 ears) with uniformity in ear size, erect leaves for developing maize varieties for high population density, top bearing habit and drooping tassel to ensure effective fertilization [26]. The ears are small, slender and cylindrical or sometimes tapering, 6-12 cm in length, 8-12 irregular rows, creamish to yellow and have round popcorn kernels with pointed beaks. It stays green after maturity, thus it is also good for fodder purposes. It is resistant to stalk rot and has tremendous stem strength which prevents lodging.

The placement of ear is one of the most important characteristics of SP. Ears in SP occupy positions in the upper one-third of the plant height rather than the middle so that photosynthate is translocated right from subtending and upper leaves to the sink (cob). The middle leaves and lower leaves are unable to photosynthesize because of chlorophyll breakdown and loss of functional chloroplasts. Also, SP maize has erect leaves near the top of the canopy that can synthesize food more rapidly than modern maize in which leaves are oriented horizontally [26].

Factors shaping the diversity of maize landraces

One of the most important issues in maize evolution is how to explain the extraordinary morphological and genetic diversity that exists among the maize landraces [4]. A number of regions in Mexico are home to a great collection of maize landraces. Chiapas, Chihuahua, Durango, Guanajuato, Guerrero, Jalisco, Oaxaca, Puebla top the list in maintaining this variety. A maize landrace is defined by the farmer in terms of ear characteristics; ear type will be maintained by the farmers through conservative selection in spite of considerable gene flow [27, 28]. In addition to farmer management, the biology of the species also plays a major role in structuring the maize populations [29].

Diversity among landraces is influenced by several factors. Geographical landscapes, climatic conditions, agriculture ecosystems, biological diversity, and endemic and ethical cultures are some of those. Geographical divergence arises from centuries of human selection, isolation and relatively infrequent migration between populations. Perales *et al*. [30] analyzed diversity of maize among ethno-linguistic groups in Chiapas Mexico, and observed distinct maize populations among communities of two groups.

There are various reasons attributed to a farmer's selection, maintenance and retention of landraces; these include storage, cooking, nutritional, and processing qualities, besides historical and cultural factors, such as dietary diversity and use in traditional foods or religious ceremonies. Another important reason is the agronomic advantage: some landraces are considered more suited to traditional intercropping patterns, have longer or shorter growing cycles, or are more resistant to local biotic and abiotic stresses. Yield stability is also a critical factor for retaining the folk varieties or landraces. For example, the Hopi tribe in South America still retains the blue maize landraces because these are adapted to drought, have a short growing season and meet their cultural requirements.

Utility of maize landraces in breeding strategies

There are several studies demonstrating the utility of landraces of maize in breeding programmes, with respect to development of broad-based pools and improved cultivars [31, 32], possessing various agronomically and nutritionally important traits, as exemplified below, particularly in the Indian context.

Agronomic performance

Using Suwan-1, a popular OPV from Thailand, a composite "Parbhat" has been developed at Punjab Agricultural University, Ludhiana, which shows multiple disease resistance, high yield and stability in performance [31, 32]. Several inbred lines and hybrids that are well adapted to hill areas have been derived at Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, Uttarakhand, using landraces from Jammu & Kashmir as well as the Uttarakhand states in India. The popular hybrids include Him-129 (yellow, flint, 85-90 days maturity, highly tolerant to leaf blight); Him-128 and several 'Vivek' hybrids.

In India, multi-location phenotypic trials with a diverse set of maize landrace accessions were undertaken under the ICAR National Fellow Project during 2005-2008 at Tadong (in Sikkim), Almora (in Uttarakhand), Bajaura (in Himachal Pradesh), Delhi and Hyderabad (in Andhra Pradesh) for grain yield and related traits, besides prolificacy (multiple ears per plant), turcicum leaf blight resistance (at Naganahalli in

Karnataka and Hawalbagh in Uttarakhand), total carotenoid content and kernel micronutrient (Fe and Zn) concentration [15, 33]. These studies led to identification of several promising landraces, including accessions from Himachal Pradesh, Jammu & Kashmir, Uttarakhand, Gujarat, Arunachal Pradesh, Jharkhand, Bihar, West Bengal, Rajasthan, Madhya Pradesh, Andhra Pradesh, Karnataka and Sikkim. Six different pools, including Specialty Corn pools, have also been formulated using such promising accessions.

In general, maize landraces are characterized by high G x E for vegetative characters. Ear and kernel traits are relatively stable over different environments. Using race structure of the collection, CIMMYT Maize Germplasm Bank has chosen core subsets of the races for sampling representative diversity within the race [14, 34], and developed a CD-ROM on the preliminary core subsets of Latin America race accessions.

Biotic stress resistance

A number of studies in maize have indicated the potential of the landraces in improving biotic stress tolerance. Reid *et al*. [35] investigated a set of the indigenous races of Mexico, differing in altitudinal adaptations and found them to be resistant to the European corn borer, *Ostrinia nubilalis*. The Nal-tel, Chapalote and Palomero landraces from Mexico shows resistance to the maize weevil, *Sitophilus zeamais* [36].

Abiotic stress tolerance

Tuxpeño maize was domesticated in the Oaxaca-Chiapas region. It is a highly productive lowland race, is well suited to fertile soils, and is widely used in maize improvement programmes. Tuxpeño Sequía is an early maturing (matures in 60 days) and a drought tolerant sub-population of Tuxpeño landrace. The early maturing feature itself has endowed Tuxpeño Sequía with a drought-escaping trait. Also, adjustment in the degree of stomatal opening could be another mechanism that controls the loss of water [37].

