

Chapter 11

Zea

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11.1 Some Important Characteristics of Wild *Zea* Species

11.1.1 Geographical Distribution and Genetic Diversity

The teosintes are annual and perennial grasses native to Mexico and Central America. Most of these wild *Zea* species and subspecies are distributed across narrow ranges and can only be found in some tropical and subtropical areas of Mexico, Guatemala, Nicaragua, and Honduras. The geographic distributions of the teosintes are listed in Table 11.1. A point worth highlighting is that wild *Zea* species do not have uniform distributions and are enormously sensitive to even the slightest environmental changes in day length and moisture (Sánchez-González and Ruiz-Corral 1997). Hence, we also list in Table 11.1 some specific climate, elevation, and soil conditions under which teosintes are found.

Two subspecies of *Z. mays* (*parviglumis* and *mexicana*) harbor high levels of molecular genetic variation and show genetic substructuring along geographic lines (Doebley et al. 1984). Phylogeographic and diversity analysis demonstrated that *parviglumis* originated from the eastern part of Central Balsas, Mexico, and spread from east to west, while *mexicana* originated from the Central Plateau of Mexico and spread along multiple paths to the north and east

(Fukunaga et al. 2005). Other species of wild *Zea* (*Z. luxurians*, *Z. diploperennis*, *Z. perennis*, *Z. mays* ssp. *mexicana*, and *Z. nicaraguensis*) show less diversity, as expected from their very narrow geographic distributions.

11.1.2 Morphology

As the wild ancestor of modern maize, the plant architecture and general growth forms of teosinte are similar to maize. A typical teosinte plant usually has a main stalk that typically contains a series of nodes and elongated lateral branches at most nodes. The internodes can reach up to 20–30 cm in length. The ears occur in clusters of 1–5 (or more) at each node along the branch. The essential morphological features of the teosintes are given in Table 11.2.

The main morphological differences between teosinte and maize are their branches and inflorescences. Teosinte plants contain more branches and smaller female inflorescences than maize. For wild *Zea* species, the inflorescences can only form 5–10 triangular or trapezoidal black or brown seeds with a hard fruitcase. By comparison, maize usually has 100 or more naked seeds (Fig. 11.1). Recently, it was reported that pollen, starch grain, and phytoliths can also be used for differentiating between teosintes and maize (Holst et al. 2007).

11.1.3 Cytogenetics

Zea species have been one of the most favored models for extensive cytogenetic studies since the 1950s.

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Table 11.1 Geographic distribution of the wild *Zea* species (teosinte)

Populations	Distribution	Growing environment
<i>Z. mays</i> ssp. <i>parviglumis</i>	Southwestern Mexico	Middle altitude (400–1,800 m), subhumid temperate
<i>Z. mays</i> ssp. <i>huehuetenangensis</i>	Northwestern Guatemala	Low elevation (900–1,650 m)
<i>Z. mays</i> ssp. <i>mexicana</i>	Central and northern Mexico	High elevation (1,700–2,600 m), dry environment
<i>Z. diploperennis</i>	Sierra de Manantlán biosphere reserve region, Mexico	Highlands (1,900–2,100 m), average annual temperature about 16°C
<i>Z. perennis</i>	Western Mexico	Highlands (1,500–2,000 m), moderate climate (average annual temperature of 16–27°C)
<i>Z. luxurians</i>	Southeastern Guatemala; Honduras and Nicaragua	Altitudes between sea level and 1,100 m
<i>Z. nicaraguensis</i>	Southwestern Nicaragua (Pacific coast)	Coastal plain and very mesic environment (some grow in water)

Data are from Sánchez-González and Ruiz-Corral (1997); Fukunaga et al. (2005)

Here, we briefly summarize the cytology of *Zea* chromosomes. Additional descriptions of cytogenetic studies on wild *Zea* species are presented in Sect. 11.4.

11.1.3.1 Basic Chromosome Number and Size

All the annual wild *Zea* species are diploid with $2n = 20$ chromosomes. One perennial species, *Z. diploperennis*, is also diploid and has $2n = 20$. The other perennial species *Z. perennis* (also called *Z. tetraploperennis*) is a tetraploid with 40 chromosomes (Molina and Naranjo 1987). Generally, the teosintes have larger chromosomes than those found in cultivated maize where they have an average length of 11.2 μm . Among the wild species, *Z. nicaraguensis* has the largest chromosomes, with an average length of 19.6 μm (Ellnskog-Staam et al. 2007). *Z. perennis* has the smallest average chromosome size and the smallest heterochromatic blocks (Tito et al. 1991).

11.1.3.2 Chromosome Knobs

Chromosome knobs are enlarged structures consisting of condensed heterochromatin on mitotic and meiotic chromosomes. They can be used for taxonomic studies in the genus *Zea* because they exhibit fixed numbers and positions on chromosomes within a specific accession, but vary between accessions (Kato 1976). In most cases, the main chromosome knobs in maize are internal or subterminal. In contrast, the chromosomes of *Z. nicaraguensis*, *Z. luxurians*, *Z. diploperennis*, and *Z. perennis* (Section *Luxuriantes*) have

many terminal chromosome knobs and lack internal knobs. The main cytological difference between the *Z. nicaraguensis* and *Z. luxurians* genomes is that chromosome 10 of *Z. nicaraguensis* is knobless (Ellnskog-Staam et al. 2007). On the other hand, all of the subspecies of *Z. mays* (Section *Zea*), in general, have many internal knobs and few or no terminal knobs except *Z. mays* ssp. *huehuetenangensis*, which has many terminal chromosome knobs (Wilkes 1967). These chromosomal knobs contain thousands to millions of tandem 180 bp and 350 bp repetitive DNAs that have high frequencies of preferential segregation as a result of meiotic drive (Buckler et al. 1999).

11.1.4 Agricultural Status

The wild species within the genus *Zea* are considered by many maize farmers as harmful weeds in some parts of Mexico, while in other areas, they are regarded as beneficial companion plants that can hybridize with maize. Currently, a good deal of interest is focused on delivering beneficial teosinte traits, such as insect resistance, perennialism, and flooding tolerance, to cultivated maize. However, this is difficult due to linked deleterious traits in the teosinte genomes. Some wild *Zea* species are also used locally for food and/or forage in Guatemala and Honduras. Although the wild *Zea* species may provide an important germplasm resource for the improvement of cultivated maize, several populations of teosinte are either threatened or endangered. *Z. diploperennis* exists in a very

Table 11.2 Morphological features of the wild *Zea* species

	<i>Z. mays</i> ssp. <i>parviglumis</i>	<i>Z. mays</i> ssp. <i>huehuetenangensis</i>	<i>Z. mays</i> ssp. <i>mexicana</i>	<i>Z. diploperennis</i>	<i>Z. perennis</i>	<i>Z. luxurians</i>	<i>Z. nicaraguensis</i>
Habit	Annual	Annual	Annual	Perennial	Perennial	Annual	Annual
Height	2–5 m	up to 5 m	1.5–4 m	2–2.5 m	1.5–2 m	3–4 m	2–5 m
Tassel	Slender, 20–100 branches	Slender, 20 or more branches	Slender, 10–20 branches	Thicker, lax, 2–10 branches	Thicker, erect, 2–8 branches	Thicker, erect, 4–20 branches	Slender, lax, 27–38 branches
Male spikelet	Paired, 4.6–7.2 mm long, smaller size pedicels (4.6–7 mm)	Paired, longer pedicels (5–7 mm)	Paired, longer pedicels (6–10 mm)	Paired, 10 mm long, shorter pedicels (1.5–3.5 mm)	Shorter pedicels	Shorter pedicels (1.5–3.8 mm)	Paired, shorter pedicels (1.5–3.5 mm)
Female spikelet	Slender and distichous	Slender and distichous	Slender and distichous	Slender and distichous	Slender and distichous	Slender and distichous	Slender and distichous
Blooming date	September through October	Late November to January	September through October	September through October	September through October	September through October	Mid-October to early November
Fruit cases	Small, blunt, triangular (30–80 mg)	Small, triangular (30–60 mg)	Large, acute triangular (60–95 mg)	Trapezoidal (68–75 mg)	Trapezoidal (70–83 mg)	Trapezoidal (76–99 mg)	Trapezoidal (76–99 mg)
Rhizomes	Absent	Absent	Absent	Slender, both cord-like and tuber-like with short internodes (2–6 mm)	Slender, cord-like with long internodes (1–6 cm)	Absent	Absent

Data are from Ilitis et al. (1979); Ilitis and Doebley (1980); Doebley (1983); Ilitis and Benz (2000)

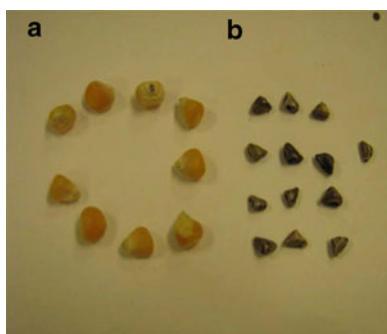


Fig. 11.1 Maize and teosinte seeds. (a) maize; (b) *Z. mays* ssp. *parviglumis*

narrow area in Mexico and could go extinct in the wild if not protected (Sanchez-Velasquez et al. 2002). *Z. nicaraguensis* appears to be restricted to an area of 200 × 150 m in Chinandega, Nicaragua (Iltis and Benz 2000).

11.1.5 Summary

In summary, the teosintes are naturally distributed in Mexico, Guatemala, Honduras, and Nicaragua. The morphology and cytogenetics of the wild *Zea* species are quite similar to maize but have significant differences in branches, inflorescences, and ears, and in chromosome length and knob distributions. The distributions of wild *Zea* species are very limited. Fortunately, the Mexican and Guatemalan governments have taken action in recent years to protect wild *Zea* populations, using both in situ and ex situ conservation methods.

11.2 Conservation Initiatives

Teosintes have been generally accepted as the closest relatives of domesticated maize and thus are expected to exhibit many of the wild traits that were present in the ancestor(s) of maize. The natural distributions of teosintes covered more than a thousand square kilometers in Mexico and Guatemala in the 1960s (Wilkes 1967). However, in the last 40 years, teosinte populations have declined dramatically. Once an abundant element of rural field margins and fallow plots, two of

the wild teosintes in Mexico (*Z. mays* ssp. *mexicana* and *Z. mays* ssp. *parviglumis*) have now become fragmented and scattered. Today, the current distributions of teosintes in Mexico have shrunk to 50% of their size in 1900 (Wilkes 1997). Teosinte in Guatemala (*Z. luxurians* and *Z. mays* ssp. *huehuetenangensis*) is near extinction and is expected to be eliminated within the next decade if no immediate conservation efforts are undertaken (Wilkes 2007). Three of the annual teosinte populations (*Z. nicaraguensis*, *Z. luxurians* and *Z. mays* ssp. *huehuetenangensis*) are considered “rare,” occurring at single locations, while others are considered “vulnerable” according to the terms of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources (IUCN) in Switzerland (National Research Council US 1993).

Several factors appear to have hastened the decline of the teosintes (1) *Genetic erosion* has greatly affected the genetic diversity of wild *Zea* species. Teosintes often hybridize with cultivated maize, especially on roadsides and in the populations growing on the borders of maize fields (FAO 2002). The hybrids may lose some unique characteristics of their teosinte parents and lead to the extinction of some rare species. (2) *Increasing human population* is an important factor for the decline of wild teosintes. The rate of teosinte disappearance will accelerate with an increase in the number and size of roads and houses. (3) *Overgrazing* is another principal threat to teosinte. Teosintes are a good source of forage for livestock. Livestock relish the sweet, green leaves and stems of teosinte that appear after summer rains (Tuxill and Nabhan 2001). (4) *Replacement of maize* with coffee or other crops that are not compatible with the teosinte growth environment can also lead to the eradication of wild *Zea* germplasm (Wilkes 2007).

11.2.1 Evaluation of Genetic Erosion in Teosinte Germplasm

Genetic erosion is the loss of genetic diversity, both the loss of individual genes and the loss of particular combinations of genes. Crop plants and their wild relatives can interbreed because of their close evolutionary relationship. Gene flow from the wild *Zea*

species is predicted to often be beneficial for maize improvement and hybrid development. However, gene flow from the vast populations of highly inbred maize into the wild *Zea* species inevitably causes genetic erosion and thereby restricts the genetic diversity of teosinte. The frequency of gene flow is quite different between maize and different species and subspecies of teosinte. A 3-year field experiment showed that maize and *Z. mays* ssp. *parviglumis* hybridized at a high rate, while *Z. mays* ssp. *mexicana* and *Z. luxurians* show cross-incompatibility with maize (Evans and Kermicle 2001; Ellstrand et al. 2007). In addition, allozyme comparison of *Z. luxurians* and *Z. diploperennis* revealed that there is an extremely low level of introgression from maize to these wild teosinte species (Doebley 1990b). Maize has coexisted and coevolved with its wild relatives over thousands of years. It is likely that maize and *Z. mays* ssp. *parviglumis* have been exchanging genes for the last several thousand years. It is reported that crossing between maize and *Z. mays* ssp. *parviglumis* usually occurs in September to October when the flowering period of teosinte plants overlaps the end of the flowering period of maize (Baltazar and Schoper 2002). But further attempts to exactly predict the extent of genetic erosion is not possible because ancestral contributions cannot be demarcated clearly (Smith and Goodman 1981).

The isolated teosinte populations that survive in or near maize fields may be genetically “swamped” and replaced by maize. In the major areas of teosinte distribution in Mexico, where maize also predominates, 10% or more of the teosinte may actually be hybrids (Doebley 1984). Teosintes have a dispersal advantage because they possess articulate seed cases. However, they usually lose this ability after hybridization with maize, thus leading to the possible extinction of small teosinte populations.

11.2.2 Attempts at In Situ and Ex Situ Conservation

Conservation of plant genetic resources can be achieved by protection of populations in nature (in situ) or by preservation of samples in germplasm banks and botanical gardens (ex situ). As early as the 1960s, the principal zones of teosinte distribution in Mexico and Guatemala were surveyed (Wilkes 1985).

In 1985, the government of Guatemala prohibited the collection of wild *Zea* species and their removal from the country. Over the last 40 years, with financial support from the Rockefeller Foundation, the United States Department of Agriculture (USDA), and the governments of Mexico and Guatemala, in situ and ex situ conservation efforts have been expanded for the wild *Zea* species.

11.2.2.1 In Situ Conservation

In situ (on site) conservation is to protect, monitor, and manage the selected species in their natural habitats, including where they have evolved. The benefits of in situ conservation of crop wild relatives (CWR) have been well acknowledged (Prescott and Prescott 1981). In the mid-1980s, Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), and Instituto de Ciencia y Tecnología Agrícolas (ICTA) came together to start a periodic and long-term in situ monitoring program on each teosinte population with the participation of local farmers. The intention was to check the status of each teosinte population and take preservation actions whenever a population appeared to be in danger of extinction (Eltringham 1984). However, besides the in situ monitoring program, the establishment of in situ protected areas has been slow to emerge due to various social and economic reasons (FAO 1997). So far, the only successful in situ conservation project is that of *Z. diploperennis* in Mexico's Sierra de Manantlan Biosphere Reserve (Fig. 11.2). This 1,396 km² reserve area was established in 1987 and encompasses 12.5 km² of the *Z. diploperennis* germplasm distribution (Benz 1988). In Guatemala, attempts to establish large in situ conservation areas were unsuccessful and there was only some small village-level protected areas established by ICTA. Researchers in Nicaragua are planning to start an in situ conservation program funded by USDA (Meilleur and Hodgkin 2004).

11.2.2.2 Ex Situ Conservation

Ex situ (off site) conservation is to remove the genetic materials from their native habitats and maintain them in managed, easily accessible locations such as

botanical gardens, nurseries, or germplasm banks. Ex situ is a complementary method to in situ conservation with a relatively low cost, facile establishment, and easy access for users (Hawkes et al. 2000). About 80% of the extant genetic diversity in the teosintes is believed to have been sampled and conserved ex situ through various methods (Smith et al. 2004). A few teosintes are cultivated in some important botanical gardens such as the Brooklyn Botanic Garden and the Aztec Botanical Garden in the USA and Mexico, respectively. Most wild *Zea* species are conserved through germplasm banks.

The major teosinte collections in germplasm banks are those of CIMMYT and INIFAP in Mexico. The other two germplasm banks with large numbers of teosinte accessions are the North Central Regional Plant Introduction Station (NCRPIS) of the USDA at

Iowa State University and at the University of Guadalajara, Mexico. Antonio Narro, Autonomous Agrarian University in Mexico, also preserves a small number of teosinte accessions. In 2003, ICTA constructed an active germplasm storage facility for teosinte and maize in Guatemala. Teosinte accessions collected in the main germplasm banks are listed in Table 11.3.

