Chapter 3
Tropical Maize (*Zea mays* L.)

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3.1 Introduction

3.1.1 History and Origin

Maize is the main tropical crop that has been domesticated in the American continent. Its origin has been the subject of much debate. Theories linking teosinte, *Tripsacum*, an unspecified common wild relative, and teosinte × *Tripsacum* crosses (tripartite hypothesis), have been proposed for the route taken by maize from wild plants to the domesticated crop that now depends on human intervention for survival (Wilkes 2004). In the last decade, molecular markers and ^{14}C analysis of maize cobs recovered from excavations in Mexico and Guatemala clarified the origin of maize (Matsuoka et al. 2002; van Heerwaarden et al. 2011). These indicated that maize was already cultivated as early as 8700 BP and perhaps originated from a single domestication event of the annual Balsas teosinte (*Zea mays* subspecies *parviglumis*) in mid-altitude areas of South-Central Mexico. From here maize diversified into the Mexican highlands by crossing with a highland-adapted teosinte, *Zea*

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mays subspecies *mexicana* (Warburton et al. 2011; Mir et al. 2013). Maize was therefore entirely tropical in origin. Its spread was via two major paths. The first was through northern Mexico to the southern USA and northward into the long photoperiods and short seasons of northern USA and Canada. The second path was through the lowlands of Mexico to Central America, the Caribbean, and thence to the Andes (Matsuoka et al. 2002). Peru has very similar geography/environments and diversity of maize races as highland Mexico and is often considered a subcenter of origin (Wilkes 2004).

The amazing diversity of maize in the Americas made its adaptation to other continents relatively rapid. The first introduction of Caribbean maize into Europe was in 1493, where it was used initially as a garden curiosity. It was joined there by northern US flints (Mir et al. 2013). McCann (2005) cites evidence that maize was present in Egypt in 1517, just 25 years after Columbus. The Portuguese were active in introducing maize to Africa through their colonies and trading posts in the Azores, Angola, Mozambique, Mombasa, and Zanzibar. Slavers and missionaries introduced maize to West Africa in the seventeenth century (McCann 2005). From these points of introduction, maize spread all over Africa and became a main staple.

The Turks and the Portuguese disseminated maize in the Asian continent. The crop was probably introduced to southern China in the late seventeenth century and spread to northern China in the late eighteenth century. Now in China, maize has overtaken rice in terms of cropped area, making it the second largest maize producer in the world after the USA.

The outcome of this global migration has been an extraordinary diversity of landraces with different ear shapes, ear sizes, grain colors, and textures and with a diversity of food, feed, and industrial uses. Maize is now found adapted to diverse environments from sea level to 4000 masl, from latitudes 0 to 57°, on soils with pH from 4 to 8, and in areas with annual rainfalls of 400–2500 mm.

3.1.2 Importance of Maize in the Tropics

Major maize production zones where tropically adapted germplasm is used (countries producing >100,000 tons of maize annually at latitudes <35°) are shown in Table 3.1. Around 30% of global maize production is from tropical areas and from tropically adapted germplasm, but occupying 49% of the global area planted to maize. Yields in temperate environments, led mainly by the USA, Europe, and China, averaged 7.2 t/ha vs. 3.3 t/ha for tropical regions. Nonetheless, yields have been increasing at about the same rate (74–75 kg/ha/year) in both ecologies, which translates to an annual increase of 1% in temperate and 2.3% in tropical regions (Fig**.** 3.1). Averages hide large variations in yield gain by regions, with the South American Cone and SE Asia leading the way (128–142 kg/ha/year) and the smallest increases in yield (27–40 kg/ha/year) being reported from the three African subregions and the Central American and Caribbean regions. Low yields in South Asia and sub-Saharan Africa (SSA) reflect lack of use of improved varieties, inadequate

Table 3.1 Maize production, area and yield, and time trends (2000–2014) in area and yield, for major production areas in tropical maize-producing regions, compared with the temperate world