Studies carried out at VPKAS, Almora, Uttarakhand, using 15 'Sikkim Primitives' collected from the NEH region revealed that these landraces could serve as a potential source for abiotic and biotic stress tolerance in maize breeding programmes due to their long history of survival under unfavourable conditions [38]. Rodriguez *et al*. [39] analyzed the physiological aspects of Tuxpeño maize with relevance to its drought tolerant characteristics.

Quality traits

The '100 generations of corn' experiment for selecting maize strains with high and low kernel oil and protein content at the University of Illinois, was started using the 'Burr's white' landrace of the Burr's county, Illinois, USA [40, 41]. The selected strains were further used to detect QTLs for protein, oil, and fatty acid composition [42].

Micronutrient enrichment in the major staple food crops is an important breeding goal in view of the extensive problem of 'hidden hunger' caused by micronutrient malnutrition. A study by Daood *et al*. [43] identified promising landraces with high concentrations of carotenoids, from the germplasm collection at the Institute for Agrobotany, Hungary. Another study undertaken by Prasanna *et al*. [33] led to identification of some highly promising landrace accessions of India for kernel-Fe and kernel-Zn concentrations.

Molecular markers for characterization of maize landraces

Understanding the phenotypic variation in landraces is important for their effective utilization for certain traits; however, not much of the genetic variation present in natural germplasm can be exploited for crop improvement through this strategy [2]. The advent of molecular marker technology to detect naturally occurring polymorphisms at the DNA level has become an invaluable and revolutionizing tool for both basic and applied studies using a more scientific and technically advanced platform. A vast potential lies in their ability to identify the structure of genetic diversity present within and among accessions and provide a direct measure of genetic diversity which go beyond the measures based on agronomic traits or geographic origin [44].

Different marker types have been used to study genetic diversity of maize populations/landraces, including the Restriction Fragment Length Polymorphisms (RFLPs) [45, 46], Random Amplified Polymrophic DNA (RAPD) [47], Simple Sequence Repeats (SSRs) [6, 48-55], and more recently, Single Nucleotide Polymorphisms (SNPs) [56]. Among the diverse range of molecular marker techniques available for evaluating genetic diversity, SSRs are well-known for their potentially high information content and versatility. Thousands of SSR or microsatellite markers have been developed in maize, and their chromosomal location and polymorphism levels have been determined (MaizeGDB; www.maizegdb.org). These markers are well-suited for genetic diversity analysis [57].

SNPs are considered ideal markers in marker-assisted management of genetic resources because of their high genomic abundance, locus-specificity, codominance, simple documentation and potential for high throughput analysis. Moreover, detection of SNP variation is not highly dependent on the detection platform used, which makes them particularly useful for projects involving analysis in more than one laboratory. Compared with the genomes of other cultivated plant species, SNP frequency in maize is high, with one SNP being found every 28–124 bp [58, 59]. A database and resource for SNP discovery and trait dissection has been established for maize in which genotype, phenotype and polymorphism data can be accessed for diverse maize inbreds and populations [56; http://www.panzea.org]. Several high throughput genotyping platforms have been developed that allow rapid and simultaneous genotyping of up to a million SNP markers. In addition, a custom GoldenGate assay containing 1536 SNPs has been developed based on public SNP information for maize [60, 61]. Most SNPs used in this GoldenGate assay appear to be equally useful for diversity analysis, marker-trait association studies, and marker-aided breeding.

Studies using molecular markers have provided new insights into geographic distribution of genetic variation of widely distributed species, identification of wild ancestors of crop species, divergence within cultivated gene pools, tracking the migration routes of crops like maize from the centers of origin, fate of genetic diversity during domestication and adoption of advanced breeding procedures, etc. Some of the salient applications of molecular diversity analysis of maize are as follows.

Understanding the patterns of genetic diversity in the global maize germplasm

Several studies have also been undertaken in the recent past focusing on the characterization of natural germplasm at molecular level, beginning with isozyme markers and more extensively, using DNA-based markers. A study by Bhat and Chandel [62] using isozyme markers revealed that maize landraces grown by the tribal people of Northeastern India bear a close resemblance to Nal-tel, a Mexican landrace. The analysis indicated a wider diversity among the maize accessions collected from Tripura, Uttar Pradesh, Nagaland, Sikkim and Meghalaya (the four forming one sub-cluster). Using bulk RFLP analysis, Gauthier et al. [46] undertook molecular characterization of 488 European maize populations. Populations from Eastern Europe (Poland, Austria, Germany, etc.) showed a lower genetic diversity, a lower number of unique alleles and a higher percentage of fixed loci than populations from Southern Europe, where the first maize populations are thought to have been introduced. A correlation between allelic frequencies at some loci and latitude/longitude was observed. Such tendencies may reflect the direction of gene flow between different races of maize; for instance, in the past, North American (Northern flint) and Caribbean populations were introduced, to northern and southern Europe, respectively.

Rebourg *et al*. [45] showed how the pattern of polymorphism varies with the high differentiation of populations using RFLPs. A higher polymorphism was observed for American populations than for European populations, which found to be consistent with earlier studies. This polymorphism is particularly high in Central America, suggesting a gradual reduction of maize polymorphism as the geographic distance from the centre of domestication increases. It also suggests a "bottleneck" effect occurring during the introduction of maize in Europe and/or a possible loss of diversity due to a selective adaptation to European conditions.