In addition to seed storage, an international cooperative project to regenerate the endangered wild *Zea* species has been in progress since 1993 (Taba 2003). Several teosinte accessions are grown annually at CIMMYT and planted in their field plots located in El Batán and Tlaltizapan, Mexico. ICTA (Guatemala) is planning to replenish seed stocks of all the new accessions of *Z. mays* ssp. *huehuetenangensis* as well as some of the accessions at CIMMYT in the next several years.

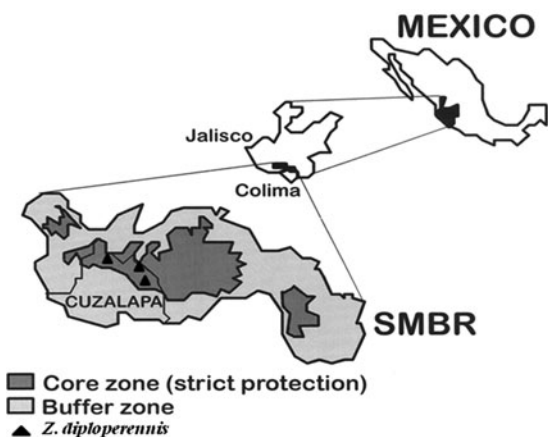


Fig. 11.2 Map of Sierra de Manantlán Biosphere Reserve (SMBR). Figure is adapted from Louette et al. (1997)

11.2.3 Modes of Preservation and Maintenance

Different germplasm banks often preserve seeds in different quantities and under varied conditions. For short storage (less than 10 years), the materials are usually stored in glass jars or plastic boxes at 4–15°C and 10–30% humidity. This is the so-called “active bank” where the seeds are frequently used by researchers and farmers. For medium-term storage (up to 30 years), the temperature is maintained at 0–4°C. Of the teosinte germplasm banks, only CIMMYT and USDA-ARS NCRPIS have adequate and specialized long-term storage (more than 30 years) facilities for

Table 11.3 Number of teosinte accessions held in germplasm banks

Species/number	Germplasm banks			
	INIFAP	CIMMYT	USDA (GRIN)	University of Guadalajara
<i>Zea mays</i> ssp. <i>parviglumis</i>	135	50	216	96
<i>Zea mays</i> ssp. <i>mexicana</i>	103	65	74	57
<i>Zea mays</i> ssp. <i>huehuetenangensis</i>	0	1	7	0
<i>Zea perennis</i>	4	2	10	1
<i>Zea luxurians</i>	0	1	20	0
<i>Zea diploperennis</i>	7	2	13	2
<i>Zea nicaraguensis</i>	0	1	1	0

Data are from the USDA Germplasm Resources Information Network (GRIN) online database, <http://www.ars-grin.gov/> (Cited 12 Oct; Global Crop Diversity Trust 2007)

teosinte. A small part of the maize collection (including some teosinte seed) is preserved in INIFAP's long-term storage facilities.

Cryopreservation is also used for long term storage of teosinte seed at CIMMYT. CIMMYT's long-term storage facility has 240 m³ of storage space for maize and teosinte seed, and it was constructed in 1996. The teosinte seed are packaged in aluminum-laminated bags containing 1–1.5 kg of seed per bag on movable shelves to optimize use of the available space (Pardey et al. 2001). This storage area is maintained at a temperature of –18°C and at a relative humidity of 6–8%. Germplasm bank employees monitor the teosinte seed viability periodically to ensure that the viability remains between 85 and 100%.

11.2.4 Summary

Because of their small and isolated populations, most of the teosintes are threatened with extinction, and their germplasm is continually being eroded, largely by gene flow from domesticated maize. Specific efforts to conserve teosinte alleles of *Zea* genes are needed. Both in situ and ex situ conservation methods are important. They have distinct advantages and disadvantages and therefore are complementary approaches for teosinte protection. In situ conservation of teosinte germplasm has only recently been initiated, and this only in two regions of Central America, so additional populations need to be protected. Ex situ conservation is more advanced, with germplasm banks established for short, medium, and long-term storage at a number of facilities around the world.

11.3 Role in Elucidation of the Origin and Evolution of Maize

Most, perhaps all, crop plants have wild relatives that currently exist in natural populations. Since the end of the nineteenth and the beginning of the twentieth century, the relationships between the teosintes and other plant species have been controversial. Initially, some taxonomists proposed that teosintes were closely related to rice (reviewed in Doebley 2004). Subsequently,

fertile hybrids crossed between maize and some types of teosinte were noted, leading some scientists to consider one or more teosintes to be plausible candidates as the ancestor(s) of maize (Schuman 1904; Collins 1919). In 1939, Beadle proposed the “teosinte hypothesis,” which stated that modern maize was derived directly from a teosinte (Beadle 1939). The idea itself was not new; however, Beadle was the first person who successfully used experimental results to verify this hypothesis and pointed out five key mutations from teosinte to maize (Beadle 1939, 1972, 1980). A recent commentary has described the accumulated and overwhelming evidence in support of wild *Zea* species being the sole progenitors of maize (Bennetzen et al. 2001). To help understand the important role of the teosintes in the origin of maize, we outline the morphotaxonomy and chemotaxonomy of teosinte and maize. Additionally, the outcomes of molecular marker analyses used to elucidate the issues on the origin and evolution of maize are summarized. At the end of this part, we briefly describe some useful statistical techniques and computer software.

11.3.1 Morphotaxonomy of Teosinte and Maize

The latest taxonomic relationships of teosinte and maize were discussed in the first part of this chapter. Here, we discuss the classification outcomes for the *Zea* species based on morphological traits.

Although maize has held the interest of botanists and taxonomists for several hundred years, little attention was paid to teosinte taxonomy and phylogeny. The first formal taxonomic study on the teosintes was attempted by Gilly in the 1940s (Table 11.4). However, in his taxonomy, teosintes were only classified by their geographic distributions (Iltis and Doebley 1980). Subsequently, Wilkes presented a landmark monograph on a system of classification for the teosintes (Wilkes 1967). Unlike Gilly, Wilkes carried out long-lasting field explorations of teosinte species in Mexico, Guatemala, and Honduras several times. He described the geographic distribution of the teosintes clearly and divided *Z. mexicana* into six allopatric races (Table 11.4). However, Wilkes relied heavily on geographical distribution for classification and

Table 11.4 Taxonomic studies of the teosintes and maize and their proposed classification systems

Gilly (unpublished specimen annotations, 1948)	Wilkes (1967)	Iltis and Doebley (1980); Doebley (1983)
<i>Zea</i>	Section <i>Zea</i>	Section <i>Zea</i>
<i>Zea mays</i> L	<i>Zea mays</i> L	<i>Zea mays</i> L.
<i>Euchlanea</i>	Section <i>Euchlanea</i>	ssp. <i>mexicana</i>
<i>Euchlanea mexicana</i>	<i>Zea mexicana</i>	Race Chalco
Phase Durango	Race Nobogame	Race Central Plateau
Phase Bajio	Race Central Plateau	Race Nobogame
Phase Chalco	Race Chalco	ssp. <i>parviglumis</i>
Phase Barranca	Race Balsas	ssp. <i>huehuetenangensis</i>
Phase Guerrero	Race Guatemala	ssp. <i>mays</i>
Phase Huehuetenango	Race Huehuetenango	Section <i>Luxuriantes</i>
	<i>Zea perennis</i>	<i>Zea diplorennis</i>
		<i>Zea perennis</i>
		<i>Zea luxurians</i>
		<i>Zea nicaraguensis</i>

Adapted from Doebley et al. (1984); Doebley (1990a)

paid little attention to floral morphology (Doebley 1983). Thus, some of his races are clearly distinct, while others are differentiated narrowly. Moreover, he did not accept Beadle’s teosinte hypothesis for the origin of maize (Wilkes 1985). Further taxonomic study of the genus *Zea* was published by Iltis and Doebley, establishing a new hierarchical system of classification based on the morphology of the male inflorescence (tassel) and spikes (Doebley and Iltis 1980; Iltis and Doebley 1980). Furthermore, Doebley stated that morphological distinctions made it clear that the genus *Zea* should be divided into two sections: *Luxuriantes* and *Zea* (Doebley 1983). In section *Zea*, cultivated maize (*Z. mays* ssp. *mays*) was grouped together with several teosintes (*Z. mays* ssp. *mexicana*; *Z. mays* ssp. *parviglumis*; and *Z. mays* ssp. *huehuetenangensis*). Section *Luxuriantes* includes *Z. diplorennis*, *Z. perennis*, and *Z. luxurians*. This taxonomic system placed the taxa in a sequence that is believed to reflect their phylogenetic origins. More importantly, the morphological data provided strong evidence that *Z. mays* ssp. *parviglumis* and/or ssp. *mexicana* was a direct ancestor of maize.

Because of the striking differences in the female inflorescence (ear) between maize and teosinte (Fig. 11.3), there is still a lot of debate about the evolution of maize. Iltis carefully evaluated the ear morphology of teosinte and maize and used these observations to construct his catastrophic sexual

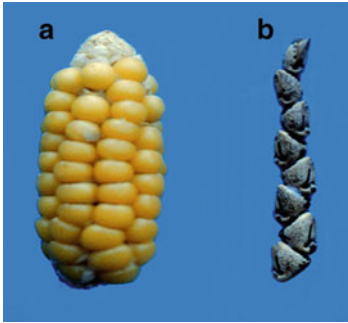


Fig. 11.3 Extreme differences between maize and teosinte ears. (a) A short maize ear; (b) Ear of pure teosinte (*Z. mays* ssp. *parviglumis*). Figure is adapted from Doebley (2004). Reprinted, with permission from the *Annual Review of Genetics*

transmutation theory (CSTT; Iltis 1983). In the CSTT model, Iltis proposed that teosinte is the ancestor of maize and that the maize ear evolved from the teosinte ear through a one-step feminization of the tassel. Iltis has now replaced his CSTT model with his sexual translocation theory (STLT) to explain the ear evolution from teosinte to maize. STLT theory proposed that maize ear has evolved from teosinte tassels through one or two homeotic sexual translocations, which replace the primary and secondary branch tassels with a female structure (Iltis 2000). The female inflorescence development of teosinte was also investigated in other published studies (Benz and Iltis 1992;

Orr and Sundberg 1994; Orr et al. 2002). Their results further supported Ittis's STLT model and confirmed the important role that teosinte played in the origin of maize.

11.3.2 Chemotaxonomy Based on Biochemical Markers

Chemotaxonomy or chemosystematics classifies organisms by chemical or biochemical methods. Because of the importance of the taxonomy of *Zea*, a considerable amount of work using biochemical markers (enzymes, other proteins, and amino acids) has been undertaken. The electrophoretic patterns of zeins and reduced alcohol-soluble glutelins in teosintes were found to be quite similar to those in maize, but those of the sister genus *Tripsacum* were found to exhibit marked differences (Paulis and Wall 1977). Similar banding patterns were found on denaturing acrylamide gels for water-soluble seed proteins in comparisons between maize and Mexican teosinte. These results further suggested that a Mexican teosinte was an ancestor of maize (Smith and Lester 1980). Subsequently, influential work on the chemotaxonomy of the genus *Zea* was done by Doebley and his colleagues through the analysis of isozymes (Doebley et al. 1984, 1985, 1987a). In their work, 13 enzyme systems were investigated in 56 populations of teosinte and 99 populations of maize from Mexico and Guatemala, and a phylogenetic tree was constructed (Fig. 11.4). The classification on the basis of isozyme data agreed well with previous studies based on morphological traits.

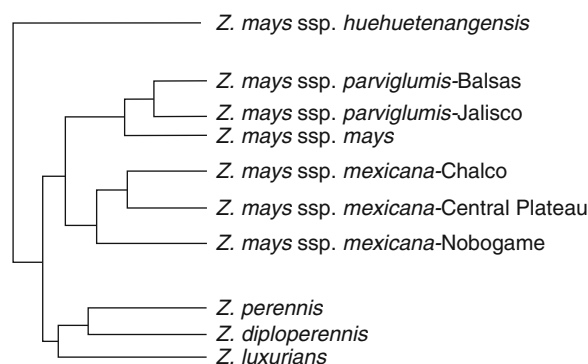


Fig. 11.4 The phylogenetic tree of teosinte and maize based on isozyme data. Figure is adapted from Doebley et al. (1984)

Several morphologically similar species (*Z. mays* ssp. *mexicana* and ssp. *parviglumis*, *Z. perennis*, and *Z. diploperennis*) were well-differentiated based on these data. This study also indicated that one of the Mexican annual teosintes, *Z. mays* ssp. *parviglumis*, was the closest relative of maize because it had indistinguishable isozyme allele frequencies from those of maize.

11.3.3 Molecular Markers and Their Application for the Study of Maize Origins and Evolution

Because of their abundance, ease of use, high level of polymorphism, robustness, and constantly decreasing cost, the development of DNA-based markers has provided an excellent opportunity to evaluate the relationship between the teosintes and modern maize. Molecular markers have been used to examine the mitochondrial, chloroplast, and nuclear DNA of teosinte and maize.

In early molecular analyses, chloroplast and mitochondrial DNA fragments produced by restriction enzyme digestion were used to characterize relatedness in the genus *Zea*. Using this method, maize, a perennial teosinte, and six races of annual teosinte (Guatemala, Huehuetenango, Balsas, Central Plateau, Chalco, and Nobogame) were characterized (Timothy et al. 1979). The results showed that the annual teosinte Guatemala and the perennial teosinte differed markedly from maize, while the other five races of annual teosinte were similar to maize, suggesting a common ancestry. Phylogenetic analysis based on chloroplast DNA digestion patterns split the genus *Zea* into two sections that were fully congruent with those revealed by isozyme analysis and morphology (Doebley et al. 1987b). These results supported the hypothesis that maize is a domesticated form of either *Z. mays* ssp. *mexicana* or *Z. mays* ssp. *parviglumis*. However, distinguishing between these two teosinte subspecies as an ancestor was not possible at this low level of resolution.

Two common types of molecular markers that have been used in maize and teosinte research are restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) or microsatellite markers.

(1) *RFLPs*: Due to their inherent properties (numerous and codominant), RFLP markers have been used with great success in many genomic characterizations, including identification and mapping of quantitative trait loci (QTL) controlling traits (Phillips and Vasil 1994). A total of 52 genes that might be related to the major morphological differences between maize and *Z. mays* ssp. *mexicana* were investigated. The results demonstrated that these differences are controlled by five chromosomal regions, each with major effects on one or more of these traits (Doebley et al. 1990; Doebley and Stec 1991). These studies provided new evidence on maize evolution that were further supported by Szabo and Burr's experiments investigating maize and *Z. mays* ssp. *parviglumis* (Szabo and Burr 1996). Multiple QTL controlling morphological differences between *Z. diploperennis* and *Z. mays* ssp. *parviglumis*, and between *Z. mays* ssp. *parviglumis* and *Z. mays* ssp. *mexicana*, were also analyzed by RFLP markers (Westerbergh and Doebley 2002, 2004; Lauter et al. 2004). Based on QTL results, two genes were identified as strong candidates to be responsible for the major differences in plant morphology between maize and teosinte. These two genes, *teosinte branched1* (*tb1*) and *teosinte glume architecture1* (*tga1*), were cloned and functionally characterized by the Doebley laboratory (Doebley et al. 1997; Wang et al. 2005). More details about the function of these genes will be discussed in Sect. 5. (2) *SSRs*: Microsatellite or SSR markers are a series of tandemly repeated units (1–6 bp each) commonly found in eukaryotic genomes. SSRs have been exploited as tools to assess the genetic relatedness and diversity of crop plants because they are highly polymorphic, codominant, easy to assay, and relatively inexpensive (Bruford and Wayne 1993). More than 2,000 SSR markers have been developed for maize (www.maizegdb.org/ssr.php) and some of them can be applied to different teosinte species (Lubberstedt et al. 1998). Using SSR markers, the phylogenetic tree of the genus *Zea* was constructed with good resolution and the results revealed that *Z. mays* ssp. *parviglumis* is most closely related to the progenitor of maize (Matsuoka et al. 2002a). Moreover, the microsatellite-based study also indicated that maize originated from a single domestication event in southwestern Mexico about 8,700 years ago, which is consistent with the archaeological and paleoecological records such as phytoliths, starch grains, and pollen (Piperno and Flannery 2001;

Matsuoka et al. 2002b; Piperno et al. 2009; Ranere et al. 2009). Recently, phylogeny and population structure were investigated in a comprehensive study that genotyped numerous maize and teosinte accessions from the American continent using 93 microsatellite markers (Fukunaga et al. 2005). These results provide a solid framework for further studies of the genetic origins of maize domestication and diversification.