	Average 2012–2014			Annual rate of increase	
Region	Production (Mt)	Area (Mha)	Yield (t/ha)	Area (%)	Yield (kg/ha/year) [%]
World	973.2	182.0	5.34	1.97	80 [1.49]
Temperate[a]	675.6	93.6	7.21	2.09	74 [1.02]
Tropical	294.5	89.1	3.30	1.89	75 [2.26]
Andean	8.1	2.2	3.72	0.70	76 [2.05]
Cent. America and Caribbean	27.1	9.4	2.87	−0.13	40 [1.40]
South. Cone	111.4	20.7	5.37	2.08	142 [2.65]
South Asia	33.5	11.7	2.86	2.10	86 [3.01]
Southeast Asia	38.6	9.2	4.18	1.37	128 [3.05]
N. Africa to W. Asia	10.2	1.7	5.88	1.15	38 [0.65]
W. and C. Africa	22.3	13.5	1.66	3.23	27 [1.64]
E. and S. Africa	43.3	20.2	2.14	2.05	42 [1.94]

Source: FAOSTAT (2016)

[a] Includes Mainland China

The definition of regions follows that of Pingali (2001)

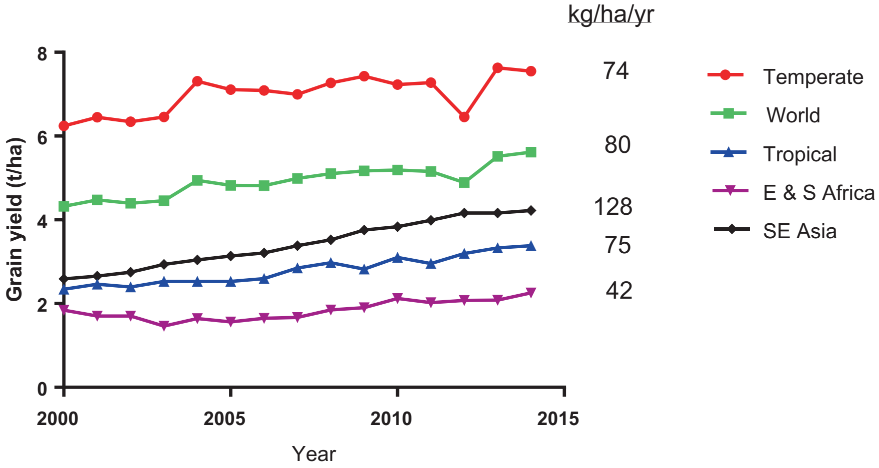

Fig. 3.1 Maize yields vs. time from 2000 to 2013 for the world, for temperate countries (including China), for tropical countries, for eastern and southern Africa, and for Southeast Asia. Corresponding linear gains with time are shown as numbers beside each graph (FAOSTAT 2016)

nutrients (poor soil fertility), and frequent occurrence of drought (Fischer et al. 2014), since more than 80% of maize in these areas is rainfed. Nonetheless, the contribution of yield increases in tropical areas to the 1 billion tons of maize grain produced annually has been significant. This is despite lower average yields, and the

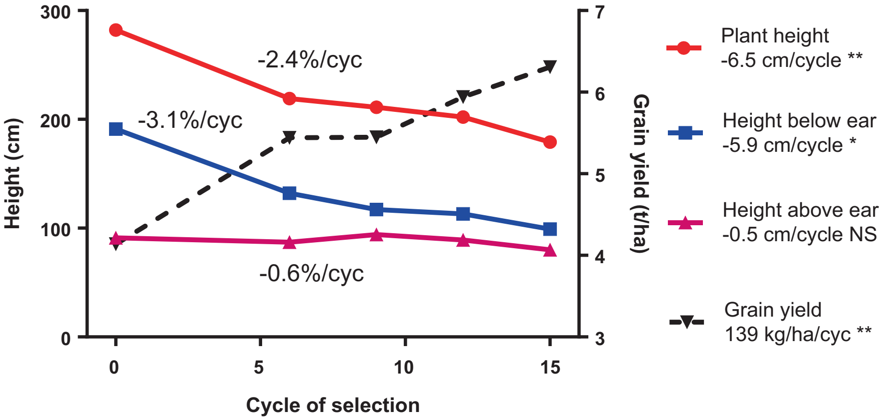

Fig. 3.2 Response of plant and ear height, stem height above the ear, and grain yield at or near the optimum plant density to 15 cycles of visual selection for reduced plant height in Tuxpeño Crema I (Johnston et al. 1986)

smaller research investment in tropical maize vs. temperate maize, especially by the private sector.