Warburton *et al*. [50] analysed 218 maize inbreds using RFLP markers, and reported that the tropical and subtropical germplasm, developed in the center of origin for maize in the central valleys of Mexico, was more diverse both phenotypically and genetically than the temperate maize, and this diversity was reflected in the marker data. This highlights the importance of judiciously using tropical germplasm in temperate maize breeding programmes and vice versa.

Vigouroux *et al*. [63] analysed how domestication bottlenecks and artificial selection shaped the amount and distribution of genetic variation in the genomes of modern crops. By analyzing diversity at 462 simple sequence repeats spread throughout the maize genome and comparing the diversity observed at these SSRs in maize to that present in its wild progenitor, teosinte, they concluded a modest genome-wide deficit of diversity in maize relative to teosinte.

Camus-Kulandaivelu *et al*. [48] evaluated the genetic basis of maize adaptation to temperate climate, particularly in Europe. They related population structure of the European landraces to flowering time using SSR markers. The study also revealed strong effects of both historical and modern selection on population structure in European maize and clear relationships with geographical origin.

The analysis of genetically heterogeneous populations has been until recently very expensive and time consuming because variation tends to be partitioned within, rather than between, maize populations, and levels of variation can be very high. This means that at least 15 individuals must be characterized in order to adequately represent the allelic diversity present in a population. A new method for SSR analysis of pools of individuals from a population has proved to be much more efficient than genotyping multiple individuals per population, and much more accurate than genotyping only one individual per population [64]. DNA fingerprinting (and thus, distinguishing) open-pollinated varieties (OPVs) is possible using SSR markers based on a population bulk DNA fingerprinting strategy [51].

Using this 'population bulk DNA fingerprinting' strategy, nearly 200 maize landraces in India have been so far characterized by the Maize Genetics Unit, IARI, New Delhi, under the ICAR National Fellow Project using microsatellite/SSR markers. The study revealed significant intra-population and inter-population diversity in the Indian maize landraces, especially those from the NEH region, besides highlighting the genetic distinctiveness of 'Sikkim Primitives' from the rest of the accessions [53-55].

Another important study undertaken recently was the characterization of global maize populations and tracking the maize migration routes from the centre of origin, carried out under the Generation Challenge Programme, involving researchers from CIMMYT (Mexico), INRA (France), IITA (Nigeria) and national programmes of Africa and Asia (India, China, Indonesia, Thailand and Vietnam). Using the population bulk DNA fingerprinting strategy based on SSR markers, a set of ~600 maize landraces (including ~170 from Asia), besides some teosinte accessions from Mexico, were characterized. This comprehensive study led to determination of genetic relationships among landraces/populations within each country, between different countries, and also a comparison of the diversity in Asian maize landraces with representative sets of landraces from the rest of the world [53]. The study also provided interesting insights on the gene flow from teosinte into the domesticated maize gene pool [52] and the complex migration routes of maize from the centre of origin in Mexico. The genetic characterization data could aid in utilizing these populations in genomic studies and breeding efforts to create new maize varieties.

Population structure analyses

The organization of genetic diversity in maize holds tremendous importance with relevance to identifying genetic resources to be used in breeding programs and to define heterotic groups for hybrid breeding. The knowledge of allelic properties of a population facilitates to measure the level of allelic diversity. *F*-statistics, given by Wright [65], can be applied to infer the population structure by comparing the actual allelic diversity to that expected under random mating. *F*-statistics measures fixation caused by drift or selection that causes substructure of a population. They make use of the correlation of alleles within individuals and their calculations are based on allele frequencies. If alleles are drawn randomly from the population to be combined in an individual, they can describe the amount of fixation effects in a given population: within the entire population (F_{IT}), within subpopulations (F_{ST}), and within individuals (F_{IS}). *F*-statistics indicate the deviation of allele frequencies from the expectations under Hardy-Weinberg Equilibrium (HWE) and they can also be interpreted as a measure of correlation between allele frequencies in different hierarchical levels of a given population [66].

Population structure analysis is an important component of association mapping. With a quantitative or probabilistic clustering method, implemented through software such as STRUCTURE, there has been a great advancement in combining these two kinds of information in a unified analysis of population structure [67]. Factorial analyses (principal component analysis and principal coordinate analysis) can also lead to a quantitative positioning of the accessions and understanding of the population structure.

STRUCTURE is a model-based clustering software and uses a Bayesian approach. Allele frequencies, estimated in each population at a series of unlinked loci, are used to compute the likelihood that a given genotype originated in each population. The estimated log probability of data $Pr(X/K)$ for each value of K allows the estimation of the likely number of populations. Q matrix is also generated which gives the ancestry coefficients or probability values which can assign individuals to their known cluster of origin.

This method of clustering can group individuals into their appropriate populations, even using a smaller number of loci. The accuracy of assigning individuals into populations depends on the number of individuals, the number of loci, the amount of admixture, and the extent of allele frequency differences among populations. The model-based clustering method can be useful (i) for identifying populations when there is little or no information about population structure; (ii) for identifying "cryptic" population structure (population structure that is difficult to detect using visible characters, but may be significant in genetic terms); (iii) for defining populations based on non-genetic information such that the underlying genetic structure is reflected [67]. Due to their high allelic diversity, SSR loci have been widely used for the study of population structure in maize (e.g., 68]. However, SNP information can also be effectively utilized to understand genetic structure of maize germplasm, including inbred lines and landraces.