11.3.4 Relevant Statistical Techniques and Computer Software

Research on the origin and evolution of maize increasingly produce a large quantity of information that includes morphological data, molecular marker data, gene expression data, and protein-level data. A series of classical and modern statistical techniques have been widely used to allow more power and flexibility in handling these impressive sets of data. Here, we briefly describe some important statistical tools and computer programs used for the classification of the genus *Zea* and for elucidating the contributions of the teosintes to maize evolution. The software and their platforms, uses, and online links are summarized in Table 11.5.

11.3.4.1 Statistical Tools for Classification Within the Genus *Zea*

A clear taxonomy of the genus *Zea* is the backbone for the elucidation of the role played by teosintes in origin and evolution of modern maize. Principal component analysis (PCA), canonical analysis, and cluster analysis have been the three most widely used statistical methods. (1) *PCA*: PCA is an ordination statistical technique invented by Karl Pearson in 1901. In taxa classification, it is utilized to generate a 2- or 3-dimensional scatter plot on which the geometrical distances among the dots reveal the genetic distance and the aggregations of dots reflect the genetic similarity among the individual samples (Warburton and Crossa 2000; Mohammadi and Prasanna 2003). For example, on a PCA plot, *Z. perennis*, *Z. diploperennis*, and *Z. mays* ssp. *huehuetenangensis* were separated from each other and from the remaining annual Mexican

Table 11.5 Some widely used computer software for maize origin and evolution research

Name	Recent version	Platforms	Brief description	Useful online link
SAS/SAT	9.2	Windows (2003, xp, Vista); Linux; Solaris	PCA and canonical analysis; clustering analysis	http://www.sas.com
VisuMap	2.6	Windows (2000/xp)	PCA, especially for 3D maps	http://www.visumap.net
XLSTAT	2008	Windows (9x~vista); Mac, based on Excel	PCA and canonical analysis	http://www.xlstat.com
CANOCO	4.5	Windows; DOS	Canonical analysis	http://www.microcomputerpower.com
Microsat	1.5	Dos; Mac	Genetic distance measurement	http://hpgl.stanford.edu/projects/microsat/
LCDMV	–	Windows or Linux based on SAS (6.10 and above version)	Genetic distance measurement	http://www.cimmyt.org/english/docs/manual/lcdmv/contents.htm
STRUCTURE	2.2	Windows (9x/xp/2000/NT); Linux; Mac	Model-based method clustering; population structure	http://pritch.bsd.uchicago.edu/structure.html
PHYLIP	3.68	Linux; Mac; Windows; (9x/NT/me/2000/xp/vista)	Phylogenetic tree construction	http://evolution.genetics.washington.edu/phylip.html
PAUP*	4.0	DOS; Linux; Mac ; Windows (9x/me/2000/NT/xp)	Phylogenetic tree construction	http://paup.csit.fsu.edu/
MacClade	4.08	Windows; Mac	Phylogenetic tree construction	http://macclade.org/macclade.html
Mapmaker	3.0	Windows; Web-based	Linkage map	http://www.broad.mit.edu/science/software/software
QTL Cartographer	1.17	Windows; Mac; Linux	QTL mapping	ftp://statgen.ncsu.edu/pub/qtlcart/
Windows QTL Cartographer	2.5	Windows (9x, NT/ME/2000/xP)	QTL mapping	http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

teosintes. *Z. mays* ssp. *mays* completely overlapped with *Z. mays* ssp. *parviglumis* (Doebley et al. 1984; Smith et al. 1984). As a 100-year-old method, it is not surprising that PCA can be performed with different softwares such as SAS, VisuMap, and XLSTAT. (2) *Canonical analysis*: Canonical analysis, also called multiple discriminant analysis, is an elegant multivariate statistical model for studying the relationships between two or more variables in a data set. This method was used to assess relationships within the genus *Zea* (Doebley and Iltis 1980). The function and the output graph of canonical analysis are both quite similar to PCA. However, canonical analysis is more suitable than PCA for taxonomic analysis because PCA is based on the variability among individuals and does not efficiently separate taxa that show few intrataxa differences (Doebley 1983). The software of choice for this type of analysis is CANOCO. Mathematically, this software is excellent. However, its ease of use and the graphics are not the best. Additionally, XLSTAT and SAS can also do the

canonical analysis. (3) *Cluster analysis*: Cluster analysis or typological analysis refers to a common statistical technique whose purpose is to classify objects into groups based on the characteristics they possess so that individuals in the same group are more similar than objects in a different group. There are two types of cluster analysis methods: the distance-based method and the model-based method. Distance-based phylogenetic tree construction has predominated in maize origin and evolution studies. The most commonly used algorithms for the construction of phylogenetic trees are neighbor joining (NJ) and Fitch-Margoliash. An important step in producing a distance-based phylogenetic tree is to measure the genetic distance. Different genetic distance methods may produce different phylogenetic trees. The commonly used approaches for measuring genetic distance are Nei's standard distance (D_s) and the modified Rogers' distance (D_r). Trees constructed using four different genetic distance methods (D_s ; D_r ; Cavalli-Sforza and Edwards's chord distance

and Nei's chord distance) were compared (Matsuoka et al. 2002a). The results showed that the tree based on D_s is congruent with the expected tree based on Doebley and Iltis's classical taxonomy of *Zea* (Doebley and Iltis 1980), while the other three trees failed to show a monophyletic cluster for *Z. mays* ssp. *mexicana*. The genetic distance is usually calculated by MICROSAT, but this software is no longer updated. LCDMV, a software developed by CIMMYT, is also used to estimate genetic distance. To use this software, a recent version of SAS (later than version 6.10) is required. PHYLIP, PAUP*, and MacClade are the three most frequently cited computer programs for phylogenetic analysis.

11.3.4.2 Statistical Tools for QTL Mapping

QTL mapping is a powerful method for determining the number of genomic regions that control specific traits (Kao et al. 1999). A number of QTL that determine the morphological differences between teosinte and maize have been identified (Doebley and Stec 1993; Doebley et al. 1995; Lauter et al. 2004). There are two steps to make a QTL map: one is to construct the linkage map with the molecular markers and the other is to use quantitative phenotyping to map the QTL. The linkage map is usually made by a computer program called MAPMAKER, based on the maximum likelihood method (Table 11.5). In maize evolution research, interval mapping (IM) and composite interval mapping (CIM) are two widely used statistical techniques for mapping QTL. Theoretically, CIM should be more precise because CIM combines IM with multiple regressions so that the effects of QTL outside the test interval are included in the model (Zeng 1993). QTL Cartographer is one of the most commonly used QTL mapping programs. However, updated packages have not been released since 2004. Recently, a new computer program called Windows QTL Cartographer was developed on the basis of the original QTL Cartographer (Wang et al. 2006).

11.3.5 Summary

A wide set of morphological, biochemical, diversity, and mapping analyses have been employed to study

the origin and evolution of modern maize and its teosinte relatives. The results of these studies support the idea that a specific teosinte (*Zea mays* ssp. *parviglumis*) was the ancestor of modern maize, and that a small number of genetic events gave rise to the major morphological differences that distinguish these species. A wealth of software is available to assist these analyses, but many of these tools are not easy to use and/or have not been recently updated.

11.4 Role in the Development of Cytogenetic Stocks and Their Utility

The basic cytogenetics of the wild *Zea* species was discussed in the first part of this chapter. In the genus *Zea*, all species are diploid with a basic set of ten chromosomes ($2n = 20$), except *Z. perennis*, a tetraploid with $2n = 40$. Due to their unique karyotypic and morphological features, the teosintes have played an important role in the development of cytogenetic stocks of maize. These stocks include substitution lines, haploids, polyploids, and aneuploids. These cytogenetic materials have been widely used in genetics research.

11.4.1 Substitution and Introgression Lines

A chromosome substitution line is generated by replacement of a single chromosome in a host plant by a chromosome from a donor parent. Introgression lines contain genomic fragments from a similar species. The teosintes are the closest wild relatives of maize, and they have a similar ($n = 10$) chromosomal composition. Thus, the teosintes have been used to produce maize–teosinte substitution and introgression lines by wide crosses followed by backcrossing to the maize inbred parents. These substitution and introgression lines have mainly been used for trait mapping and introgression of desirable traits from teosinte into the maize germplasm. (1) *Linkage map construction and QTL identification*. QTL have been identified by phenotypic and statistical analyses in F_2 populations or backcross populations derived from maize–teosinte crosses (Doebley and Stec 1991; Doebley et al. 1995,

1997). Compared to F_2 populations, maize–teosinte backcross populations (introgression lines) have been more informative because of their larger population size and resultant higher number of recombination events (Briggs et al. 2007). (2) *Maize improvement*. To transfer desirable characters from teosinte to maize, maize–teosinte substitution and introgression lines were created. For example, several maize \times annual teosinte (*Z. mays* ssp. *mexicana* and *Z. luxurians*) substitution lines with high grain yield were generated (Cohen and Galinat 1984). Lines with superior disease resistance were developed by hybridization and backcrossing of maize in projects with two different perennial teosintes (Wei et al. 2003; Tang et al. 2005a).

11.4.2 Haploids

Haploids containing only one half of the chromosome number present in somatic cells provide a shortcut to develop inbred lines. The generation of maize haploids through in vitro (anther and microspore culture) and in vivo (genetic induction) approaches has been routine for quite some time (Coe and Sarkar 1964; Chase 1969; Kindiger and Hamann 1993; Tang et al. 2005b; Zhang et al. 2008). However, there are no reports of teosinte haploid production, probably due to the unclear cytogenetic and genetic background. However, several teosintes have been used as pollen sources to recover wheat haploids. High frequencies of haploid production in wheat were obtained through intergeneric hybridization with teosinte, following the elimination of teosinte chromosome during the development of the embryo (Ushiyama et al. 1991; Ushiyama and Yoshida 2008). The relative efficiencies of nine teosinte lines and several elite maize lines for the production of wheat haploids were evaluated by Suenaga et al. (1998). The results showed that *Z. mays* L. ssp. *mexicana* had a significantly higher efficiency of haploid production compared to *Z. mays* L. ssp. *mays* and to other teosinte species.

11.4.3 Polyploidy and Aneuploidy

With the exception of the tetraploid *Z. perennis*, crosses between maize and different teosintes will

produce fertile F_1 hybrids at the diploid level ($2n = 20$). In contrast, *Z. perennis* is an excellent parent for the production of teosinte–maize polyploids. Triploid stocks were obtained by crossing *Z. perennis* ($2n = 40$) with *Z. mays* L. ssp. *mays* ($2n = 20$) or *Z. perennis* with other teosintes. The triploids are completely sterile due to the high number of univalents and trivalents produced in gametes (Poggio et al. 1990). One can produce tetraploid hybrids ($2n = 40$) from *Z. perennis* \times *Z. mays* L. *mays* hybrids by doubling the chromosome number of maize with colchicine treatment before crossing (Molina and Garcia 1999). Additionally, a fertile tetraploid hybrid from the combination of an unreduced gamete of *Z. diploperennis* ($2n = 20$) with a normal gamete of *Z. perennis* ($2n = 20$) was generated by Molina (1983). Polyploid stocks of maize and teosinte have been used mainly for cytogenetic research to investigate ancestral relationships and taxonomic classification in the genus *Zea*. Different meiotic behavior was found between a triploid hybrid (*Z. perennis* \times *Z. luxurians*) and a diploid hybrid (*Z. diploperennis* \times *Z. luxurians*) that can be used for differentiating *Z. luxurians* from other teosintes (Poggio et al. 1999). The meiotic configuration of several triploid and tetraploid stocks provided important evidence regarding the ancestral allotetraploid nature of maize (Molina and Naranjo 1987; Poggio et al. 1999; Molina et al. 2004).

Aneuploids have extra or missing chromosomes, including monosomics ($2n - 1$) and trisomics ($2n + 1$). Maize monosomic and trisomic lines are usually produced by the r-X1 deficiency system and are extremely useful in mapping genes to linkage groups and specific chromosomes (Helentjaris et al. 1986; Weber 1986; Zhao and Weber 1988). At this date, there is no record of teosinte aneuploids.

11.4.4 Summary

A series of maize–teosinte substitution and introgression lines were created and used for maize improvement. Teosintes have been used to generate wheat haploids. Teosinte polyploids were used to investigate ancestral relationships in genus *Zea*. To date, no teosinte aneuploids have been reported.

11.5 Role in Classical and Molecular Genetic Studies

Maize and its wild relatives provide a tractable model system for both classical and molecular genetic research because maize and the teosintes are fully interfertile. The first maize–teosinte hybrid experiments can be traced back to the year 1896 (Harshberger 1896). In the past 100 years, through hybridization of maize with teosinte or teosinte with teosinte, a series of genetic linkage maps was constructed. These populations were also employed in the identification of important genes and polygenic clusters. The effects of ploidy level, the details of physiological pathways, and the properties of various host–parasite interactions were also investigated. These classical and molecular genetic studies of the teosintes and maize have made a major contribution to our understanding of plant biology.

11.5.1 Use in Classical Genetic Studies

Classical genetic studies on the teosintes were mainly focused on the inheritance of morphological traits (dominant, recessive, or intermediate) in maize–teosinte or teosinte–teosinte hybrids. These investigations helped to explain the origin of maize before any molecular studies were undertaken. Mexican agronomist José Segura first pointed out that the teosintes were more closely related to maize than previously thought based on his investigations of maize–teosinte hybrids (Harshberger 1896). Pioneering work on inheritance in maize and the teosintes was carried out in the early twentieth century (Collins and Kempton 1920; Beadle 1939; Mangelsdorf and Reeves 1939). As mentioned above, Beadle's classical genetic experiments (Beadle 1939) led to his hypothesis of a teosinte origin for maize that has been widely accepted by modern maize geneticists (Bennetzen et al. 2001). In his experiment, Beadle crossed one maize line with a teosinte and observed that the teosinte ear reappeared in the F_2 populations at a frequency of about one in 500. From these data, he concluded that a teosinte was the sole progenitor of maize and that only four or five loci controlled the major morphological differences between maize and teosinte (Beadle

1939). Subsequently, the inheritance patterns of other traits (tassel branch, male spikelets, bloom date, prolificity, etc.) were also investigated. The genetic behaviors of these traits in F_2 populations are summarized in Table 11.6. Some of these traits are inherited in a similar manner and are closely linked, as seen in the cases of TBN (tassel branch number) and TBAL (tassel branching axis length) and of VBL (number of veins between primary lateral veins) and TV (total vein number).

Teosintes were also used in classical cytogenetic research that investigated genome origins within the genes *Zea*. DeWet and Harlan's cytogenetic studies provide strong evidence that maize and teosinte (*Z. mays* ssp. *mexicana*) are conspecific and that teosinte could not be derived from a hybrid of maize and *Tripsacum*, as has been hypothesized by Mangelsdorf (Mangelsdorf and Reeves 1939; Dewet and Harlan 1976).