Time trends in grain yield (Fig. 3.1) also highlight fluctuations in yield with time, the most variable being in temperate regions, mainly due to the severe 2012 drought in the USA (Boyer et al. 2013) (Fig. 3.2).

3.1.3 Broad Patterns of Adaptation: Megaenvironments

In order to provide a systematic structure to maize breeding programs, the target environments have been broadly characterized into megaenvironments (MEs). An ME can be defined as an area growing at least 1 m ha of maize within which cultivar × environment interactions are relatively minor. They are often defined by temperature, altitude (e.g., highland vs. lowlands), rainfall (dry vs. wet and humid), and daylength, since tropical maize is usually very photoperiod sensitive and poorly adapted to latitudes >30°. CIMMYT has defined six major maize MEs for tropical environments for sub-Saharan Africa, but the descriptions apply also to other regions. Two temperate MEs at latitudes >30° can be added to these (Table 3.2). From a breeding perspective, each of these classes has been subdivided further by crop duration, incidence of specific diseases and pests, grain color, kernel texture, and protein quality.

The tropical and subtropical MEs differ mainly in the nature of disease pressure. Highland maize is unique – it grows exceptionally well at low temperatures, has a lower optimum temperature for development than maize of other adaptation classes (Ellis et al. 1992), and has a distinct morphology (Eagles and Lothrop 1994).

Table 3.2 Major maize megaenvironments showing approximate proportional areas, stratified by altitude, rainfall[a], temperature[b], and daylength[c]

Megaenvironment	Altitude (masl)	Proportion total area %	Potential yield t/ha	Example areas
MME1: Highland tropical	>2000	3	11	Ethiopia, Mexico, Andes
MME2: Wet upper mid-altitude subtropical	1600–2000	3	13	Ethiopia, Kenya, South Africa, Central America
MME3: Wet lower mid-altitude subtropical	1200–1600	5	13	Uganda, Kenya, Indian subcontinent (winter)
MME4: Dry mid-altitude subtropical	1200–2000	8	9	Tanzania, Western Kenya, central Mexico, Nepal
MME5: Wet lowland tropical	0–1200	15	9	Thailand, Nigeria, coastal Central America
MME6: Dry lowland tropical	0–1200	14	6	Coastal East Africa, Central America, India, NE Brazil
MME7: Wet temperate	0–1500	35	14	Corn Belt of the USA, western Europe, Argentina
MME8: Dry temperate	0–1500	17	9	Western USA, eastern Europe, northwest China

Adapted from Fischer et al. 2014

Potential yield is an estimate based on temperature and radiation receipt, as outlined by Muchow et al. (1990)

[a] Rainfall in growing season (the five consecutive months with the greatest P/PET ratio. For MME1, >350 mm; for MME4, 350–600 mm; for MME6, 350–800 mm; for MME8, 300–600 mm

[b] Average daily maximum temperature for the middle 70% of the growing season: MME1, 18–24 °C; MME2, 24–28 °C; MME3, 28–30 °C; MME4, 24–30 °C; MME5, 30–34 °C; MME6, 30–36 °C; MME7, 26–34 °C; and MME8, 26–36 °C.

[c] Daylength associated with the longest day during a summer growing season. MME1–MME6 are mainly in latitudes of <30°, while MME7 and MME8 are in latitudes 30–57°.

3.2 Tropical Maize Germplasm: Races, Landraces, and Germplasm Exchanges

3.2.1 Races of Maize: Packaging Diversity by Adaptation and Grain Type

A total of 285 maize races have been described (e.g., Wellhausen et al. 1952), though Hallauer and Miranda (1988) considered that perhaps only 130 were distinct entities. Of these, 71% originated in South America, 24% in Central America and Mexico, 2% in the Caribbean, and 3% in the USA (Serratos 2009). Adaptation of these can be considered 50% lowland tropical (<1200 masl), 10% mid-altitude (1200–1800 masl), and 40% highland (1800–2900 masl). In terms of grain type, around 40% are floury, 30% flints, 20% dent, 10% popcorns, and 3% sweet corn, and most are white grained (Pandey and Gardner 1992).

Molecular markers have largely confirmed racial groupings initially based on numerical taxonomy (e.g., Wellhausen et al. 1952). Reif and coworkers (2006) genotyped 497 plants drawn from 24 Mexican races with 25 SSR markers and grouped the accessions based on multivariate analysis. The accessions averaged almost eight alleles (range 4–14) per locus and so were highly variable. They reported that an average of 1.3 alleles per locus were unique to each accession. This and other studies on Mexican races have confirmed that there is a high level of variation within a race, and within individual landraces, and less among races and landraces (Warburton et al. 2002; Reif et al. 2004).