Formulating core collections

From the commodity or crop improvement perspective, assessment of the amount of variation present in a wide spectrum of the genepool, including traditional varieties, improved lines and wild relatives is an important requirement. Plant breeders are, however, interested in working with a manageable number of genotypes that possess or are likely to possess the traits needed in their breeding programs. The concept of 'core collection' was suggested by Frankel and Brown [69], which was defined as "a representative sample of a collection where the diversity is retained as much as possible with minimum of redundancy". This provides a means for application of more expensive characterization activities on representative subsets than would be practical on large collections.

Core collections can be developed from passport data, characterization data, evaluation data, biochemical and molecular marker data or a combination of one or more types of these data. In most cases characterization and evaluation data in combination with passport data, provide most representative core subsets. Core collections are particularly useful for small breeding programs, where fewer accessions and wide diversity are needed or where initial exchange between countries of a representative sample of diversity is requested. Several researchers have attempted to establish core collections of maize germplasm, primarily using phenotypic and/or passport data [e.g., 34, for the Caribbean germplasm; 70, for the Uruguayan maize landraces; 71, for the maize landraces in China National Genebank).

Maize genome sequencing, allele mining and association mapping

A major challenge in modern biology is to understand the genetic basis and molecular mechanisms of the naturally occurring genetic variation in a species at the nucleotide, protein and cellular level [72]. The capability to sequence the whole genomes or part of the genome and to isolate novel and useful alleles of genes from a wide range of genotypes will open avenues for plant breeders to improve an array of traits including productivity, biotic stress resistance, abiotic stress tolerance, enhanced nutrient use efficiency and improved quality, including human nutrition [73].

The cost and speed of genome sequencing have improved dramatically in the recent years. The availability of genome sequence of a popular maize inbred B73 is an important milestone [74]. Another major maize sequencing project that was undertaken recently at the Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV, Mexico), was genome sequencing of the Mexican popcorn landrace 'Palomero' using the bulked plants [75]. Palomero is an ancient popcorn landrace of the Central and Northern Highlands of Mexico. This maize landrace has 22% lesser DNA and is phylogenetically closer to teosinte than B73. Structural and functional analysis of the Palomero genome has also revealed a large number of unreported sequences, suggesting that the ancient landraces contain a large pool of unexplored genetic diversity that could be useful in generating novel varieties as well as for studying the evolution and domestication of maize and other cereals.

Several high throughput genotyping platforms have also been developed in recent years, allowing rapid and simultaneous genotyping of up to a million SNP markers, and whole-genome scanning for identification of favorable allelic variants in crop plants like maize [e.g., 76]. Finding such unknown valuable alleles in a germplasm collection is called "allele mining". The part of the genome which should be explored for mining new alleles depends on which parts of the genome determine agronomic traits of interest. Once these putative 'candidate genes', which are believed to have a functional significance, are identified, new alleles can be discovered from a set of highly distinctive accessions. Proper choice of the genetic material is a key factor to the success of allele mining [73]. Traditional varieties form a good material for looking for novel alleles because they have had an independent history of domestication for centuries of years, and are therefore more likely to show differences across the whole genome [77]. The strategy of allele mining involves PCR-based amplification and sequencing of different versions of genes found in inbred lines, varieties, landraces and wild relatives. Variation in gene sequence is then correlated with the trait or performance of the accession, to enable identification of the favorable alleles for future experiments.

Association mapping or linkage disequilibrium (LD) mapping focuses on the identification of the association or linkage of particular molecular marker variants (alleles) with a heritable phenotypic trait. It utilizes ancestral recombination events in natural populations to make marker-phenotype associations. Association methods evaluate whether certain alleles within a population are found with specific phenotypes more frequently than expected. These approaches are fast, can provide high resolution and can evaluate wide range of alleles rapidly [78, 79].

The applicability of association mapping is influenced by the forces generating and conserving linkage disequilibrium (LD), *viz*., population stratification, relatedness, selection, mutation, genetic drift, and linkage [80]. Except for linkage, all other forces may cause spurious marker-trait associations in population-based association mapping approaches. Population structure is another important issue in association analyses. The complex breeding history of most crops and limited gene flow in most wild plants creates population stratification within the germplasm. In crop species, the underlying population structure can result in serious false associations between genes and phenotypes [81]. The effects of false positives in association analysis due to population structure can be corrected by using a large number of independent genetic markers across the genome and by using appropriate statistical models [82, 83]. However, if the distribution of functional alleles is highly correlated with population structure, statistically controlling for population structure can result in false-negatives, particularly for small sample sizes. A large unstructured natural population provides an ideal pool for association genetics. LD is expected to be limited in such populations. Association studies are best carried out in independent populations with a large sample size [84].

Conclusions

The current revolution in DNA technologies is having a profound impact on the understanding of genetic relationships, diversity and evolution of plant species. DNA fingerprinting has rendered genotype characterization highly efficient enabling reliable distinction of even closely related accessions. Molecular marker based diversity analysis is providing new

Fig. 1. Ear diversity in the Latin American maize landraces conserved in the CIMMYT Maize Gene bank

information on the extent and distribution of genetic variation among populations and subpopulations. Array-based, high throughput DNA markers, coupled with rapid advances in genome sequencing, will further revolutionize genotyping in crops like maize. New SNP assays in maize [Illumina GoldenGate array of 1536 SNPs; Infinium array with up to 1 million SNPs; Genotyping-by-sequencing; 61, 85] enable quick estimation of genetic structure in populations, and linkage disequilibrium (LD) structure in genomes, which in turn, will greatly speed up the identification and use of new and useful alleles for maize improvement.