11.5.2 Use of Teosintes as Parents in Interspecific/Intergeneric Crosses for Construction of Classical and Molecular Genetic Linkage Maps

Genetic linkage maps were constructed using the progenies of teosinte–maize or teosinte–teosinte crosses (F_1 , F_2 or backcross). The development of these linkage maps has been useful for mapping QTL responsible for the morphological, developmental, and stress tolerance differences between maize and teosinte or between different teosinte species. It has also been helpful for marker-assisted selection (MAS) for maize improvement. (1) *Genetic linkage maps and QTL related to morphological evolution in the genus Zea*. In 1947, Mangelsdorf showed that the inflorescence differences between maize and Nobogame teosinte (*Z. mays* ssp. *mexicana*) were linked with markers on chromosomes 1, 3, 4, 8, 9, and 10 (Mangelsdorf 1947). However, these early linkage map results on the chromosomal regions controlling the morphological differences between maize and teosinte were contradictory. For instance, the single pistillate spikelet of *Z. mays* ssp. *parviglumis* was associated with different chromosomes by different research groups, as summarized by Szabo and Burr

Table 11.6 Genetic behavior of some traits in F₂ population from a cross between maize and teosinte

Trait	Description	Cross	Genetic behavior of F ₂ population	Reference
TBN	Tassel branch number	<i>Zea perennis</i> × <i>Zea mays</i> L. ssp. <i>mays</i> (Gaspe)	Transgressive inheritance and high TBN is dominant (traits from maize)	Palacios and Magoja (1984)
TBAL	Tassel branching axis length	<i>Zea perennis</i> × <i>Zea mays</i> L. ssp. <i>mays</i> (Gaspe)	Transgressive inheritance and long TBAL is dominant (traits from maize)	Palacios and Magoja (1984)
LTBIL	Lateral tassel branch internode length	<i>Zea perennis</i> × <i>Zea mays</i> L. ssp. <i>mays</i> (Gaspe)	No significant difference from mean parental value	Palacios and Magoja (1984)
PGZ	Pollen grain size	<i>Zea perennis</i> × <i>Zea mays</i> L. ssp. <i>mays</i> (Gaspe)	No significant difference from mean parental value	Magoja and Palacios (1984)
EUN	Number of ears in the uppermost node	<i>Zea mays</i> ssp. <i>parviglumis</i> × <i>Zea mays</i> L. ssp. <i>mays</i>	Transgressive inheritance and high number is dominant (from teosinte)	Corcuera (1991)
ET	Number of ears per tiller	<i>Zea mays</i> ssp. <i>parviglumis</i> × <i>Zea mays</i> L. ssp. <i>mays</i>	Transgressive inheritance and high number is dominant (from teosinte)	Corcuera (1991)
PN	Number of productive nodes per tiller	<i>Zea mays</i> ssp. <i>parviglumis</i> × <i>Zea mays</i> L. ssp. <i>mays</i>	Transgressive inheritance and high number is dominant (from teosinte)	Corcuera (1991)
BD	Bloom date	<i>Zea perennis</i> × <i>Zea mays</i> L. ssp. <i>mays</i>	No significant difference from mean parental value	Magoja and Benito (1981)
NL	Number of leaves	<i>Zea perennis</i> × <i>Zea mays</i> L. ssp. <i>mays</i>	No significant difference from mean parental value	Magoja and Benito (1983)
VBL	Number of veins between primary lateral veins	<i>Zea diploperennis</i> × <i>Zea mays</i> L. ssp. <i>mays</i>	Quantitative inheritance and low VBL is partially dominant (from maize)	Corcuera and Magoja (1991)
TV	Total vein number	<i>Zea diploperennis</i> × <i>Zea mays</i> L. ssp. <i>mays</i>	Quantitative inheritance and low TV is partially dominant (from maize)	Corcuera and Magoja (1991)

(1996). This disparity apparently was caused by the relatively small population sizes investigated and the absence of any statistical analysis. The application of molecular markers combined with statistical analysis made the genetic linkage maps more accurate. Based on maize–teosinte or teosinte–teosinte molecular genetic linkage maps, (a) the chromosome loci and QTL (major and minor) involved in maize morphological traits are distributed across all ten chromosomes (Doebley 2004). (b) Most morphological differences between maize and teosinte are controlled by relatively few chromosomes with large effects (five chromosome regions and about 50 QTL) plus several other regions with small effects. The morphological differences between different teosinte species are mainly controlled by several QTLs with small effect and no single QTL of strikingly large effect (Westerbergh and Doebley 2002, 2004). (c) The major differences in morphological traits between maize and the teosintes are located in a few chromosomal regions. A region on chromosome 3 is strongly associated with differences in kernel weight (Doebley et al. 1994). The cupulate fruitcase and the degree of glume induration are

mainly controlled by a region on chromosome arm 4S. The differences in ear rank (the number of grain-bearing cupules) are determined primarily by chromosome 2S. A region on chromosome 1L accounts for the major differences in plant architecture such as primary lateral branches and female inflorescences (Doebley et al. 1990, 1995; Doebley and Stec 1991, 1993; Bomblies and Doebley 2006). (2) *Genetic linkage maps and QTL for maize improvement*. Some teosintes have additional important characteristics (flood tolerance, pest resistance, etc.) that could be of use in maize improvement. On the basis of current genetic linkage maps, it should be possible to efficiently introgress these unique characters into maize by a marker-assisted selection (MAS) approach. Recently, a genetic linkage map was constructed using an F₂ population generated from a cross of maize inbred B64 with the teosinte *Z. nicaraguensis*. This map employed 88 SSR markers and identified four QTL related to root aerenchyma (tissue with large airy compartments for gas exchange and storage) formation (Mano et al. 2007). These results could provide the genetic basis for the development of flooding-tolerant maize lines.

11.5.3 Mapping of Genes and Polygenic Clusters

Although genetic linkage map and QTL studies provided some evidence about the genetic foundations of the differences between maize and specific teosintes, they do not provide information about the genes underlying the QTL. For example, mapping data do not indicate whether a QTL for a trait represents a single major gene or a block of linked genes. To definitively resolve this issue, it is necessary to clone the genes responsible for the quantitative traits. In maize and teosinte research, several methods such as genetic complementation, association mapping, and human selection have been applied to identify the genes responsible for specific QTL (Mackay 2001; Yamasaki et al. 2005). By these approaches, a number of genes related to maize evolution and domestication have been identified, though most of their functions are not well characterized. These results demonstrated multifactorial inheritance of the key morphological traits associated with maize domestication from teosinte. However, these traits were not necessarily highly polygenic, except kernel weight, which exhibits oligogenic inheritance (Doebley et al. 1990, 1994; Barton and Keightley 2002). Here, we place these genes into three classes (some overlapping) according to their functions. The known genes and their roles in maize evolution and domestication or natural variation within the teosintes are listed in Table 11.7, and the details of their discovery and functional characterization are discussed below.

(1) *Genes controlling key morphological differences (threshold traits) between maize and teosinte.* As discussed in the first part of Sect. 11.1.2 on Morphology, the most obvious phenotypic differences between maize and the teosintes are that the wild *Zea* species have many lateral branches and the ears have cupulate fruitcases. Several genes controlling these morphological differences have been identified. The first such gene is *teosinte branched 1 (tb1)*, responsible for the QTL on chromosome 1L (Doebley et al. 1995). The *tb1* gene encodes a predicted helix–loop–helix DNA-binding protein. This protein, a probable transcriptional regulator, controls lateral branch growth and acts as a repressor of apical growth (Doebley et al. 1997). Maize with a *tb1* null mutation exhibits an outgrowth of axillary buds and loses apical dominance, causing it to resemble a teosinte in vegetative growth pattern (Doebley et al. 1995). Furthermore, insertional mutants of *tb1* indicate that this gene reduces the growth of axillary organs and aids in the development of female inflorescences (Doebley et al. 1997). Two other genes that control key morphological differences between maize and the teosintes are *teosinte glume architecture 1 (tga1)* and *barren stalk1 (bal)*. The *tga1* gene controls the development of the cupulate fruitcase and the degree of glume induration (Dorweiler et al. 1993). Developmental analysis has shown that the allele of *tga1* in teosinte is responsible for increasing the length of inflorescence internodes and increasing the length, thickness, and curvature of the glume (Dorweiler and Doebley 1997). The function of *bal* is similar to that of *tb1*. It also encodes a predicted helix–loop–helix protein that regulates

Table 11.7 Genes that contributed to maize domestication and/or natural variation within the teosintes

Gene	Chromosomal location ^a	Trait(s) influenced	References
<i>teosinte branched1 (tb1)</i>	1L	Lateral branches and female inflorescences	Doebley et al. (1995, 1997)
<i>teosinte glume architecture 1 (tga1)</i>	4S	Development of cupulate fruitcase	Dorweiler and Doebley (1997)
<i>barren stalk1 (bal)</i>	3L	Lateral meristems	Gallavotti et al. (2004)
<i>zea floricaula/leafy2 (zfl2)</i>	2S	Ear rank and inflorescence structure; plant height in the teosintes	Bombliet et al. (2003); Weber et al. (2007)
<i>teosinte crossing barrier1 (tcb1)</i>	4	Crossing ability between maize and teosinte	Evans and Kermicle (2001)
<i>terminal ear1 (te1)</i>	3L	Internodes number in the teosintes	Weber et al. (2007)
<i>tassel-replaces-upper-ear1 (tru1)</i>	3L	Growth of lateral branches	Doebley et al. (1995)
<i>ramosa1 and 2 (ra1 and ra1 2)</i>	–	Ear structure variation in the teosintes	Weber et al. (2008)
<i>zea agamous-like1 (zag1)</i>	–	Ear shattering variation in the teosintes	Weber et al. (2008)

L long arm; S short arm

the development of the vegetative lateral meristem (Gallavotti et al. 2004).

(2) *Genes with agronomic importance as signatures of selection during maize domestication.* Maize has experienced strong selective pressure directed at genes controlling traits of agronomic importance during its domestication from an ancestral teosinte. As a result, these agronomically important genes bear strong signatures of selection, where the nucleotide diversity of these genes is low compared to genes that are not under equally powerful selection. Using various gene mapping approaches, several candidate genes were identified and their functions were confirmed by comparing the nucleotide sequences of the target genes in maize to those in teosintes. The “domesticated alleles” of two genes discussed above that control key morphological differences, *tb1* and *ba1*, have undergone selective sweeps during maize domestication (Wang et al. 1999; Gallavotti et al. 2004). A MADS box gene of unknown function also displayed a selective sweep based on genomic scanning of 501 maize genes for signatures of selection (Vigouroux et al. 2002). Furthermore, eight genes with agronomic importance related to nutritional quality, maturity, and productivity were also identified by sequencing 1,095 maize genes and comparing sequence diversity with *Z. mays* ssp. *parviglumis* (Yamasaki et al. 2005). These genes encode for protein products, which are likely to be involved in auxin response, circadian clock, signal transduction, amino acid transport, and mediating protein–protein interactions.

(3) *Genes controlling natural genetic variation between and within the teosintes.* Natural variation, the genetic variation within wild species, has been considered as the main resource for evolutionary change of a species in response to its environment. Identification of genes associated with natural variation is one of the principal goals in evolutionary genetics (Shindo et al. 2007). Some key genes controlling the growth and development of maize are also involved in natural variation within teosinte species. For instance, three maize regulatory genes (*zfl2*, *zap1* and *tb1*) play important roles in the observed phenotypic variation within the *Z. mays* ssp. *parviglumis* germplasm (Weber et al. 2007). In addition, *Terminal ear1* (*te1*), a gene responsible for key morphological differences between maize and teosinte, was found also to be involved in natural variation within *Z. mays*

ssp. *parviglumis* (Doebley et al. 1995; White and Doebley 1999; Weber et al. 2007). Studies on *Zea floricaula/leafy2* (*zfl2*) showed that this gene determines both ranks of cupules on the teosinte ear (a key difference between maize and teosinte) and the variation of plant height within teosinte species (Bomblies et al. 2003; Weber et al. 2007). Recently, 15 genes responsible for natural genetic variation within the teosintes were identified (Weber et al. 2008). Among them, a MADS-box gene, *zag11*, was associated with teosinte ear shattering. Furthermore, variation in teosinte ear structure was found to be linked with two *ramosa* genes (*ra1* and *ra2*) that are transcription factors associated with inflorescence architecture in maize (Vollbrecht et al. 2005; Bortiri et al. 2006).

11.5.4 Assessment of Gene Action

Genetic studies usually involve two topics: one is the identification of genes that perform specific functions and the other is the determination of the means by which genes accomplish their tasks. Here, we will discuss assessment of gene action. Additive, dominant (recessive), partially dominant, overdominant, and epistatic gene actions are the five models for general gene function in any species, including maize and teosinte. (1) *Additive, dominant, partially dominant and overdominant gene action:* Genetic analysis of F_2 populations derived from maize crosses to teosintes has shown that most of the QTL involved in the domestication of maize exhibit additive gene action because F_2 values of the traits controlled by these QTL are usually close to the mid-parent value (Doebley et al. 1990, 1994; Doebley and Stec 1993; Table 11.6). A few QTLs, which control leaf length, leaf width, kernel weight, and lateral branch internode number, behave in a fully dominant/recessive fashion. Interestingly, teosinte alleles tended to be dominant in F_2 populations from the cross between *Z. mays* L. ssp. *mays* and *Z. mays* ssp. *mexicana* or *Z. mays* ssp. *parviglumis*, while these same traits were more likely to be maize-like in F_2 populations from a cross between *Z. mays* L. ssp. *mays* and *Z. diploperennis* (Doebley and Stec 1993; Doebley et al. 1994; Srinivasan and Brewbaker 1999). Besides, in maize–*Z. diploperennis* F_2 populations, maize alleles are partially dominant

for traits of total vein number and number of veins between lateral veins of male spikelet outer glume, which are two important characteristics for the taxonomy of genus *Zea* (Doebley and Iltis 1980; Corcuera and Magoja 1991). However, the modes of gene interaction for QTL controlling the differences among different teosinte species did not show the same relationships. For example, of the 38 QTL found to be responsible for differences in growth habit between *Z. diploperennis* and *Z. mays* ssp. *parviglumis*, 10 exhibited dominant gene action, 11 were inherited in an additive manner, and the remaining 17 exhibited overdominance (Westerbergh and Doebley 2004).

(2) *Epistatic gene action*: Epistasis is a term that connotes a phenotypic interaction between different genes. Studies on the action of *tb1* showed that it involves a complex network of gene interactions with at least one QTL that may be an upstream regulator (Lukens and Doebley 1999). Epistatic effects were also detected between the large-effect QTL and other QTL controlling the inheritance of two leaf sheath characters used to distinguish *Z. mays* ssp. *mexicana* and *Z. mays* ssp. *parviglumis* (Lauter et al. 2004).

11.5.5 Physiological Pathways

Dissecting the physiological pathways that lie between genes and traits is the fundamental challenge for developmental and evolutionary genetics (Nadeau and Dunn 1998). Here, the contributions of teosinte research to the understanding of two well characterized pathways, those for anthocyanin and starch biosynthesis, will be described.

(1) *Anthocyanin biosynthesis*. Anthocyanins are flavonoids found as water-soluble pigments within the vacuole. Anthocyanins are found in floral and vegetative tissues of both maize and teosinte, and they range in color from red to blue (Fig. 11.5). Some maize landraces and varieties also have anthocyanin-pigmented kernels while teosinte kernels are colorless and enclosed by hard fruit cases with unidentified brown pigments on their external surface. The anthocyanin biosynthetic pathway in maize has been well characterized, with the identification of eight structural genes (*a1*, *a2*, *bz1*, *bz2*, *c2*, *chi*, *pr* and *whp*) and six regulatory genes (*b*, *c1*, *pl*, *r*, *a3* and *vp1*) (Coe et al. 1988; Holtont and Cornish 1995;

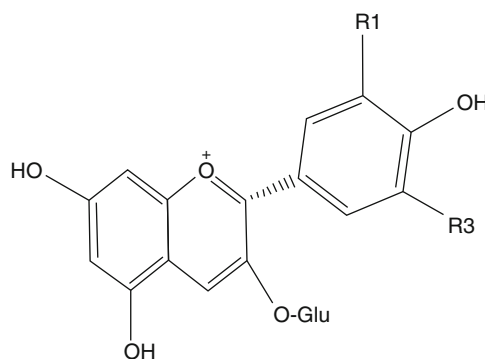


Fig. 11.5 Chemical structures of anthocyanins. Pigmentation depends on the presence of specific side groups. *Red color*: R1 = H, R3 = H; *Purple color*: R1 = OH, R3 = H; *Blue color*: R1 = OH, R3 = OH

Robinett et al. 1995) plus a number of less well characterized modifier genes. The role of both structural and regulatory genes in the anthocyanin pathway was investigated in *Z. mays* ssp. *parviglumis* (Hanson et al 1996). The results showed that the *Z. mays* ssp. *parviglumis* genome possesses competent versions of all of the structural genes required for the synthesis of anthocyanins. Therefore, the colorless kernels in *Z. mays* ssp. *parviglumis* are probably the result of non-functional regulatory genes. Subsequently, in order to confirm this conclusion, the regulatory gene *c1* was investigated and differences in *cis*-regulatory elements of this gene were found between maize and *Z. mays* ssp. *parviglumis*. This finding was proposed to explain why *Z. mays* ssp. *parviglumis* has the C1 functional protein in aleurone but only during germination and not during kernel maturation. A recessive (non-functional) allele of another maize anthocyanin pathway regulatory gene, *r*, is also presumed to be present in *Z. mays* ssp. *parviglumis* because the red anthocyanin color that was lacking from seed (regulated by *R*) was found to be present in vegetative tissues (where *R* is not required and *B* fills the same role) (Hanson et al. 1996). Lauter et al. (2004) investigated anthocyanin biosynthesis in two teosintes, *Z. mays* ssp. *Mexicana*, which has red leaf sheaths and *Z. mays* ssp. *Parviglumis*, which has green leaf sheaths. The results indicated that two regulatory genes, *b* and *a3*, were not functional in the leaf sheaths of the *Z. mays* ssp. *parviglumis* line analyzed (Lauter et al. 2004).

(2) *Starch synthesis pathway*. The properties of two enzymes (soluble starch synthases and starch branching enzyme) related to starch synthesis in

Z. diploperennis were examined and found to be similar to those in maize by a chromatographic method (Boyer 1982). The study of nucleotide diversity of six key genes of maize starch biosynthesis in 30 maize inbred lines and 10 accessions of *Z. mays* ssp. *parviglumis* found that the diversity in these teosintes is much higher than in maize, especially for *su1*, *bt2*, and *ae1* (Whitt et al. 2002). These results suggest that these genes underwent extreme human (artificial) selection during the domestication and improvement of maize from its teosinte ancestor.