The large majority of tropical maize races are very sensitive to daylength and, when grown in photoperiods of greater than 14 h, become tall, unwieldy, late to flower, slow to dry down, and with much reduced grain yield (Stevenson and Goodman 1972; Edmeades et al. 2000a; Edmeades et al. 2000b). Since landraces are difficult to phenotype or use in crossing nurseries, temperate maize breeders have struggled to introgress tropical germplasm directly into Corn Belt maize, and a more staged approach has been proposed (Gerrish 1983; Holland et al. 1996). Private and public institutions in temperate regions have devoted considerable resources in adapting tropical and subtropical germplasm to temperate regions.

In summary, racial diversity is greatest where diverse natural selection environments are also found. Unique microclimates and pockets of disease in geographically isolated valleys, such as those found in Mexico and the Andean region, have resulted in a rich diversity of genotypes. The majority of this variation now resides in germplasm banks. In the interim, CIMMYT and several national breeding programs have captured a significant proportion of this variation in the most productive backgrounds in the form of pools, populations, and inbred lines that are now widely grown (Warburton et al. 2008).

3.2.2 The Search for Superior Alleles and Their Concentration: The Mexican Experience

Because of proximity to the center of origin of maize, there has been considerable focus on Mexican races. Climatic adaptation of Mexican races ranges from 0 to 2900 masl, an average annual temperature of 11–27 °C, from 400 to 3500 mm of seasonal rainfall, and average daylengths during the crop season of 12.5–13.5 h (Corral et al. 2008). Tuxpeño is reported by Corral et al. (2008) to have the greatest adaptability among Mexican races. Reif et al. (2006) studied 24 representative Mexican races using multivariate analysis of molecular marker data. They identified three racial complexes from (a) the high elevation, (b) medium to low elevation, and (c) narrow-eared races from NW Mexico. Highland tropical races (e.g., Chalqueño, Cacahuacintle, Palomero Toluqueño, Arrocillo Amarillo) typically have few tassel branches and are adapted to cool conditions. The medium to low elevation group includes Celaya, the popular, diverse, and more modern race Tuxpeño, and the long-eared Jala, Zapalote Chico and Grande, Bolita, Nal-Tel, and Pepitilla. The NW

Mexico group possesses long, thin eight-rowed ears from races such as Harinoso de Ocho and include Chapalote, Reventador, and Maíz Dulce adapted to slightly longer days and lower elevations. These have generally been shown to be the highest-yielding races (Crossa et al. 1990) and are the foundations on which CIMMYT's breeding program was originally based. Their analysis, based on molecular marker data and an earlier study using morphometric similarities (Goodman and Brown 1988), supports the proposal that parental races in Mexico are Chalqueño, Cónico Norteño, Bolita, and Celaya.

CIMMYT has tested and incorporated leading landraces from the Americas in pre-breeding gene "pools" and elite populations. Pools were later systematically arranged to address general requirements for adaptation (highland, lowland tropical, subtropical, and temperate), maturity (early, intermediate, late), grain color (yellow, white) and grain texture, and protein quality (flint, dent, and QPM[1]). Elite populations addressed similar niches, though some were direct introductions from national programs (e.g., Population 32, ETO Blanco from Colombia – Chavarriaga 1966) or composites of collections or varietal crosses from regions (e.g., Population 35, Antigua × Republica Dominicana) (Pandey and Gardner 1992). Pools were open ended, and pre-tested components were sown ear to row as females in a half-sib recurrent breeding scheme where the male pollinator was a balanced bulk of female entries. Though this scheme broke genetic linkages and mixed germplasm components under mild selection pressure for yield and agronomic traits, the flow of new materials into pools was relatively small, and limited attention was paid to heterotic groupings. Grain yields were rarely assessed directly but rather determined from an assumed shelling percentage of 80%, a practice that eliminated selection pressure for increased shelling percentages. These can be 5–8% greater in temperate hybrids vs. tropical hybrids when compared in a disease- and stress-free environment.