There is a need for wider adoption of high throughput molecular tools, coupled with phenotypic evaluation, for better understanding, evaluation and management of the rich diversity of maize germplasm. This would have greater chances of contributing to the development of new and improved varieties, with potential for more direct use by farmers and appropriate for specific agro-ecologies. The process would also lead to identification of diverse sources of gene donors in breeding programs that would ultimately lead to higher levels of genetic diversity in cultivated varieties and agricultural systems. CIMMYT's new initiative, titled the 'Seeds of Discovery', which aims to discover the extent of allelic variation in the genetic resources of maize and wheat, formulate core sets based on genotyping and phenotyping, and utilize marker assisted breeding to bring those rare useful alleles into breeding programmes for developing novel genotypes, shall be an important step in that direction.

Molecular markers and modern genomic technologies have provided us powerful means for assessing the levels and patterns of genetic diversity in landraces and mining favorable alleles for various target traits. While high throughput genotyping in crops like maize is already a reality, there is a distinct need to establish global high throughput and precision phenotyping facilities for comprehensive and efficient characterization of genetic resources and breeding materials for an array of target traits, particularly for biotic and abiotic stress tolerance and nutritional quality. This would significantly accelerate genomics-assisted breeding, diversification of the genetic base of elite breeding materials, creation of novel varieties and countering the effects of global climate changes.

Acknowledgement

I express my thanks to ICAR for providing financial assistance through the National Fellow Project during 2005-2010; the farmers of Sikkim, for conserving the unique maize landraces and sharing the same for the work done under the Project; the National Bureau of Plant Genetic Resources (NBPGR) for providing seed material of several maize landrace accessions from the National Gene Bank; collaborators from different Institutions in India and abroad, as well as the present and former members of the Maize Genetics Unit, IARI, especially Dr Rajesh Kumar, Dr Firoz Hossain, Ms. Lata Sharma, Dr. Samanthi K. Wasala and Dr. Avinash Singode, for their dedicated support.

References

1. **Teshome A., Fahrig L., Torrence J. K., Lambert J. D., Arnason T. J. and Baum B. R.** 1999. Maintenance of sorghum (*Sorghum bicolor*, Poaceae) landrace diversity by farmers' selection in Ethiopia. Econ. Bot., **53**: 79-88.
2. **Tanksley S. D. and McCouch S. R.** 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science, **277**: 1063-1066.
3. **Reif J., Warburton M. L., Xia X. C., Hoisington D. A., Crossa J., Taba S., Muminovic J., Bohn M., Frisch M. and Melchinger A. E.** 2006. Grouping of accessions of Mexican races of maize revisited with SSR markers. Theor. Appl. Genet., **113**: 177-185.
4. **Matsuoka Y., Vigouroux Y., Goodman M. M., Sanchez G. J., Buckler E. and Doebley J. F.** 2002. Single domestication for maize shown by multilocus microsatellite genotyping. Proc. Natl. Acad. Sci. USA, **99**: 6080-6084.
5. **Taba S., Shands H. L. and Eberhart S. A.** 2005. The growth of CIMMYT's maize collection with the introduction of Latin American maize landrace accessions through the cooperative regeneration project. *In*: Taba S. (ed.) Latin American Maize Germplasm Conservation: Regeneration, In Situ Conservation, Core Subsets and Prebreeding, Proceedings of a Workshop at CIMMYT (7–10 April 2003). CIMMYT, Mexico D.F., pp. 1–8.
6. **Warburton M. L., Reif J. C., Frisch M., Bohn M., Bedoya C., Xia X. C., Crossa J., Franco J., Hoisington D., Pixley K., Taba S. and Melchinger A.E.** 2008. Genetic diversity in CIMMYT non temperate maize germplasm: landraces, open pollinated varieties, and inbred lines. Crop Sci., **48**: 617-624.
7. **Harlan J. R.** 1975. Our vanishing genetic resources. Science, **188**: 618–621.
8. **Zeven A. C.** 1998. Landraces: a review of definitions and classifications. Euphytica, **104**: 127-139.
9. **Wellhausen E. J., Roberts L. M. and Hernández X. E.** 1952. Races of Maize in Mexico, Their Origin, Characteristics, and Distribution. Bussey Institution,

Harvard University Press, Cambridge.

10. **Anderson E. and Cutler H. C.** 1942. Races of *Zea mays*: I. Their recognition and classification. Ann. Mo. Bot. Gard., **29**: 69–89.

11. **Paterniani E. and Goodman M. M.** 1977. Races of maize in Brazil and adjacent areas. CIMMYT, Mexico City.

12. **Mangelsdorf P. C.** 1974. Corn: Its Origin, Evolution and Improvement. The Belknap Press, Harvard University Press, Cambridge, MA.

13. **Goodman M. M. and Brown W. L.** 1988. Races of corn. *In*: Sprague G. F. and Dudley J. W. (eds.) Corn and Corn Improvement. Agronomy Monograph No. 18, 3rd edition, pp. 33–79.