11.5.6 Host–Parasite Interactions

The coevolution of host–parasite interactions is arguably the single most important source of biodiversity on earth and thus has a dramatic impact on ecological and agricultural fitness (Rausher 2001). Plants have evolved multiple defense strategies in response to the invasion of pathogens. Some traditional genetic studies in plant pathology have aimed to elucidate the genetic mechanisms and the major genes involved in both the host (resistance genes, *R*) and the parasite (avirulence genes, *Avr*). Many but not all genetic relationships between the host and the parasite can be explained by the gene-for-gene model, in which the specific *R* gene expressed by the host has a corresponding *Avr* gene that is expressed by the pathogens (Flor 1955; Thompson and Burdon 1992). In addition, a plant immunity system also has a series of defense genes encoding some proteins, enzymes, and secondary metabolites, which directly or indirectly inhibit the growth, reproduction, or infection of parasite (Nimchuk et al. 2003; Moeller and Tiffin 2005). Gene-for-gene *R* loci have not been extensively studied in the teosintes, but the evolution and selection patterns of several defense genes have been characterized by examining their nucleotide diversity in maize and the teosintes. Geneticists chose teosinte because it was suitable to investigate the long-term evolutionary dynamics and the genetic effect of domestication on these genes (Zhang et al. 2002). These studies showed that the defense genes *wpl*, *wip1*, *mpi*, *chiA*, *chiB*, and *chiI* all had a neutral evolutionary history and that their diversity was maintained by either balancing selection or episodic selection (Tiffin and Gaut 2001; Tiffin 2004; Tiffin et al. 2004). In contrast, the sequence

diversity distribution of two *R* genes that are not of the gene-for-gene class, *hm1* and *hm2*, was inconsistent with neutral evolution (Zhang et al. 2002). The results on the molecular evolution of 16 plant innate immunity genes (such as *chiA* and *chiB*) indicated that geographically variable selection had little influence on the nucleotide variation of these genes (Moeller and Tiffin 2008).

11.5.7 Detection of Duplicated Genomic Regions and Precise Ploidy Level

Cytogenetic studies on maize and the teosintes, such as those investigating the structure and meiotic behavior of chromosomes, have been a hot topic in both classical and molecular genetics since the beginning of the last century. In previous sections of this chapter, we have already discussed the basic chromosome number (Sect. 11.1.3) and various cytogenetic stocks (Sect. 11.4). Here, we will focus on the detection of duplicated genomic regions in the chromosomes of wild *Zea* species because they play important roles in confirming the precise ploidy level, in designing chromosome markers, in clarifying the taxonomy of the genus *Zea*, and in elucidating the evolution of maize.

In an early molecular study, a family of moderately repetitive sequences isolated from *Z. diploperennis* showed the same genomic organization patterns and similar copy numbers in all other *Zea* species investigated (Raz et al. 1991). These results further supported the long held idea that maize and the teosintes were derived from the same ancestral *Zea* population. An 180-bp maize-knob-repeat sequence was found to be present in all teosinte species except *Z. perennis*, and further studies showed that *Z. luxurians* contains not only this 180-bp repeat but also other species-specific repetitive sequences (Dennis and Peacock 1984; Poggio et al. 1999, 2000). This characteristic in *Z. luxurians* has been used as a species-specific marker to differentiate chromosomes from other species by genomic in situ hybridization (GISH) (Poggio et al. 2000).

The ancestral ploidy level and chromosomal origins within the genus *Zea* has been a subject of some controversy. Early mapping studies suggested that maize contained extensive regions of colinear genes (Rhoades 1951), suggesting a tetraploid origin, and

this hypothesis has been confirmed by numerous molecular mapping and segmental sequencing studies (Helentjaris et al. 1988; Gaut and Doebley 1997; Ilic et al. 2003; Lai et al. 2004; Swigonová et al. 2004). Although they currently show normal diploid pairing and inheritance, it is believed that maize and the other wild *Zea* species except *Z. perennis* are derived from a common ancestral allotetraploid ($n = 5$) and that *Z. perennis* originated from an ancient allooctoploid (Molina and Naranjo 1987; Poggio et al. 1990). Comparative sequence analysis of the retained homoeologous regions from this *Zea* allotetraploid indicated that it originated in the last few million years from the fusion of two ancestral genomes that themselves diverged about 12 million years ago (Swigonová et al. 2004). Since that time, more than 50% of the genes duplicated in the polyploid event have been lost by a process of accumulated small deletions and point mutations (Ilic et al. 2003; Lai et al. 2004). The cytogenetic detection of duplicated genomic regions in teosinte genomes has also supported this hypothesis (Poggio et al. 1999; Takahashi et al. 1999; Gonzalez et al. 2006).

11.5.8 Identification and Use of Potentially Useful Teosinte Alleles for Maize Improvement

As discussed in Sect. 11.1.4, teosinte populations with unique characteristics can serve as reservoirs of favorable alleles for the improvement of maize (Galinat 1985). Genetic studies of wild *Zea* species have already identified a series of potentially useful alleles for yield, flooding tolerance, and perennialism. Using advanced backcross QTL (AB-QTL) analysis, valuable alleles for higher yield were identified and transferred from *Z. perennis* to maize (Harjes et al. 1999). Perennial plants might help address many environmental and agricultural problems including soil erosion, nutrient loss, and abiotic stress (Cox et al. 2006). *Z. perennis* and *Z. diploperennis*, two perennial species in the genus *Zea*, have the potential to contribute to the generation of new, more environmentally friendly, maize lines (Moffat 1996). Cluster of QTL controlling the perennialism of teosinte were identified on chromosomes 2 and 6 (Westerbergh and Doebley 2004). Furthermore, four QTL from *Z. nicaraguensis*

were identified for increased flooding tolerance in maize (Mano et al. 2007). More details about the application of these favorable alleles on maize breeding will be discussed in Sect. 11.6 below.

11.5.9 Summary

Maize–teosinte hybrids have been used to construct genetic maps, assess gene actions, and identify potentially useful alleles for maize improvement. Teosinte studies have also played an important role in elucidating physiological pathways and host–parasite interactions.

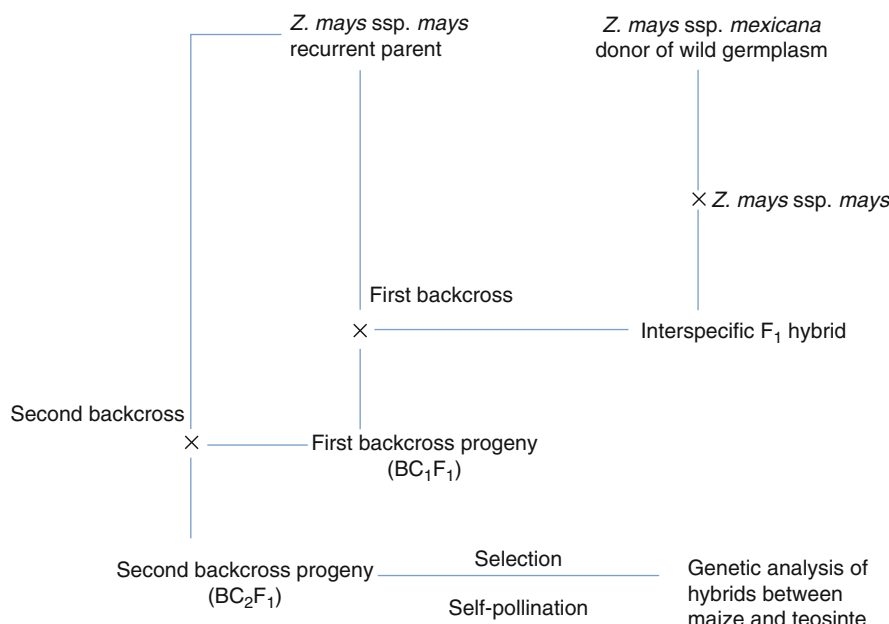
11.6 Role in Crop Improvement Through Traditional and Advanced Tools

In previous sections, we discussed the place of teosinte in the evolution of maize and the role of teosinte studies in understanding maize genetics. Teosinte has been proposed to be of practical use for maize breeding (Reeves 1950). Various teosinte species have been used as germplasm sources for breeding maize with traits such as higher nutritional quality, higher yield, and increased stress resistance (Tabata 1995). Here, we introduce some traditional and molecular marker-assisted breeding efforts for maize improvement using wild *Zea* species.

11.6.1 Introgression of Wild Germplasm into Maize

Introgression is the incorporation of genes or chromosomal regions from one species or accession into another species or accession. The natural introgression between maize and teosinte that may lead to genetic erosion has been discussed in Sect. 11.2.1. Here, we describe the introgression practiced by maize breeders to transfer valuable traits from wild *Zea* species to maize. This “artificial” introgression is usually conducted by recurrent backcrossing of the elite maize line with the initial interspecific hybrid (Fig. 11.6) to select genetically superior varieties. At the end of this process, the presence of a whole chromosome or a

Fig. 11.6 Schematic diagram showing introgression of genes or chromosomal regions from wild germplasm into maize. Figure is adapted from Wang et al. (2008b)



segment from an introgressed teosinte chromosome is confirmed by fluorescence in situ hybridization (FISH), GISH, or molecular markers (Kato and Sanchez 2002; Tang et al. 2005a). Several introgression lines were created using various teosinte parents including *Z. perennis*, *Z. diploperennis*, *Z. mays* ssp. *mexicana*, and *Z. luxurians* (Pischedda and Magoja 1985; Ray et al. 1999; Wei et al. 2003; Wang et al. 2008b). The interspecific cross between maize ($2n = 20$) and *Z. perennis* ($2n = 40$) mostly produced infertile F_1 hybrids ($<5\%$ fertile) with $2n = 30$ due to a strong incompatibility barrier (Tang et al. 2005a). F_2 seeds were obtained from F_1 hybrids by using short day length and treatment with gibberellic acid (Tang et al. 2005a). An intergeneric cross introduced a disease resistance gene allele (Rpl^{TD}) from *Tripsacum dactyloides* into maize, conferring resistance to strains of *Puccinia sorghi* that were virulent on maize with only the Rpl^D allele (Bergquist 1981).

11.6.2 Breeding for Heterosis

Heterosis (or hybrid vigor) is the phenomenon where the performance of an F_1 hybrid is genetically superior to either parent. In the decades since Shull's original description of heterosis, agronomists have been widely utilizing heterosis as a means for improving crop

productivity (Shull 1948; Kallo et al. 2006). A basic requirement for heterosis breeding is to find suitable parents that produce hybrids with a sufficient level of heterosis. In maize breeding, much of the heterotic vigor is attributed to introgressive hybridization from its closest relative, teosinte (Wilkes 1972). Heterotic hybrids with higher yield were obtained through crossing *Z. mays* ssp. *mays* and *Z. mays* ssp. *mexicana* (Cohen and Galinat 1984). Other hybrids such as maize–*Z. perennis*, maize–*Z. diploperennis*, maize–*Z. mays* ssp. *parviglumis*, and maize–*Z. luxurians* also show such heterotic traits as vigorous development and high prolificity (Magoja and Benito 1982; Magoja and Palacios 1987; Corcuera 1991). Different maize–teosinte heterotic hybrids were compared and hybrids using teosinte belonging to Section *Luxuriantes* were found to have much higher prolificity and vigorous plant traits than those obtained using accessions belonging to Section *Zea*, with maize–*Z. perennis* hybrids being the most heterotic (Aulicino and Magoja 1991).

11.6.3 Somatic Hybridization and Genetic Transformation

Although some teosinte accessions have been used to enrich domesticated maize germplasms for the

purposes of maize breeding, most of these valuable genetic resources are sitting unused in the germplasm banks. The major reason is the difficulty in identifying useful characters in these materials and transferring the desired genes to maize (Hoisington et al. 1999). Fortunately, somatic hybridization techniques can overcome the constraints related to hybridization incompatibility between maize and some teosinte species (Garcia and Molina 2001). Somatic hybridization operates through the regeneration of somatic hybrids that were generated by the fusion of protoplasts from two different species. Through the fusion process, the genomes from both parents are mixed in the hybrid cells and the desired genes or traits will be transferred (Carlson et al. 1972; Liu et al. 2005). For instance, using this technique, hybrids of maize and *Z. perennis* were obtained, which otherwise are cross-incompatible (Molina and Garcia 1999). The chromosomal composition of somatic hybrids and the gene transfer are usually confirmed by FISH with molecular probes such as labeled repetitive elements and large genomic clones (Kato et al. 2006; Lamb et al. 2007).

11.6.4 Alleles for High Yield and Resistance to Biotic and Abiotic Stresses

Based on the development of traditional and modern breeding techniques, several maize hybrids were created with various desirable characters transferred from teosinte including high yield and various stress resistances. (1) *High quality*. One of the major objectives of maize breeders is to develop high yielding maize germplasm with improved nutritional quality. The hybridization of perennial teosinte and maize was observed to produce some progeny with high protein content in the kernel (Perini et al. 1991). The kernel protein content in some maize populations with introgression from *Z. diploperennis* was up to 50% higher than that observed in standard commercial maize (Perini and Magoja 1988). Recently, a new introgression line was obtained through the intraspecific cross between *Z. mays* ssp. *mexicana* and maize (Wang et al. 2008a). Amino acid analysis of endosperm from progeny of this cross exhibited a relatively high concen-

tration of lysine. Low lysine content is a significant nutritional problem when using maize as a food or feed. (2) *Biotic and abiotic stress resistances*. Biotic and abiotic stresses cause significant decreases in maize production and quality. Many teosinte species are resistant to some important diseases and pests of maize and some can survive in a number of harsh environments (Wilkes 1977). These beneficial traits are available to improve modern maize. The infection frequency of maize stalk rot, rough dwarf disease, and brown spot in *Z. mays* ssp. *mexicana* introgression populations of maize was much lower than standard maize inbred lines (Wang et al. 2008b). Some of the alloplasmic lines with resistance to *Helminthosporium turcicum* and *Helminthosporium maydis*, two important fungal pathogens of maize, were created from *Z. mays* ssp. *mays* × *Z. diploperennis*, in which a part of the cytoplasmic genomes of maize was replaced by cytoplasmic segments from *Z. diploperennis* (Wei et al. 2003). *Z. nicaraguensis* grows in frequently flooded lowlands and has special root aerenchyma adapted to flooding (Iltis and Benz 2000). This character was transferred to maize by advanced backcross-QTL mapping and could be useful in developing flooding-tolerant hybrids (Mano and Omori 2008).

11.6.5 Summary

Compared to the huge potential value of teosinte genes for maize improvement, it is still a largely “untapped” genetic resource. The first efforts to introduce teosinte alleles for traits that influence heterotic potential, disease resistance, seed protein content, and flooding tolerance have been quite promising. Further introgression of teosinte alleles into maize germplasm is highly warranted.

11.7 Genomics Resources Developed

There are large differences in some morphological traits and physiological characters (yield, biotic and abiotic resistance, etc.) between modern maize and teosinte. Genomic research on maize and teosinte is needed to determine the genetic foundations of these

differences. To date, genome sequence data, expressed sequence tags (ESTs), full length cDNA sequence, protein sequences, and metabolomic and other data have been generated primarily for maize. Here, we summarize genomic resources for teosinte.

11.7.1 Genomic and Protein Sequences

Raw genomic and protein sequences for teosinte have been stored at NCBI (<http://www.ncbi.nlm.nih.gov>). Several species-specific or data-type-specific databases also provide access to teosinte genome data such as the Maize Genetics and Genomics Database (MaizeGDB) (<http://www.maizegdb.org>) and the Plant Genome Database (PlantGDB) (<http://www.plantgdb.org>). Various teosinte protein and genomic sequence data are listed in Table 11.8, including genome survey sequences (GSSs), sequence tagged sites (STSs), and ESTs. Compared to maize genomic sequence data, the information on teosinte is very limited. Among the wild *Zea* species, most of the sequence data are from *Z. mays* ssp. *parviglumis*, the proposed ancestor of maize. There is still no sequence information on *Z. nicaraguensis*, a new teosinte named in 2000 that has few samples available for research (Iltis and Benz 2000).

11.7.2 Transcripts

PlantGDB regularly assembles unique transcripts using ESTs plus full-length and partial cDNA sequences collected from NCBI GenBank (Dong et al. 2004).

Figure 11.7 shows the total number of assembled transcripts in different teosinte species. These teosinte transcripts, when compared to comparable transcripts from maize, can provide some idea of differences in the transcribed component of the genome.