CIMMYT populations, on the other hand, were largely closed, with occasional introductions from matched genetic pools. From 1972 through the mid-1990s, they were improved through recurrent full-sib selection in which 250 families from each cycle were evaluated in a lattice design in two replications evaluated at six international locations. Selection was mild with the superior 40% families recombined, and cycles took 2 years to complete. During the 1990s within-family S_1 improvement occurred while progeny testing was underway, thereby reducing the load of undesirable recessives. Again, little attention was paid to heterotic group formation. Varieties were formed by recombining the ten best full-sib families at each test location or across locations and were supplied as open-pollinated varieties (OPVs) to National Agricultural Research System (NARS) scientists. During the 1990s, the populations were increasingly used to generate inbreds and resulted in a number of released CIMMYT maize lines (CMLs) that now number almost 600.

CIMMYT products, now mainly hybrids, inbred lines, and a small proportion of OPVs are available on request. Molecular data suggest that there is more genetic

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[1] QPM is quality protein maize, a grain type with elevated levels of lysine and tryptophan caused by the presence of the *opaque-2* recessive gene, and improved for kernel hardness through the accumulation of modifier genes.

variation within landraces and populations than among them (Warburton et al. 2002; Reif et al. 2004), and the genetic variation among the CMLs is considered to encompass most of the variation present in the entire tropical gene pool (Warburton et al. 2008).

A recent initiative, Seeds of Discovery (SeeD) (http://seedsofdiscovery.org/), undertaken by CIMMYT and funded by the Government of Mexico, aims to characterize the genetic diversity of maize landraces with high-density SNP markers linked to phenotypes of landraces obtained in environments to which they are reasonably well adapted. In order to make this variability more useful, bridge populations are being developed where rare but useful alleles from landraces are being crossed into elite maize germplasm using markers to reduce linkage drag from remaining alleles.

3.2.3 Global Movements of Germplasm: Developing Other Sources

Tropical maize has moved successfully to latitudes, altitudes, and rainfall regimes similar to those where it originated. Examples would be lowland/mid-altitude Tuxpeños from Mexico introduced to Brazil and West Africa; Caribbean germplasm introduced into Thailand, the Philippines, Indonesia, and Brazil; and the Andean landrace Montana introduced in the highlands of Kenya. It was also transferred to higher latitudes, e.g., US Southern Dents introduced in southern Africa (Mir et al. 2013). However, successful movement across wide latitudes has only occurred when germplasm is essentially photoperiod insensitive as with temperate germplasm.

Breeders are always interested in adding useful genetic diversity to their breeding populations. That interest is especially acute among breeders of temperate germplasm in the USA where a large proportion of the hybrids sold today trace back to seven inbred lines developed ~50 years ago (Butruille et al. 2015). There have been systematic attempts to widen the genetic base of temperate maize using tropical germplasm, though photoperiod sensitivity has slowed this process. Two USDA-supported programs, the Latin American Maize Program (LAMP) and its successors GEM and G2F, have played a key role in linking tropical landraces with the mainstream temperate breeding programs. Under LAMP the USA and collaborating national institutions in 11 Latin American countries evaluated more than 12,000 accessions from 328 races from the Americas, systematically reducing these at successive stages of testing to 3000 and then to a core subset of 270 (Salhuana and Pollak 2006). Under GEM these elite landraces were crossed with elite US commercial germplasm to provide commercially useful germplasm. Other programs such as that at North Carolina State have used LAMP germplasm and commercial hybrids from the Caribbean and South America to generate adapted tropical populations. This was done by systematically crossing to temperate germplasm to introduce daylength insensitivity followed by backcrossing to the tropical source and line extraction (Holland et al. 1996; Goodman 1999). The result has been hybrids that have performed competitively with adapted commercial Corn Belt hybrids and production of inbreds that are essentially 100% tropical (Uhr and Goodman 1995). Others such as the Hallauer group at Iowa State University selected recurrently for earliness to flower for ten or more generations in tropical populations such as ETO, Tusón, Tuxpeño, and Suwan. By doing so, they steadily increased the frequency of alleles for daylength insensitivity in these broadly based populations (Teixeira et al. 2015). The breeding program based at the University of Hawaii in the subtropics, led by Dr. J Brewbaker for the past 50 years, produced a number of inbreds with excellent resistance to a number of tropical diseases (Brewbaker 2009), with some lines serving as sources of disease resistance in temperate zones as well. One such inbred (KS23-6) has been identified as resistant to maize chlorotic mottle virus (MCMV), one of the two important viruses in the devastating maize lethal necrosis (MLN) virus complex in eastern Africa. Clearly, tropical populations can be tamed for use in temperate breeding programs, but it is a long-term effort.