14. **Ortiz R., Taba S., Chavez-Tovar V. H., Mazzalama M., Xu Y., Yan J. and Crouch J.** 2010. Conserving and enhancing maize genetic resources as global public goods – a perspective from CIMMYT. Crop Sci., **50**: 13-28.

15. **Prasanna B. M. and Sharma L.** 2005. The landraces of maize (*Zea mays* L.): diversity and utility. Indian J. Plant Genet. Resour., **18**: 155-168.

16. **Grant U. J. and Wellhausen E. J.** 1955. A study of corn breeding and production in India. Ministry of Food and Agriculture, Govt. of India.

17. **Wellhausen E. J.** 1965. Exotic germplasm for improvement of Corn Belt maize. Proc. Annu. Corn Sorghum Res. Conf., **20**: 31–45.

18. **Wilkes H. G.** 1981. Maize in India. Maize Genet. Coop. Newslett., **55**: 13-15.

19. **Anderson E.** 1945. Maize in the New World. *In*: Wilson C. M. (Ed.) New Crops for the New World, Macmillan, New York.

20. **Stonor C. R. and Anderson E.** 1949. Maize among the hill peoples of Assam. Ann. Mo. Bot. Gard., **36**: 355–404.

21. **Singh B.** 1977. Races of Maize in India. Indian Council of Agricultural Research, New Delhi.

22. **Suto T. and Yoshida Y.** 1956. Characteristics of the oriental maize. *In*: Kihara H. (ed.). Land and Crops of Nepal Himalaya, Vol. 2. Fauna and Flora Research Society, University, Kyoto, Japan, pp. 375–530.

23. **Dhawan N. L.** 1964. Primitive maize in Sikkim. Maize Genet. Coop. Newslett., **38**: 69-70.

24. **Wilkes H. G and Peeters J. P.** 1982. Sikkim Primitive, Maize Genet. Coop. Newslett., **56**: 27-28.

25. **Sachan J. K. S., Sarkar K. R. and Payak M. M.** 1984. Studies on distribution of constitutive heterochromatin in relation to origin, evolution and diffusion of maize (*Zea mays* L.). *In*: Advances in Cytogenetics and Crop Improvement, Kalyani Publishers, New Delhi, pp. 41-48.

26. **Sachan J. K. S and Sarkar K. R.** 1982. Plant type of 'Sikkim Primitives'. Maize Genet. Coop. Newslett., **56**: 122-124.

27. **Louette D., Charrier A. and Berthaud J.** 1997. *In situ* conservation of maize in Mexico: genetic diversity and maize seed management in a traditional community. Econ. Bot., **51**: 20-38.

28. **Louette D. and Smale M.** 2000. Farmers' seed selection practices and traditional maize varieties in Cuzalapa, Mexico. Euphytica, **113**: 25-41.

29. **Pressoir G. and Berthaud J.** 2004. Patterns of population structure in maize landraces from the Central Valleys of Oaxaca in Mexico. Heredity, **92**: 88-94.

30. **Perales H. R., Benz, B. F. and Brush S. B.** 2005. Maize diversity and ethnolinguistic diversity in Chiapas, Mexico. Proc. Natl. Acad. Sci. USA, **102**: 949-954.

31. **Dhillon B. S. and Prasanna B. M.** 2001. Maize. *In*: Chopra V. L. (ed.) Breeding Field Crops. Oxford & IBH, New Delhi, pp. 147-189.

32. **Dhillon B. S., Vasal S. K. and Prasanna B. M.** 2002. Maize. *In*: Chopra V. L. and Shyam Prakash (eds.) Evolution and Adaptation of Cereal Crops. Oxford & IBH, New Delhi, pp. 99-133.

33. **Prasanna B. M., Majumdar S., Chakraborti M., Hossain F., Manjaiah K. M., Agarwal, P. K., Guleria S. K. and Gupta H. S.** 2010b. Genetic variability and genotype x year interactions for kernel iron and zinc concentrations in maize (*Zea mays* L.) genotypes. Indian J. Agric. Sci. (in press).

34. **Taba S., Diaz J., Franco J. and Crossa J.** 1998. Evaluation of Caribbean maize accessions to develop a core subset. Crop Sci., **38**: 1378-1386.

35. **Reid L., Arnason J. T., Nozzolillo C. and Hamilton R. H.** 1990. Resistance of maize to the European corn borer, *Ostrinia nubilalis,* as related to the geographical origin of maize. Can. J. Bot., **68**: 311-316.

36. **Arnason J. T., Baum B., Gale, J., Lambert J. D. H., Bergvinson D., Philogene B. J. R., Serratos J. A., Mihm J. and Jewell D. C.** 1994. Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. Euphytica, **74**: 227-236.

37. **CIMMYT.** 1997. Development of New Stress-Resistant Maize Genetic Resources (UNDP Project GLO/90/003). CIMMYT Maize Programme Special Report, Mexico, D.F.

38. **Mani V. P., Jotshi H. C. and Koranne K. D.** 1987. Preliminary observations on drought tolerance in Sikkim Primitive maize. Maize Genet. Coop. Newslett., **61**: 2.

39. **Rodriguez M. G., Miguel-Chavez R. S. and Larque-Saavedra A.** 1998. Physiological aspects in Tuxpeno maize with improved drought tolerance. Maydica, **43**: 137-141.

40. **Hopkins C. G.** 1899. Improvement in the chemical composition of the corn kernel. Illinois Agr. Expt. Sta. Bul., **55**: 205-240.