11.7.3 Mutants

The information about mutations affecting maize and teosinte phenotypes that was originally stored in ZmDB has now been transferred to Maize GDB (Lawrence et al. 2004). At this point in time, there are only three records related to teosinte in the mutation and phenotype database of Maize GDB. These pertinent entries are for teosinte branched, teosinte glume architecture, and teosinte glumes.

11.7.4 Summary

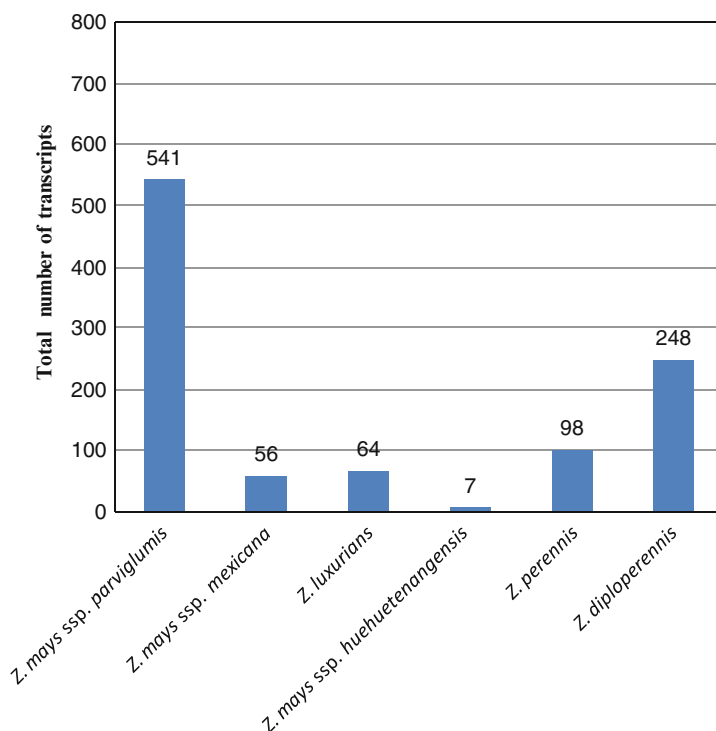
There is very little information on the structure, evolution, or genetic composition of teosinte genomes. Additional sequences, mutational characterization, and expression tools are needed for this wild germplasm in order to enhance its usefulness for maize improvement. With the ever-decreasing costs of the so-called “next generation” sequencing (Mardis 2008), it will be appropriate to “resequence” the genomes of several *Zea* species in the next few years and use the impending full genome sequence of maize inbred B73 (Tian et al. 2009) as the framework to assemble these teosinte sequences.

Table 11.8 Various types of sequence data available for teosintes

Species	Total number of each sequence type				
	Protein	GSSs	STSs	ESTs	Other DNA sequences
<i>Z. mays</i> ssp. <i>parviglumis</i>	873	1,593	17,972	3	2,884
<i>Z. mays</i> ssp. <i>mexicana</i>	74	0	102	0	431
<i>Z. luxurians</i>	83	0	0	0	143
<i>Z. mays</i> ssp. <i>huehuetenangensis</i>	12	0	1	0	33
<i>Z. perennis</i>	69	0	0	1	122
<i>Z. diploperennis</i>	126	0	43	7	296
<i>Z. nicaraguensis</i>	0	0	0	0	0

EST data were obtained from NCBI’s GenBank; others were from the PlantGDB database, the latest access: June 27, 2009

Fig. 11.7 PlantGDB-assembled Unique Transcripts (PUTs) of teosinte species. Latest access: June 27, 2009



11.8 History and Scope for Domestication and Commercialization

Some teosintes have proven to be a good quality and reliable forage source. In the nineteenth century, *Z. luxurians* was cultivated specifically for forage in some southern states of the United States (Schmidt and Colville 1963). Due to the natural pest-resistance of *Z. diploperennis*, the aqueous extract of its leaves has been proposed as a control agent for fall armyworm (Farias-Rivera et al. 2002). We are not aware of any reports regarding the use of teosinte in the herbal drug or perfume industries. Today, people seldom eat teosinte except as an ingredient of some salads (Coe 1994). Teosinte is not considered as an alternative crop because of the very low yield and bad taste of its grain. These observations make one wonder why and how maize was domesticated by pre-Columbian Amerindians from this almost useless and unattractive teosinte species. Anyone familiar with teosinte will appreciate this vexing question, especially due to the hard cupulate fruitcase covering the teosinte grains.

There are two competing hypotheses regarding the impetus for teosinte domestication into maize. One is George Beadle's "popcorn" hypothesis (Beadle 1939). Beadle proposed that popping attracted the pre-Columbian Amerindians when they accidentally threw teosinte seed into cooking or camp fires, and thereby produced a processed grain form that was suitable for consumption. Subsequently, when the hunter-gatherers settled down to fixed dwellings, they probably began to plant the teosinte seeds to help provide a dependable food source. If this hypothesis is true, there should be some phytoliths of teosinte fruitcases, but none have been found (Piperno 2006). An alternative hypothesis is "first for sugar and then for grain" (Iltis 2000). Iltis proposed that, initially, teosinte was harvested for its sugary stalks. His idea came from the observation that, even today, children in some areas of Mexico chew the raw pith of teosinte stalks (like chewing sugar cane). Iltis's hypothesis appears to be supported by some archeological evidences found in Honduras (Webster et al. 2005). However, recent archeological activities did not find any stalk phytoliths in Xihuatoxtla shelter, located in Central Balsas Valley of Mexico, the most likely region of

maize domestication. In contrast, some new maize cob phytoliths and starch grains from this area indicate that teosinte was not consumed for its stalks (Piperno et al. 2009). More evidence is needed to fully resolve the origin of maize domestication and the involvement of other factors such as the abundance of teosinte, the availability of other alternative resources, and the involvement of local culture (Piperno and Pearsall 1999; Hastorf 2009).

We are not aware of any redomestication of teosintes other than a proposed project from Doebley (reviewed in Doebley 2004) to see if he can recreate the “catastrophic transformation” of *Z. mays* ssp. *parvaglium* into something resembling domesticated maize. It would be interesting, however, to see if biomass production for stalk sugars and/or lignocellulose could be further improved in one or more teosintes so that an additional *Zea* other than maize could be used as a crop for biofuel production. This approach might be most appropriate with one or more of the perennial species.

11.8.1 Summary

Today, the wild *Zea* species are mainly used for forage by small landholders in Central America. Teosinte (*Z. mays* ssp. *parvaglium*) may have first attracted human attention because of their sugary stalks. Teosintes have played an important role in maize domestication. Some of the teosintes produce a good deal of biomass that might be improved for commercial development as a bioenergy fuel source.

11.9 Teosinte as a Possible Problem Weed

In previous sections, we discussed the roles played by teosinte in maize evolution and improvement, in genetic and genomic studies, and in the development of cytogenetic stocks. In this section, we will discuss some potentially harmful characteristics of the teosintes; after all, farmers usually regard them as weeds. It is unlikely that any current teosinte will become an invasive species because teosinte populations are limited and some of them are endangered, as discussed

earlier. However, attention has to be paid to the potential consequences of gene flow between transgenic maize and teosinte species. One possible problem that we have discussed in Sect. 11.2.1 is that gene flow may result in the extinction of teosinte species. The other concern is that gene flow may increase weediness and create “super weeds” if some resistance genes in transgenic maize enter into teosinte (Ellstrand 2003). Here, we outline recent progress on the conceptualization and analysis of possible enhanced weediness in the teosintes.

The risk evaluation of a potential enhanced weediness of teosinte usually involves three questions. (1) The first issue is whether the natural gene flow between transgenic maize and teosinte can occur. The spontaneous hybridization between such teosintes as *Z. mays* ssp. *parvaglium* and maize has been confirmed by several methods, including comparison of morphological traits (Wilkes 1977), use of molecular markers (Doebley 1990b; Blancas et al. 2002), and examination of pollen movement (Aylor et al. 2005). The gene flow rate was also measured by a 3-year field experiment (Ellstrand et al. 2007). These results showed that the natural hybridization rate between maize and *Z. mays* ssp. *mexicana* is low due to an incompatibility barrier, while maize and *Z. mays* ssp. *parvaglium* hybridized at a high rate. Hence, any transgene present in fertile maize will eventually find its way into some teosinte germplasm. (2) The second question is whether the transgene will persist. According to population genetics theory, and confirmed by many studies, an allele will stay in a population for a reasonable period of time even if the allele is neutral or moderately disadvantageous (Wright 1951; Varvio et al. 1986). Studies on the relative fitness of transgenic maize \times *Z. mays* ssp. *mexicana* hybrids indicated that the hybrids have higher vigor than their teosinte parents (Guadagnuolo et al. 2006). (3) The third consideration is whether the transgene will enhance weediness. In theory, most transgenes that are beneficial for domesticated maize (e.g., those controlling herbicide tolerance or insect resistance) would be expected to benefit a teosinte for the same (if less intense) reasons, and would thus enhance weediness. However, some field fitness experiments did not support this idea (Guadagnuolo et al. 2006). One possible explanation is that these transgenes may have some unknown negative effect(s) on teosinte fitness (Ellstrand 1997).

From what is currently known, it is reasonable to expect that gene flow from transgenics may somewhat increase the weediness of some teosintes. Still, the likelihood of any wild *Zea* species becoming a problematic invasive seems relatively low. First, the genetic resource bases of teosintes are limited and scattered (Wilkes 2007). Moreover, *Z. mays* ssp. *mexicana* and *Z. luxurians* have significant sexual incompatibility with maize (Evans and Kermicle 2001). In the case of *Z. mays* ssp. *parviglumis*, it is separated from maize by temporal (different flowering date) and spatial factors (few sympatric regions) (Doebley 1990b). Second, teosinte has coexisted and coevolved with maize over thousands of years and has still maintained its distinct genetic constitution (Fukunaga et al. 2005).

11.9.1 Summary

The outcomes of gene flow between maize and teosinte seem to be quite different from that observed for some other crops and their wild relatives (Boudry et al 1993). Additional specific studies are needed regarding the flow and persistence of transgenes between maize and the teosintes, especially with respect to their possible contributions to weediness and to the possible in situ extinction of teosinte germplasm. These studies should also include analysis of seed dispersal from maize–teosinte hybrids. In combination, these proposed future experiments would provide a much more complete analysis of any possible threat associated with teosinte as a potential invasive weed.

11.10 Recommendations for Future Action

Although tremendous progress has been made in the last several decades on the protection, study, and utilization of wild *Zea* species, more research is needed. Also, more effort is needed to increase public awareness of the value of the teosintes as a part of the natural environment and a source of genes for future maize improvement.

CWRs play a role in maintaining ecosystem health as components of natural and agricultural systems.

About 30 years ago, scientists identified wild *Zea* species as a target for enhanced conservation efforts (Frankel 1970). The current status and some achievements in teosinte conservation have already been discussed in this chapter. Here, we offer recommendations for additional teosinte conservation and use. (1) *Creating public awareness of the importance of teosinte*. Teosinte is a part of the natural heritage of humankind. However, little is known by the general public about the wild *Zea* species. Even some farmers in current regions of teosinte distribution regard it as a useless weed. Because efficient and comprehensive conservation requires efforts across the broad growth range of the teosintes, dealing with complex international policy and administrative elements will be necessary. Hence, it would be appropriate to establish a special non-profit organization that could educate, negotiate, and monitor. Staff of this non-profit would be responsible for training programs (producing and disseminating public awareness materials about teosinte) and coordination in Mexico, Guatemala Nicaragua, and Honduras (as a kind of “Wildlife Trust, US”) (Meilleur and Hodgkin 2004), with the target audience being local populations, as well as government officials in these regions. (2) *Establishing more in situ conservation sites*. Until now, the only established in situ conservation site for teosintes is in the Sierra de Manantlan Biosphere Reserve, Mexico. The absence of other designated preserves may be due primarily to their high cost (natural capital, human capital, and social capital). Wilkes proposed an alternative low cost idea to separate teosinte populations into small plots (home garden size or bigger) and hire local farmers to maintain these plots. They would be allowed to harvest the maize introgression lines in the sympatric regions of maize and teosinte to help reward their service (Wilkes 2007). Equally important, farmers that protected teosinte could receive some prestige from their communities, if the heritage and importance of teosinte were common knowledge (Castineiras et al. 2000). Of course, this sympatric growth model has the potential to increase the rate of decay of teosinte germplasm novelty by encouraging gene flow from adjacent maize, but it might not be a dramatic effect because teosinte has a long history of growth alongside domesticated maize.

Regardless of the in situ preservation strategy employed, immediate efforts are needed to negotiate their creation, and this would be an appropriate task for

the aforementioned non-profit organization. This organization would also be needed to serve as an independent monitor of the status of preservation efforts. The sites that merit immediate in situ conservation are in southern Guatemala and Balas, Mexico (Wilkes 1997, 2007). However, in the long run, all current sites of teosinte growth need some level of protection, and planting of the rarer teosintes at new locations would be justified as an insurance against local extinction.

11.10.1 Summary

Public awareness of the significance and tenuous survival of teosinte is needed to help justify its preservation. This conservation will not happen by chance, but it needs the dedicated efforts of a small cohort of interested individuals. Billions of people worldwide now benefit from maize as a food, feed, forage, or industrial commodity. It is fully appropriate for the world to take an interest in teosinte conservation, to guarantee that it will continue to play an important role in maize research and improvement.

References

- Aulicino MB, Magoja JL (1991) Variability and heterosis in maize-Balsas teosinte and maize- Guatemala teosinte hybrids. *Maize Genet Coop Newsl* 65:43–44
- Aylor DE, Baltazar BM, Schoper JB (2005) Some physical properties of teosinte (*Zea mays* subsp. *parviglumis*) pollen. *J Exp Bot* 56:2401–2407
- Baltazar BM, Schoper JB (2002) Crop-to-crop gene flow: dispersal of transgenes in maize, during field tests and commercialization. In: 7th international symposium on biosafety of genetically modified organisms, Beijing, China, 10–16 Oct 2002, pp 24–33
- Barton NH, Keightley PD (2002) The domestication of maize. *Nat Rev Genet* 3:11–21
- Beadle GW (1939) Teosinte and the origin of maize. *J Hered* 30:245–247
- Beadle GW (1972) The mystery of maize. *Field Museum Nat Hist Bull* 43:2–11
- Beadle GW (1980) The ancestry of corn. *Sci Am* 242:112–119
- Bennetzen J, Buckler E, Chandler V, Doebley J, Dorweiler J, Gaut B et al (2001) Genetic evidence and the origin of maize. *Lat Am Antiq* 12(1):84–86
- Benz B (1988) In situ conservation of the genus *Zea* in the Sierra de Manantlan Biosphere Reserve. In: Recent advances in the conservation and utilization of genetic resources. Proceedings of global maize germplasm workshop, CIMMYT, Mexico, pp 59–69
- Benz BF, Iltis HH (1992) Evolution of female sexuality in the maize ear (*Zea mays* L. subsp. *mays* -Gramineae). *Econ Bot* 46(2):212–222
- Bergquist RR (1981) Transfer from *Tripsacum dactyloides* to corn of a major gene locus conditioning resistance to *Puccinia sorghi*. *Phytopathology* 71:518–520
- Blancas L, Arias DM, Ellstrand NC (2002) Patterns of genetic diversity in sympatric and allopatric populations of maize and its wild relative teosinte in Mexico: evidence for hybridization. In: Gene flow workshop, Ohio State University, Ohio, 6 Mar 2002, pp 31–38
- Bomblies K, Doebley JF (2006) Pleiotropic effects of the duplicate maize *floricaula/leafy* genes *zfl1* and *zfl2* on traits under selection during maize domestication. *Genetics* 172:519–531
- Bomblies K, Wang RL, Ambrose BA, Schmidt RJ, Meeley RB, Doebley J (2003) Duplicate *floricaula/leafy* homologs *zfl1* and *zfl2* control inflorescence architecture and flower patterning in maize. *Development* 130:2385–2395
- Bortiri E, Chuck G, Vollbrecht E, Rocheford T, Martienssen R, Hake S (2006) *ramosa2* encodes a lateral organ boundary domain protein that determines the fate of stem cells in branch meristems of maize. *Plant Cell* 18:574–585
- Boudry P, Morchen M, Saumitou-Laprade P, Vernet PH, Van-Dijk H (1993) The origin and evolution of weed beets: consequences for the breeding and release of herbicide-resistant transgenic sugar beets. *Theor Appl Genet* 87:471–478
- Boyer CD (1982) Soluble starch synthases and starch branching enzymes from developing seed of teosinte. *Maize Genet Coop Newsl* 56:120–121
- Briggs WH, McMullen MD, Gaut BS, Doebley J (2007) Linkage mapping of domestication loci in a large maize-teosinte backcross resource. *Genetics* 177:1915–1928
- Bruford MW, Wayne RK (1993) Microsatellites and their application to population genetic studies. *Curr Opin Genet Dev* 3:939–943
- Buckler ES, Phelps-Durr TL, Buckler CSK, Dawe RK (1999) Meiotic drive of chromosomal knobs reshaped the maize genome. *Genetics* 153:415–426
- Carlson PS, Smith HH, Dearing RD (1972) Parasexual interspecific plant hybridization. *Proc Natl Acad Sci USA* 69(8):2292–2294
- Castineiras L, Fundora Z, Pico S, Salinas E (2000) The use of home gardens as a component of national strategy for the in situ conservation of plant genetic resources in Cuba: a plot study. *Plant Genet Resour Newsl* 123:9–18
- Chase SS (1969) Monoploids and monoploid-derivatives of maize (*Zea mays* L.). *Bot Rev* 35:117–167
- Coe SD (ed) (1994) America's first cuisines. University of Texas Press, TX, USA, pp 11–12
- Coe EH, Sarkar KR (1964) The detection of haploids in maize. *J Hered* 55:231–233
- Coe EH, Neuffer MG, Hoisington DA (1988) The genetics of corn. In: Sprague GF, Dudley JW (eds) Corn and corn improvement. American Society of Agronomy, Madison, WI, USA, pp 81–258