Today, the movements of germplasm around the world continue, though this has been significantly affected because of regulations on seed movement arising from intellectual property protection that are considered unduly restrictive by some private sector breeders (Butruille et al. 2015). CIMMYT and its sister CGIAR center in Nigeria, IITA, have played key roles in germplasm exchanges worldwide through their international testing programs. CGIAR germplasm transfers are managed under a Standard Material Transfer Agreement (SMTA) between the supplier and the recipient. Both centers have embarked on a deliberate policy of devolving international breeding programs to sub-Saharan Africa, the Andean zone, and Asia. Another important public source of elite temperate germplasm has been through inbred lines released from patent protection 20 years after their initial release in the USA and made available on request through the US National Plant Germplasm System (NPGS) (Kurtz et al. 2016). Many of these inbreds, although several decades old, are of considerable value to tropical maize breeders and carry a well-defined heterotic response.

3.3 Biology: Do Tropical and Temperate Maize Germplasm Groups Differ?

Tropical maize landraces have evolved under natural (and more recently human) selection to outcompete weeds and withstand leaf area reduction from insects and diseases. Accordingly, they are often tall, have an ear height/plant height ratio of 0.65, may tiller freely and be prolific, are excessively leafy with heavy husks to protect against insects and birds, have large tassels that ensure surplus pollen production, and have a low-moderate number of kernels (300–400) per plant that germinate and establish rapidly from a range of sowing depths. Harvest index in landraces is typically 0.25–0.40, and plants become barren under any form of stress at flowering. The process of improvement of tropical maize using modern improvement methods has brought about major changes in the morphology and partitioning of dry matter as yields and ability to withstand higher plant densities, moving the tropical phenotype in the direction of elite temperate hybrids.

Typically modern temperate maize hybrids have a HI of 0.5–0.55, exhibit vigorous ear growth, have strong stalks and small tassels and smaller but more erect leaves, are rarely barren, and have large ears with around 500–600 kernels/ear. Because of a long history of selection under high plant density and multiple test locations for stable grain yield and resistance to lodging, temperate maize hybrids seldom have more than one ear per plant at normal plant densities or become barren under stress.

3.3.1 Source/Sink Ratios, Ear Growth, and Yield Components

Early research on tropical maize populations within a decade of their formation from landraces concluded that the ratio of photosynthetic source to reproductive sink was significantly higher than that of temperate maize (Goldsworthy et al. 1974; Fischer and Palmer 1984). Ear growth in tropical varieties was not vigorous, and when grown at commercial densities, silks often emerged after pollen shed began. The trait ASI is an indicator of ear growth rate – a reflection of biomass partitioning to the developing ear. A large ASI is symptomatic of slow ear and silk growth. In fact, delayed silking is normally associated with any stress that reduces photosynthesis per plant since this reduces ear growth more than tassel growth and development (Edmeades et al. 1993, 2000a). ASI is strongly correlated with grain number per ear (Bolaños and Edmeades 1996). Delayed silking may help ensure cross-pollination, but when ASI is large (e.g., ASI >8 day), the plant aborts kernels and may become barren. In the height reduction study, ASI at optimum plant density for yield declined from 3.9 to 1 day after 15 cycles, and barrenness declined from 30% to 2% (Johnson et al. 1986). These correlated changes suggest that stem growth was competing for assimilates that promote ear growth at flowering.