41. **Dudley J. W. and Lambert R. J.** 2004. 100 generations of selection for oil and protein in maize. Plant Breed. Rev., **24**: 79-110.

42. **Willmot D. B., Dudley J. W., Rocheford T. R. and Bari, A.** 2006. Effect of random mating on marker-QTL associations for grain quality traits in the cross of Illinois high oil x Illinois low oil. Maydica, **51**: 187-199.

43. **Daood H. G., Nagy-Gasztonyi M., Vörösváry G., Holly L., Horváth L. and Nyerges M.** 2003. Analysis of carotenoids in some Hungarian maize landraces. In: Bericht über die 54. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, pp. 1-2.

44. **Dreisigacker S., Zhang P., Warburton M. L., Skovmand B., Hoisington D. and Melchinger A. E.** 2005. Genetic diversity among and within CIMMYT wheat landrace accessions investigated with SSRs and implications for plant genetic resources management. Crop Sci., **45**: 653-661.

45. **Rebourg C., Gouesnard B. and Charcosset, A.** 2001. Large scale molecular analysis of traditional European maize populations - relationships with morphological variation. Heredity, **86**: 574-587.

46. **Gauthier P., Gouesnard B., Dallard L., Redaelli R., Rebourg C., Charcosset A. and Boyat A.** 2002. RFLP diversity and relationships among traditional European maize populations. Theor. Appl. Genet., **105**: 91-99.

47. **Moeller D. A. and Schaal B. A.** 1999. Genetic relationships among native American maize accessions of the Great Plains assessed by RAPDs. Theor. Appl. Genet., **99**: 1061-1067.

48. **Camus-Kulandaivelu L., Veyrieras J. B., Madur D., Combes V., Fourmann M., Barraud S., Dubreuil P., Gouesnard B., Manicacci D. and Charcosset A.** 2006. Maize adaptation to temperate climate: relationship between population structure and polymorphism in the *Dwarf8* gene. Genetics, **172**: 2449-2463.

49. **Warburton M. L., Xianchun X., Crossa J., Franco J., Melchinger A. E., Frisch M., Bohn M. and Hoisington D.** 2002. Genetic characterization of CIMMYT inbred maize lines and open pollinated populations using large scale fingerprinting methods. Crop Sci., **42**: 1832-1840.

50. **Warburton M. L., Ribaut J. M., Franco J., Crossa J., Dubreuil P. and Betrán F. J.** 2005. Genetic characterization of 218 elite CIMMYT inbred maize lines using RFLP markers. Euphytica, **142**: 97-106.

51. **Warburton M., Setimela P., Franco J., Cordova H., Pixley K., Banziger M., Dreisigacker S., Bedoya C. and MacRobert J.** 2010a. Toward a cost-effective fingerprinting methodology to distinguish maize open pollinated varieties. Crop Sci., **50**: 1-11.

52. **Warburton M. L., Wilkes G., Taba S., Charcosset A., Mir C., Bedoya C., Prasanna B.M., Xie C. X., Hearne S. H. and Franco J.** 2010b. Gene flow between different teosinte species and into the domesticated maize gene pool. Genetic Resources & Crop Evolution (In Press).

53. **Prasanna B. M., Pixley K. V., Warburton M. and Xie C.** 2010a. Molecular marker-assisted breeding for maize improvement in Asia. Mol. Breed., Published Online DOI 10.1007/s11032-009-9387-3.

54. **Sharma L., Prasanna B. M. and Ramesh B.** 2010. Phenotypic and microsatellite-based diversity and population genetic structure of maize landraces in India, especially from the North East Himalayan region. Genetica, Published Online DOI 10-1007/s 10709-010-9436-1.

55. **Singode A. and Prasanna B. M.** 2010. Analysis of genetic diversity in the North Eastern Himalayan (NEH) maize landraces of India using microsatellite markers. J. Pl. Biochem. Biotech., **19**: 33-41.

56. **Zhao W., Canaran P., Jurkuta R., Fulton T., Glaubitz J., Buckler E., Doebley J., Gaut B., Goodman M., Holland J., Kresovich S., McMullen M., Stein L. and Ware D.** 2006. Panzea: a database and resource for molecular and functional diversity in the maize genome. Nucleic Acids Res., **34**: D725–D757.

57. **Hamblin M. T., Warburton M. L. and Buckler E. S.** 2007. Empirical comparison of simple sequence repeats and single nucleotide polymorphisms in assessment of maize diversity and relatedness. PLoS ONE **2**: e1367.

58. **Ching A., Caldwell K. S., Jung M., Dolan M., Smith O. S., Tingey S., Morgante M. and Rafalski A.** 2002. SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genet., **3**: 19-33.

59. **Vroh Bi I., McMullen M. D., Villeda H. S., Schroeder S., Gardiner J., Polacco M., Soderlund C., Wing R., Fang Z. and Coe E. H.** 2006. Single nucleotide polymorphisms and insertion-deletions for genetic markers and anchoring the maize fingerprint contig physical map. Crop Sci., **46**: 12–21.

60. **Yan J., Shah T., Warburton M. L., Buckler E. S., McMullen M. D. and Crouch J.** 2009. Genetic characterization and linkage disequilibrium estimation of a global maize collection using SNP markers. PLos One, **4**: e8451.