- Cohen JJ, Galinat WC (1984) Potential use of alien germplasm for maize improvement. *Crop Sci* 24:1011–1015
- Collins GN (1919) Structure of the maize ear as indicated in *Zea-Euchlaena* hybrids. *J Agri Res* 17:127–135
- Collins GN, Kempton JH (1920) A teosinte-maize hybrid. *J Agric Res* 19:1–38
- Corcuera VR (1991) Maize-Balsas teosinte and maize-Guatemala teosinte hybrids: inheritance of prolificity. *Maize Genet Coop Newsl* 65:79–80
- Corcuera VR, Magoja JL (1991) Diploperennial teosinte-maize hybrids: inheritance of male spikelet outer glume traits. *Maize Genet Coop Newsl* 65:81–82
- Cox TS, Glover JD, Cox TS, Van Tassel DL, Dehaan LR (2006) Prospects for developing perennial grain crops. *Bioscience* 56(8):649–659
- Dennis ES, Peacock WJ (1984) Knob heterochromatin homology in maize and its relatives. *J Mol Evol* 20:341–350
- Dewet JJ, Harlan JR (1976) Cytogenetic evidence for the origin of teosinte (*Zea mays* ssp. *mexicana*). *Euphytica* 25:447–455
- Doebley JF (1983) The maize and teosinte male inflorescence: a numerical taxonomic study. *Ann Mo Bot Gard* 70(1):32–70
- Doebley JF (1984) Maize introgression into teosinte—a reappraisal. *Ann Mo Bot Gard* 71:1100–1113
- Doebley JF (1990a) Molecular systematics of *Zea* (Gramineae). *Maydica* 35:143–150
- Doebley JF (1990b) Molecular evidence for gene flow among *Zea* species. *Bioscience* 40(6):443–448
- Doebley JF (2004) The genetics of maize evolution. *Annu Rev Genet* 38:37–59
- Doebley JF, Iltis HH (1980) Taxonomy of *Zea* (Gramineae). I: A subgeneric classification with key to taxa. *Am J Bot* 67(6):982–993
- Doebley JF, Stec A (1991) Genetic analysis of the morphological differences between maize and teosinte. *Genetics* 129:285–295
- Doebley JF, Stec A (1993) Inheritance of the morphological differences between maize and teosinte: comparison of results for two F₂ populations. *Genetics* 134:559–570
- Doebley JF, Goodman MM, Stuber CW (1984) Isoenzymatic variation in *Zea* (Gramineae). *Syst Bot* 9:203–218
- Doebley JF, Goodman MM, Stuber CW (1985) Isozyme variation in the races of maize from Mexico. *Am J Bot* 72(5):629–639
- Doebley JF, Goodman MM, Stuber CW (1987a) Patterns of isozyme variation between maize and Mexican annual teosinte. *Econ Bot* 41(2):234–246
- Doebley JF, Renfroe W, Blanton A (1987b) Restriction site variation in the *Zea* chloroplast genome. *Genetics* 117:139–147
- Doebley JF, Stec A, Wendel J, Edwards M (1990) Genetic and morphological analysis of a maize-teosinte F₂ population: implications for the origin of maize. *Proc Natl Acad Sci USA* 87:9888–9892
- Doebley JF, Bacigalupo A, Stec A (1994) Inheritance of kernel weight in two maize-teosinte hybrid populations: implications for crop evolution. *J Hered* 85(3):191–195
- Doebley JF, Stec A, Gustus C (1995) *Teosinte branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. *Genetics* 141:333–346
- Doebley JF, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. *Nature* 386:485–488
- Dong Q, Schlueter SD, Brendel V (2004) PlantGDB, plant genome database and analysis tools. *Nucleic Acids Res* 32:D354–D359
- Dorweiler JE, Doebley J (1997) Developmental analysis of teosinte glume architecture1: a key locus in the evolution of maize (Poaceae). *Am J Bot* 84(10):1313–1322
- Dorweiler JM, Stec A, Kermicle J, Doebley J (1993) Teosinte glume architecture 1: a genetic locus controlling a key step in maize evolution. *Science* 262(8):233–235
- Ellneskog-Staam P, Loaisiga CH, Merker A (2007) Chromosome C-banding of the teosinte *Zea nicaraguensis* and comparison to other *Zea* species. *Hereditas* 144:96–101
- Ellstrand NC (1997) Evaluating the risks of transgene flow from crops to wild species. In: Serratos JA, Willcox MC, Castillo F (eds) *Gene flow among maize landraces, improved maize varieties and teosinte: implications for transgenic maize*. CIMMYT, Mexico, pp 81–83
- Ellstrand NC (ed) (2003) *Dangerous liaisons? When cultivated plants mate with their wild relatives*. Johns Hopkins University Press, Maryland, USA
- Ellstrand NC, Garner LC, Hegde S, Guadagnuolo R, Blancas L (2007) Spontaneous hybridization between maize and teosinte. *J Hered* 98(2):183–187
- Eltringham SK (1984) *Wild life resources and economic development*. Wiley, New York, USA
- Evans MS, Kermicle JL (2001) Teosinte crossing barrier1, a locus governing hybridization of teosinte with maize. *Theor Appl Genet* 103:259–265
- FAO (1997) *The state of the world's plant genetic resources for food and agriculture*. FAO, Rome, Italy
- FAO (2002) *The role of women in the conservation of the genetic resources of maize (Guatemala)*. The Food and Agriculture Organization of the United Nations (FAO), Rome, Italy
- Farias-Rivera LA, Hernandez-Mendoza JL, Molina-Ochoa J et al (2002) Effect of leaf extracts of teosinte, *Zea diploperennis* L and a Mexican maize variety, criollo 'uruapeno' on the growth and survival of the fall armyworm (*Lepidoptera: noctuidae*). *Florida Entomol* 86(3):239–243
- Flor HH (1955) Host-parasite interaction in flax rust—its genetics and other implications. *Phytopathology* 45:680–685
- Frankel O (1970) Genetic conservation of plants useful to man. *Biol Conserv* 2:162–169
- Fukunaga K, Hill J, Vigouroux Y, Matsuoka Y, Sanchez J, Liu K et al (2005) Genetic diversity and population structure of teosinte. *Genetics* 169:2241–2254
- Galinat WC (1985) The missing links between teosinte and maize: a review. *Maydica* 30:137–160
- Gallavotti A, Zhao Q, Kyoizuka J, Meeley RB, Ritter MK, Doebley J et al (2004) The role of barren stalk1 in the architecture of maize. *Nature* 432:630–635
- Garcia MD, Molina MC (2001) Embryo rescue and induction of somatic embryogenesis as a method to overcome seed inviability in *Zea mays* ssp. *mays* × *Zea mays* ssp. *parviglumis* crosses. *Biol Planta* 44(4):497–501
- Gaut BS, Doebley JF (1997) DNA sequence evidence for the segmental allotetraploid origin of maize. *Proc Natl Acad Sci USA* 94:6809–6814
- Global Crop Diversity Trust (2007) *Global strategy for the ex situ conservation and utilization of maize germplasm*. Global Crop Diversity Trust: www.croptrust.org/documents/web/

- Maize-Strategy-FINAL-18Sept07.pdf. Accessed 12 Oct 2008
- Gonzalez G, Comas C, Confalonieri V, Naranjo CA, Poggio L (2006) Genomic affinities between maize and *Zea perennis* using classical and molecular cytogenetic methods (GISH-FISH). *Chromosome Res* 14:629–635
- Guadagnuolo R, Clegg J, Ellstrand NC (2006) Relative fitness of transgenic vs. non-transgenic maize \times teosinte hybrids: a field evaluation. *Ecol Appl* 16(5):1967–1974
- Hanson MA, Gaut BS, Stec AO, Fuerstenberg SI, Goodman MM, Coe EH et al (1996) Evolution of anthocyanin biosynthesis in maize kernels: the role of regulatory and enzymatic loci. *Genetics* 143:1395–1407
- Harjes CE, Smith ME, McCouch SR, Tanksley SD (1999) Advanced backcross QTL analysis and introgression of perennial teosinte alleles to maize. In: Plant and animal genome VII conference, San Diego, CA, USA, 17–21 Jan 1999, 260p
- Harshberger JW (1896) Fertile crosses of teosinte and maize. *Gard For* 9:522–523
- Hastorf CA (2009) Rio Balsas most likely region for maize domestication. *Proc Natl Acad Sci USA* 106(13):4957–4958
- Hawkes JG, Maxted M, Ford-Lloyd BV (2000) The ex situ conservation of plant genetic resources. Springer, Berlin
- Helentjaris T, Weber DF, Wright S (1986) Use of monosomics to map cloned DNA fragments in maize. *Proc Natl Acad Sci USA* 83:6035–6039
- Helentjaris T, Weber D, Wright S (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphism. *Genetics* 118:353–363
- Hoisington D, Khairallah M, Reeves T, Ribaut JM, Skovmand B, Taba S et al (1999) Plant genetic resources: what can they contribute toward increased crop productivity? *Proc Natl Acad Sci USA* 96:5937–5943
- Holst I, Moreno JE, Piperno DR (2007) Identification of teosinte, maize, and *Tripsacum* in Mesoamerica by using pollen, starch grains, and phytoliths. *Proc Natl Acad Sci USA* 104(45):17608–17613
- Holtont A, Cornish EC (1995) Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell* 7:1071–1083
- Ilic K, SanMiguel PJ, Bennetzen JL (2003) A complex history of rearrangement in an orthologous region of the maize, sorghum and rice genomes. *Proc Natl Acad Sci USA* 100:12265–12270
- Iltis HH (1983) From teosinte to maize: the catastrophic sexual transmutation. *Science* 222:886–894
- Iltis HH (2000) Homeotic sexual translocations and the origin of maize (*Zea mays*, Poaceae): a new look at an old problem. *Econ Bot* 54(1):7–42
- Iltis HH, Benz BF (2000) *Zea nicaraguensis* (Poaceae), a new teosinte from pacific coastal Nicaragua. *Novon* 10(4):382–390
- Iltis HH, Doebley JF (1980) Taxonomy of *Zea* (Gramineae). II. subspecific categories in the *Zea mays* complex and a generic synopsis. *Am J Bot* 67(6):994–1004
- Iltis HH, Doebley JF, Guzman MR, Pazy B (1979) *Zea diploperennis* (Gramineae): a new teosinte from Mexico. *Science* 203:186–188
- Kallo G, Rai M, Singh M, Kumar S, Book R (2006) Heterosis in crop plants. Researchco Book Center, New Delhi, India
- Kao CH, Zeng ZB, Teasdale RD (1999) Multiple interval mapping for quantitative trait loci. *Genetics* 152:1203–1216
- Kato YA (1976) Cytological studies of maize (*Zea mays* L) and teosinte (*Zea mexicana* Schrade Kuntze) relation to their origin and evolution. *Mass Agric Exp Sta Bull* 635:185
- Kato TA, Sanchez JJ (2002) Introgression of chromosome knobs from *Zea diploperennis* into maize. *Maydica* 47(1):33–50
- Kato A, Albert PS, Vega JM, Birchler JA (2006) Sensitive FISH signal detection in maize using directly labeled probes produced by high concentration DNA polymerase nick translation. *Biotechnol Histochem* 81:71–78
- Kindiger B, Hamann S (1993) Generation of haploids in maize: a modification of the indeterminate gametophyte (*ig*) system. *Crop Sci* 33(2):342–344
- Lai J, Ma J, Swigonová Z, Ramakrishna W, Linton E, Llaca V et al (2004) Gene loss and movement in the maize genome. *Genome Res* 14:1924–1931
- Lamb JC, Danilova T, Bauer MJ, Meyer JM, Holland JJ, Jensen MD et al (2007) Single-gene detection and karyotyping using small-target fluorescence in situ hybridization on maize somatic chromosomes. *Genetics* 175(3):1047–1058
- Lauter N, Gustus C, Westerbergh A, Doebley J (2004) The inheritance and evolution of leaf pigmentation and pubescence in teosinte. *Genetics* 167:1949–1959
- Lawrence CJ, Dong Q, Polacco ML, Seigfried TE, Brendel V (2004) Maize GDB, the community database for maize genetics and genomics. *Nucleic Acids Res* 32:D393–D397
- Liu J, Xu X, Deng X (2005) Intergeneric somatic hybridization and its application to crop genetic improvement. *Plant Cell Tiss Organ Cult* 82:19–44
- Louette D, Charrier A, Berthaud J (1997) In situ conservation of maize in Mexico: genetic diversity and maize seed management in a traditional community. *Econ Bot* 51(1):20–38
- Lubberstedt T, Dussle C, Melchinger AE (1998) Application of microsatellites from maize to teosinte and other relatives of maize. *Plant Breed* 117:447–450
- Lukens LN, Doebley J (1999) Epistatic and environmental interactions for quantitative trait loci involved in maize evolution. *Genet Res* 74:291–302
- Mackay TFC (2001) The genetic architecture of quantitative traits. *Annu Rev Genet* 35:303–339
- Magoja JL, Benito G (1981) Inheritance of some characters in maize-perennial teosinte hybrids. *Maize Genet Coop Newsl* 55:58–60
- Magoja JL, Benito G (1982) Heterosis in maize-perennial teosinte hybrids. *Maize Genet Coop Newsl* 56:104–106
- Magoja JL, Benito G (1983) Perennial teosinte-Gaspe hybrids: inheritance of the number of leaves. *Maize Genet Coop Newsl* 57:65–66
- Magoja JL, Palacios I (1984) Perennial teosinte-Gaspe hybrids: inheritance of pollen grain size. *Maize Genet Coop Newsl* 58:118–120
- Magoja JL, Palacios I (1987) Early expression of heterosis in *diploperennial* teosinte-maize hybrids. *Maize Genet Coop Newsl* 61:63–64
- Mangelsdorf PC (1947) The origin and evolution of maize. *Adv Genet* 1:161–207
- Mangelsdorf PC, Reeves RG (1939) The origin of Indian corn and its relatives. *Texas Agric Exp Sta Bull* 574:1–315