Improved tropical maize hybrids are still characterized by heavy husks that protect the ear from birds and insects, large tassels, large leaves, and a harvest index in the range of 0.4–0.45 (Zaidi et al. 2003a). In Johnson's classic experiment, the source/sink ratio changed dramatically: the ratio of grain weight to leaf area increased from 85 g/cm^2 in C_0 to 225 g/cm^2 in C_{15}. In a separate study, Fischer et al. (1987) conducted six cycles of recurrent full-sib selection in three elite lowland tropical populations for leaf area density above the ear and/or reduced tassel primary branch number. Tassel size was highly heritable, and leaf area less so, but significant reductions in both were reported. Selection resulted in increases in both yield and the optimum density for grain yield and the proportion of biomass partitioned to the ear at flowering and maturity and in a reduction in ASI. There is little doubt that these changes in morphology have changed the tropical maize phenotype in the direction of temperate maize and increased the ability of tropical maize to withstand higher plant densities. At the same time, there is considerable room for further reduction in the grain yield/leaf area ratio of tropical maize through improvements in staygreen and in HI. The HI of tropical germplasm is still low and grain yields may be only 80–85% those of comparable temperate hybrids (Zaidi et al. 2003a; Zaidi et al. 2003b). Observations suggest that staygreen in tropical hybrids is lower under stress, tassels in tropical hybrids may be 50% larger, and standability and resistance to barrenness under high plant densities are poorer than in temperate counterparts. It is in stalk strength, density tolerance, kernels/m^2, and improved staygreen and stress tolerance that modern temperate cultivars outperform tropical landraces after a century of improvement. This increase in stress tolerance through the use of steadily increasing plant densities and extensive multilocation testing was described in a number of careful conducted studies (Duvick 1997, 2005; Tollenaar and Wu 1999; Tollenaar and Lee 2002, 2011; Campos et al. 2004, 2006; Barker et al. 2005; Cooper et al. 2014). While tropical maize has undergone extensive improvement in the last 50 years, tropical test sites are fewer and more variable, resulting in lower heritabilities than those in temperate environments, thus hampering genetic gains. Unfortunately, there are few documented comparisons of modern tropical vs. temperate hybrids in locations where both are adapted. There is still a lot of room for improvement of tropical germplasm from sustained testing under high plant densities and/or drought or by introgression of yield-efficient plant traits from temperate sources.

3.3.2 Photoperiod Response

Maize is a quantitative short-day plant, and tropical maize responds strongly when photoperiods are extended from around 13 to 16 h by delaying tassel initiation and adding additional nodes and leaves below the ear leaf. This in turn delays flowering but has little or no effect on length of grain filling. While photoperiods greater than 14.5 h do not occur naturally at latitudes <30°, they are features of temperate locations (maximum daylength at 40°N is about 15.5 h and at 50°N is 17 h). Growth distortions that result from exposure to long photoperiods make phenotyping of tropical germplasm difficult. If sensitive lowland tropical lines are sown in May in latitudes of ~40 °N, delays in flowering can be more than 30 days. Furthermore, the ASI also lengthens significantly, and kernels per ear decline drastically (Edmeades et al. 2000a). There is considerable genetic variation for photoperiod sensitivity (Table 3.3), but in general the order of sensitivity is lowland tropical > subtropical > highland > temperate. In comparison the well-known hybrid B73 × Mo17 averages a sensitivity of only 0.4 leaves/h (Edmeades et al. 1992).

3.3.3 Genetics

Within the *Zea* genus, there are five recognized species, *Z. diploperennis*, *Z. perennis*, *Z. luxurians*, *Z. nicaraguensis*, and *Z. mays*. Within the species *Zea mays*, there are four subspecies – *Z. m. huehuetenangensis*, *Z. m. mexicana*, *Z. m. parviglumis*, and

Table 3.3 Linear slopes of measures of sensitivity to photoperiod extensions from 13 to 15.5 h during the tropical summer season in Tlaltizapán, Mexico, with an average $T_{\max}$ = 31 °C and $T_{\min}$ = 18 °C

Germplasm type	*N*	Sensitivity TT to AD °Cdh^{-1}	Sensitivity time to AD dh^{-1}	Range dh^{-1}	Sensitivity final leaf no. leaves h^{-1}	Range leaves h^{-1}
Lowland tropical	33	124	7.3	3.0–12.3	2.3	1.1–3.5
Subtropical	24	108	6.4	3.1–11.2	2.5	0.3–3.5
Highland	13	89	4.9	2.3–7.8	1.3	0.9–2.0
Temperate	10	54	2.9	1.0–4.6	1.1	0.3–2.2

TT to AD = thermal time (TT) in degree days to 50% anthesis (AD), and sensitivity is measured as delays in AD in TT or days and in increased leaf number over hour of additional photoperiod (Edmeades et al. 1994)

Z. m. mays. The first four species and the first three subspecies of *Zea mays* are considered to be teosintes – wild grassy relatives of maize that often cross freely with maize in the highlands of Mexico. *Z. diploperennis* and *Z. perennis* are perennials, while the rest are annuals. All but *Z. perennis* are diploids (2n = 20). Although maize is monoecious (female and male floral organs on the same plant), it is a naturally outcrossing species. It can readily be self-pollinated, and there are no major crossing incompatibilities within the species.