61. **Yan J, Yang X, Shah T., Sanchez-Villeda H., Li J., Warburton M., Zhou Y., Crouch J. H. and Xu Y.** 2010. High-throughput SNP genotyping with the GoldenGate assay in maize. *Mol. Breed.*, **25**: 441-451.

62. **Bhat K. V. and Chandel K. P. S.** 1998. Isozyme diversity in Indian primitive maize landraces. J. Pl. Biochem. Biotech., **7**: 23-27.

63. **Vigouroux, Y., Mitchell S., Matsuoka Y., Hamblin M., Kresovich S., Smith J. S. C., Jaqueth J., Smith, O. S., and Doebley J.** 2005. An analysis of genetic diversity across the maize genome using microsatellits. Genetics, **169**: 1617-1630.

64. **Dubreuil P, Warburton M. L., Chastanet M., Hoisington D. and Charcosset A.** 2006. More on the introduction of temperate maize into Europe: Large-scale bulk SSR genotyping and new historical elements. Maydica, **51**: 281-291.

65. **Wright S.** 1951. The genetical structure of populations. Ann. Eugen., **15**: 323-354.

66. **Weir B. S. and Cockerham C. C.** 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358-1370.

67. **Pritchard J. K., Stephens M. and Donnelly P.** 2000. Inference of population structure using multilocus genotype data. Genetics, **155**: 945-959.

68. **Vigouroux Y., Glaubitz J. C., Matsuoka Y., Goodman M. M., Jesus S. G., Doebley J.** 2008. Population structure and genetic diversity of new world maize races assessed by DNA microsatellites. Am. J. Bot., **95**: 1240-1253.

69. **Frankel O. H. and Brown A. H. D.** 1984. Current plant genetic resources: a critical appraisal. *In*: Genetics: New Frontiers, Vol. IV, Oxford & IBH, New Delhi, pp. 1-11.

70. **Malosetti M. and Abadie T.** 2001. Sampling strategy to develop a core collection of Uruguayan maize landraces based on morphological traits. Genet. Resour. Crop Evol., **48**: 381-390.

71. **Li Y., Shi Y., Cao Y. and Wang T.** 2004. Establishment of a core collection for maize germplasm preserved in Chinese National Genebank using geographic distribution and characterization data. Genet. Resour. Crop Evol., **51**: 845-852.

72. **Alonso-Blanco C., Mendez-Vigo B. and Koornneef M.** 2005. From phenotypic to molecular polymorphisms involved in naturally occurring variation of plant development. Int. J. Dev. Biol., **49**: 717-732.

73. **Prasanna B. M.** 2007. Allele mining. *In*: Chopra V. L., Sharma R. P., Bhat S. R. and Prasanna B. M. (eds.) Search for New Genes. Academic Foundation, New Delhi, pp. 121-143.

74. **Schnable P. S., Ware D., Fulton R. S. *et al.*** 2009. The B73 genome: complexity, diversity, and dynamics. Science, **326**: 1112-1115.

75. **Vielle-Calzada J-P., de la Vega O. M., Hernández-Guzmán G., Ibarra-Laclette E., Alvarez-Mejía C., Vega-Arreguín J. C., Jiménez-Moraila B., Fernández-Cortés A., Corona-Armenta G., Herrera-Estrella L. and Herrera-Estrella A.** 2010. The Palomero genome suggests metal effects on maize domestication. Science, **326**: 1078.

76. **Belo A. P., Zheng P., Luck S., Shen B., Meyer D. J., Li B., Tingey S. and Rafalski A.** 2008. Whole genome scan detects an allelic variant of *fad2* associated with increased oleic acid levels in maize. Mol. Genet. Genomics, **279**: 1-10.

77. **Hamilton R. S. and McNally K.** 2005. Unlocking the genetic vault. Rice Today, pp. 32-33.

78. **Buckler E. S. and Thornsberry J. M.** 2002. Plant molecular diversity and applications to genomics. Curr. Opin. Plant Biol., **5**: 107-111.

79. **Rafalski A.** 2002. Applications of single nucleotide polymorphisms in crop genetics. Curr. Opin. Plant Biol., **5**: 94-100.

80. **Stich B., Melchinger A. E., Frisch M., Maurer H. P., Heckenberger M. and Reif J. C.** 2005. Linkage disequilibrium in European elite maize germplasm investigated with SSRs. Theor. Appl. Genet., **111**: 723-730.

81. **Sharbel T. F., Haubold B., Mitchell-Olds T.** 2000**.** Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. Mol. Ecol., **9:** 2109-2118.

82. **Pritchard J. K. and Rosenberg, N. A.** 1999. Use of unlinked genetic markers to detect population stratification in association studies. Am. J. Hum. Genet., **65**: 220–228.

83. **Reich D. E. and Goldstein D. B.** 2001. Detecting association in a case–control study while correcting for population stratification. Genet. Epidemiol., **20**: 4-16.

84. **Yu J. and Buckler E. S.** 2006. Genetic association mapping and genome organization of maize. Curr. Opin. Biotechnol., **17**: 155-160.

85. **Jones E., Chu W., Ayele M., Bruggeman E., Yourstone K., Rafalski A., Smith O. S., McMullen M. D., Bezawada C., Warren J., Babayev J., Basu S. and Smith S.** 2009. Development of single nucleotide polymorphism (SNP) markers for use in commercial maize (*Zea mays* L.) germplasm. Mol. Breed. Published Online DOI 10.1007/s11032-009-9281-z.