- Mano Y, Omori F (2008) Verification of QTL controlling root aerenchyma formation in a maize teosinte "*Zea nicaraguensis*" advanced backcross population. *Breed Sci* 58:217–223
- Mano Y, Omori F, Takamizo T, Kindiger B, RMck B, Loaisiga CH et al (2007) QTL mapping of root aerenchyma formation in seedlings of a maize \times rare teosinte "*Zea nicaraguensis*" cross. *Plant Soil* 295:103–113
- Mardis ER (2008) Next-generation DNA sequencing methods. *Annu Rev Genom Hum Genet* 9:387–402
- Matsuoka Y, Mitchell SE, Kresovich S, Goodman M, Doebley J (2002a) Microsatellites in *Zea* – variability, patterns of mutations and use for evolutionary studies. *Theor Appl Genet* 104:436–450
- Matsuoka Y, Vigouroux Y, Goodman MM, Sanchez J, Buckle E, Doebley J (2002b) A single domestication for maize shown by multilocus microsatellite genotyping. *Proc Natl Acad Sci USA* 99(9):6080–6084
- Meilleur BA, Hodgkin T (2004) In situ conservation of crop wild relatives: status and trends. *Biodivers Conserv* 13: 663–684
- Moeller DA, Tiffin P (2005) Genetic diversity and the evolutionary history of plant immunity genes in two species of *Zea*. *Mol Biol Evol* 22(12):2480–2490
- Moeller DA, Tiffin P (2008) Geographic variation in adaptation at the molecular level: a case study of plant immunity genes. *Evolution* 62(12):3069–3081
- Moffat AS (1996) Agricultural research—higher yielding perennials point the way to new crops. *Science* 274:1469–1470
- Mohammadi SA, Prasanna BM (2003) Analysis of genetic diversity in crop plants—salient statistical tools and considerations. *Crop Sci* 43:1235–1248
- Molina MC (1983) Cytogenetic study of a tetraploid hybrid between *Zea diploperennis* and *Zea perennis*. *Maize Genet Coop Newsl* 57:62
- Molina MC, Garcia MD (1999) Influence of ploidy levels on phenotypic and cytogenetic traits in maize and *Zea perennis* hybrids. *Cytologia* 64:101–109
- Molina MC, Naranjo CA (1987) Cytogenetic studies in the genus *Zea* 1. Evidence for five as the basic chromosome number. *Theor Appl Genet* 73:542–550
- Molina MC, García MD, López CG, Ferrero VM (2004) Meiotic pairing in the hybrid (*Zea diploperennis* \times *Zea perennis*) \times *Zea mays* and its reciprocal. *Hereditas* 141:135–141
- Nadeau JH, Dunn PJ (1998) Genomic strategies for defining and dissecting developmental and physiological pathways. *Curr Opin Genet Dev* 8(3):311–315
- National Research Council (US) (1993) Agricultural crop issues and policies. National Academies Press, Washington, DC, USA
- Nimchuk Z, Eulgem T, Holt BF, Dangl JL (2003) Recognition and response in the plant immune system. *Annu Rev Genet* 37:579–609
- Orr AR, Sundberg MD (1994) Inflorescence development in a perennial teosinte: *Zea perennis* (Poaceae). *Am J Bot* 81 (5):598–608
- Orr AR, Mullen K, Klaahsen D, Sundberg MD (2002) Inflorescence development in a high-altitude annual Mexican teosinte (Poaceae). *Am J Bot* 89(11):1730–1740
- Palacios I, Magoja JL (1984) Perennial teosinte-Gaspe hybrids: inheritance of tassel branching traits. *Maize Genet Coop Newsl* 58:122–127
- Pardey PG, Koo B, Wright BD, Dusen MEV, Skovmand B, Tabata S (2001) Costing the conservation of genetic resources: CIMMYT's ex situ maize and wheat collection. *Crop Sci* 41:1286–1299
- Paulis JW, Wall JS (1977) Comparison of the protein compositions of selected corns and their wild relatives, teosinte and *Tripsacum*. *J Agric Food Chem* 25(2):265–270
- Perini LA, Magoja JL (1988) Effect of perennial teosinte introgression in maize on kernel protein content. *Maize Genet Coop Newsl* 62:80
- Perini LH, Pischedda G, Magoja JL (1991) Diploperennial teosinte introgressed population of maize: kernel protein content. *Maize Genet Coop Newsl* 65:40
- Phillips RL, Vasil IK (1994) DNA-based markers in plants. Kluwer, Dordrecht, Netherlands
- Piperno DR (ed) (2006) Phytoliths: a comprehensive guide for archaeologists and paleoecologists. Altamira press, PA, USA, 65 p
- Piperno DR, Flannery KV (2001) The earliest archaeological maize (*Zea mays* L.) from highland Mexico: new accelerator mass spectrometry dates and their implications. *Proc Natl Acad Sci USA* 98(4):2101–2103
- Piperno DR, Pearsall DM (1999) The origins of agriculture in the lowland neotropics. Academic, San Diego, California, USA
- Piperno DR, Ranere AJ, Holst I, Iriarte J, Dickau R (2009) Starch grain and phytolith evidence for early ninth millennium B.P. maize from the Central Balsas River Valley, Mexico. *Proc Natl Acad Sci USA* 106(13):5019–5024
- Pischedda G, Magoja JL (1985) Perennial teosinte-Gaspe hybrids: selection for maize-like characters. *Maize Genet Coop Newsl* 59:70–71
- Poggio L, Molina MC, Naranjo CA (1990) Cytogenetic studies in the genus *Zea*. 2. Colchicine-induced multivalents. *Theor Appl Genet* 79:461–464
- Poggio L, Confalonieri V, Comas C, Gonzalez G, Naranjo CA (1999) Genomic affinities of *Zea luxurians*, *Z. diploperennis*, and *Z. perennis*: meiotic behavior of their F₁ hybrids and genomic in situ hybridization (GISH). *Genome* 42: 993–1000
- Poggio L, Confalonieri V, Comas C, Gonzalez G, Naranjo CA (2000) Evolutionary relationships in the genus *Zea*: analysis of repetitive sequences used as cytological FISH and GISH markers. *Genet Mol Biol* 23(4):1021–1027
- Prescott-Allen R, Prescott-Allen C (1981) In situ conservation of crop genetic resources: a report to the international board for plant genetic resources (IBPGR). IUCN, Gland, Switzerland
- Ranere AJ, Piperno DR, Holst I, Dickau R, Iriarte J (2009) The cultural and chronological context of early Holocene maize and squash domestication in the Central Balsas River Valley, Mexico. *Proc Natl Acad Sci USA* 106(13):5014–5018
- Rauscher MD (2001) Co-evolution and plant resistance to natural enemies. *Nature* 411(14):857–864
- Ray JD, Kindiger B, Sinclair TR (1999) Introgressing root aerenchyma into maize. *Maydica* 44(2):113–117
- Raz R, Puigdomènech P, Martínez-Izquierdo J (1991) A new family of repetitive nucleotide sequences is restricted to the genus *Zea*. *Gene* 105(2):151–158
- Reeves RG (1950) The use of teosinte in the improvement of corn inbreds. *Agron J* 42:248–251

- Rhoades MM (1951) Duplicated genes in maize. *Am Nat* 85:105–110
- Robinett D, Coe E, Cone K (1995) Map location of *anthocyanin* 3. *Maize Genet Coop News Lett* 69:46
- Sánchez-González JJ, Ruiz-Corral JA (1997) Teosinte distribution in Mexico. In: Serratos JA, Willcox MC, Castillo GF (eds) *Proceedings of a forum “gene flow among maize landraces, improved maize varieties, and Teosinte: implications for transgenic maize”*. CIMMYT, Mexico, pp 18–36
- Sanchez-Velasquez LR, Ezcurra E, Martinez-Ramos M, Álvarez-Buylla E, Lorente R (2002) Population dynamics of *Zea diploperennis*, an endangered perennial herb: effect of slash and burn practice. *J Ecol* 90(4):684–692
- Schmidt WH, Colville WL (1963) Forage yield and composition of teosinte, corn, and forage sorghum grown under irrigation. *Agron J* 55:327–328
- Schuman KM (1904) Mais und Teosinte. In: Urban I, Graebner P (eds) *Ascherson fur Festschrift*. Verlag von Gebruder Borntraeger, Leipzig, Germany, pp 137–157
- Shindo C, Bernasconi G, Hardtke CS (2007) Natural genetic variation in Arabidopsis: tools, traits and prospects for evolutionary ecology. *Ann Bot* 99:1043–1054
- Shull GH (1948) What is heterosis. *Genetics* 33(5):439–446
- Smith JC, Goodman MM (1981) A comparison of chromosome knob frequencies between sympatric and allopatric populations of teosinte and maize. *Am J Bot* 68(7):947–954
- Smith JC, Lester RN (1980) Biochemical systematics and evolution of *Zea*, *Tripsacum* and related genera. *Econ Bot* 34(3):201–218
- Smith JC, Goodman MM, Stuber CW (1984) Variation within teosinte III. Numerical analysis of allozyme data. *Econ Bot* 38(1):97–113
- Smith CW, Betrán J, Runge EA (2004) Corn: origin, history, technology, and production. Wiley, NJ, USA
- Srinivasan G, Brewbaker JL (1999) Genetic analysis of hybrids between maize and perennial teosinte. I. Morphological traits. *Maydica* 44(4):353–369
- Suenaga K, Morshedi AR, Darvey NL (1998) Evaluation of teosinte lines as pollen parents for wheat haploid production. *Cereal Res Commun* 26:119–125
- Swigonová Z, Lai J, Ma J, Ramakrishna W, Llaca V, Bennetzen JL et al (2004) Close split of sorghum and maize genome progenitors. *Genome Res* 14:1916–1923
- Szabo VM, Burr B (1996) Simple inheritance of key traits distinguishing maize and teosinte. *Mol Gen Genet* 252:33–41
- Taba S (1995) Maize genetic resources. Maize Program Special Report. CIMMYT, Mexico
- Taba S (2003) Latin American maize germplasm conservation: regeneration, in situ conservation, core subsets, and rebreeding (Proceedings of a workshop). CIMMYT, Mexico
- Takahashi C, Marshall JA, Bennett MD (1999) Genomic relationships between maize and its wild relatives. *Genome* 42:1201–1207
- Tang Q, Rong T, Song Y, Yang J, Pan G, Li W et al (2005a) Introgression of perennial teosinte genome into maize and identification of genomic in situ hybridization and microsatellite markers. *Crop Sci* 45:717–721
- Tang F, Tao Y, Zhao T, Wang G (2005b) In vitro production of haploid and doubled haploid plants from pollinated ovaries of maize (*Zea mays*). *Plant Cell Tiss Organ Cult* 84:233–237
- Thompson JN, Burdon JJ (1992) Gene-for-gene coevolution between plants and parasites. *Nature* 360:121–125
- Tian F, Stevens NM, Buckler ES (2009) Tracking footprints of maize domestication and evidence for a massive selective sweep on chromosome 10. *Proc Natl Acad Sci USA* 106:9979–9986
- Tiffin P (2004) Comparative evolutionary histories of chitinase genes in the genus *Zea* and family Poaceae. *Genetics* 167:1331–1340
- Tiffin P, Gaut BS (2001) Molecular evolution of the wound-induced serine protease inhibitor *wipl* in *Zea* and related genera. *Mol Biol Evol* 18(11):2092–2101
- Tiffin P, Hacker R, Gaut BS (2004) Population genetic evidence for rapid changes in intraspecific diversity and allelic cycling of a specialist defense gene in *Zea*. *Genetics* 168:425–434
- Timothy DH, Levings CS, Pring DR, Conde MF, Kermicle JL (1979) Organelle DNA variation and systematic relationships in the genus *Zea*: teosinte. *Proc Natl Acad Sci USA* 76(9):4220–4224
- Tito CM, Poggio L, Naranjo CA (1991) Cytogenetic studies in the genus *Zea*. 3. DNA content and heterochromatin in species and hybrids. *Theor Appl Genet* 83:58–64
- Tuxill J, Nabhan GP (2001) People, plant and protected areas: a guide to in situ management. Earthscan, London, UK
- Ushiyama T, Yoshida T (2008) Response to GA and variation of the culm length in doubled haploid lines of wheat. *Plant Prod Sci* 11(2):217–222
- Ushiyama T, Shimizu T, Kuwabara T (1991) High frequency of haploid production of wheat through intergeneric crosses with teosinte. *Jpn J Breed* 41:353–357
- Varvio SL, Chakraborty R, Nei M (1986) Genetic variation in subdivided populations and conservation genetics. *Heredity* 57:189–198
- Vigouroux McMullen M, Hittinger CT, Houchins K, Schulz L, Kresovich S et al (2002) Identifying genes of agronomic importance in maize by screening microsatellites for evidence of selection during domestication. *Proc Natl Acad Sci USA* 99(15):9650–9655
- Vollbrecht E, Springer PS, Goh L, Buckler ES, Martienssen R (2005) Architecture of floral branch systems in maize and related grasses. *Nature* 436:1119–1126
- Wang RL, Stec A, Hey J, Lukens L, Doebley J (1999) The limits of selection during maize domestication. *Nature* 398:236–239
- Wang H, Wagler TN, Li B, Zhao Q, Vigouroux Y, Faller M et al (2005) The origin of the naked grains of maize. *Nature* 436:714–719
- Wang S, Basten J, Zeng ZB (2006) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, USA
- Wang L, Xu C, Qu M, Zhang J (2008a) Kernel amino acid composition and protein content of introgression lines from *Zea mays* ssp. *mexicana* into cultivated maize. *J Cereal Sci* 48:387–393
- Wang L, Yang AF, He CM, Qu M, Zhang J (2008b) Creation of new maize germplasm using alien introgression from *Zea mays* ssp. *mexicana*. *Euphytica* 164:789–801
- Warburton M, Crossa JL (2000) Data analysis in the CIMMYT applied biotechnology center for fingerprinting and genetic diversity studies. CIMMYT, Mexico

- Weber DF (1986) The production and utilization of monosomic *Zea mays* in cytogenetic studies. In: Reddy GM, Coe EH (eds) Gene structure and function in higher plants. Oxford and IBH, New Delhi, India, pp 190–204
- Weber A, Clark RM, Vaughn L, Sánchez-Gonzalez JJ, Yu J, Yandell BS et al (2007) Major regulatory genes in maize contribute to standing variation in teosinte (*Zea mays* ssp. *parviglumis*). *Genetics* 77:2349–2359
- Weber AL, Briggs WH, Ruch J, Baltazar BM, Sánchez-Gonzalez JJ, Feng P et al (2008) The genetic architecture of complex traits in teosinte (*Zea mays* ssp. *parviglumis*): new evidence from association mapping. *Genetics* 180:1221–1232
- Webster D, Rue D, Traverse A (2005) Early *Zea* cultivation in Honduras: implications for the Ittis hypothesis. *Econ Bot* 59 (2):101–111
- Wei W, Zhao W, Song Y, Liu L, Guo L, Gu M (2003) Genomic in situ hybridization analysis for identification of introgressed segments in alloplasmic lines from *Zea mays* × *Zea diploperennis*. *Hereditas* 138:21–26
- Westerbergh A, Doebley JF (2002) Morphological traits defining species differences in wild relatives of maize are controlled by multiple quantitative trait loci. *Evolution* 56(2): 273–283
- Westerbergh A, Doebley JF (2004) Quantitative trait loci controlling phenotypes related to the perennial versus annual habit in wild relatives of maize. *Theor Appl Genet* 109:1544–1553
- White SE, Doebley JF (1999) The molecular evolution of terminal ear1, a regulatory gene in the genus *Zea*. *Genetics* 153:1455–1462
- Whitt SR, Wilson LM, Tenaillon MI, Gaut BS, Buckler ES (2002) Genetic diversity and selection in the maize starch pathway. *Proc Natl Acad Sci USA* 99:12959–12962
- Wilkes HG (1967) Teosinte, the closest relative of maize. Harvard University Press, Cambridge, Massachusetts, USA
- Wilkes HG (1972) Maize and its wild relatives. *Science* 177:1071–1077
- Wilkes HG (1977) Hybridization of maize and teosinte in Mexico and Guatemala and the improvement of maize. *Econ Bot* 31:254–293
- Wilkes HG (1985) Teosinte: the closest relative of maize revisited. *Maydica* 30:209–223
- Wilkes HG (1997) Teosinte in Mexico: personal retrospective and assessment. In: Serratos JA, Willcox MC, Castillo GF (eds) Proceedings of a forum “gene flow among maize landraces, improved maize varieties, and Teosinte: implications for transgenic maize”. CIMMYT, Mexico, pp 10–17
- Wilkes HG (2007) Urgent notice to all maize researchers: disappearance and extinction of the last wild teosinte population is more than half completed. A modest proposal for teosinte evolution and conservation in situ: The Balsas, Guerrero, Mexico. *Maydica* 52:49–58
- Wright S (1951) The genetical structure of populations. *Ann Eugen* 15:323–354
- Yamasaki M, Tenaillon M, Bi IV, Schroeder SG, Sanchez-Villeda H, Doebley JF (2005) A large-scale screen for artificial selection in maize identifies candidate agronomic loci for domestication and crop improvement. *Plant Cell* 17:2859–2872
- Zeng ZB (1993) Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. *Proc Natl Acad Sci USA* 90:10972–10976
- Zhang LQ, Peek AS, Dunams D, Gaut BS (2002) Population genetics of duplicated disease-defense genes, *hml* and *hm2*, in maize (*Zea mays* ssp. *mays*) and its wild ancestor (*Zea mays* ssp. *parviglumis*). *Genetics* 162:851–860
- Zhang Z, Qiu F, Liu Y, Ma K, Li Z, Xu S (2008) Chromosome elimination and in vivo haploid production induced by Stock 6-derived inducer line in maize (*Zea mays* L.). *Plant Cell Rep* 27:1851–1860
- Zhao ZY, Weber DF (1988) Analysis of nondisjunction induced by the r-X1 deficiency during microsporogenesis in *Zea mays* L. *Genetics* 119:975–980