There is considerable evidence that that modern maize underwent tetraploidy 5–12 M years ago, since there are remnants of two complete genomes within its current genome. Over time parts of the second genome have been preferentially expelled, leaving a fraction behind (Woodhouse et al. 2010). Schnable et al. (2011) suggests that the progressive loss of duplicate genes and overexpression associated with a duplicate gene pair are responsible for the remarkable array of genetic variation in this species.

Most quantitative traits such as grain yield and drought tolerance are controlled by additive gene action, while others such as tolerance to low *N* have a larger dominance component (e.g., Betrán et al. 2003a). Resistances to a few diseases (e.g., MSV – Semagn et al. 2014) or grain texture traits (e.g., *opaque-2* – Atlin et al. 2011) are controlled by single genes and are qualitative in nature.

Maize researchers worldwide have generated numerous reports of molecular markers tagging genes/QTLs for diverse traits of agronomic and scientific interest. QTLs for several important traits affecting maize have been mapped, including resistance to several diseases (e.g., downy mildews, northern corn leaf blight/turcicum leaf blight, common smut, *Fusarium* ear rot, banded leaf and sheath blight (BLSB), aflatoxins, etc.), abiotic stresses (e.g., drought, waterlogging, low nitrogen stress, etc.), and specialty traits (e.g., high oil content).

3.4 Heterosis

Here we define heterosis (or hybrid vigor) as the increase in growth, yield, fertility, or function of a progeny over the levels found in either parent. It is usually expressed as a percentage of either the mean of the parents (midparent heterosis) or occasionally the best performing parent (high-parent heterosis). Heterosis is the foundation of the successful maize seed industry, since it results in extra yield and is lost when offspring of the hybrid are planted in the next generation. The purchase of hybrid seed each crop season provides benefits for farmers and seed producers. Midparent heterosis between open-pollinated populations or landraces for yield can reach 15–20% and that between inbred lines often exceeds 100% (Tollenaar et al. 2004). Heterosis varies significantly among parental lines, and considerable research effort has been directed toward its prediction. Initial tests of parents focus first on general combining ability using tester lines before evaluating a series of specific crosses in the search for specific combining ability and unique crosses.

Underlying causes of heterosis are not fully understood, but they directly affect the fitness of individuals. Heterosis for a trait is a function of the square of the difference in allele frequency in the parents and the degree of dominance at those loci carrying alleles that differ (Lamkey and Edwards 1999). It is therefore specific to a particular cross. Lamkey and Edwards (1999) noted that randomly mating the F1 hybrid reduces heterosis by 50% and that inbreeding depression can be considered the converse of heterosis. Differences in the collinearity of genes between the two parents (Fu and Dooner 2002) may also be a significant source of heterosis.

There is general agreement that heterosis results in greater stress tolerance, especially to drought (Betrán et al. 2003a; Makumbi et al. 2011). Modern tropical hybrids will generally tolerate stress better than the OPVs from which their lines were originally derived. Heterosis, if its causes can be better understood, provides a unique model for selection for improved yields and resource use efficiency. In a comparison of hybrids and their inbred parents, Tollenaar et al. (2004) reported that heterosis was greatest (60–167%) for grain yield, biomass, kernel number/m^2, leaf area, plant height, and percent staygreen, 53% for harvest index, 12% for weight per kernel, and <10% or slightly negative for final leaf number, for ears/m^2, and for measures of crop duration. Zaidi et al. (2003a) observed similar results in tropical maize when comparing unrelated sets of hybrids, OPVs, and inbred lines. Hybrids showed better performance under drought, a shorter ASI, and 22–25% increase in grain yield when compared with OPVs. Leaf chlorophyll level showed 16–66% heterosis in hybrids compared with OPVs and inbreds. The largest effects of heterosis were seen on capacity to capture radiation (through leaf area and staygreen) and in the use of this assimilate to establish the size of the sink (kernels per unit area). Effects were less on HI, kernel weight, and crop duration.

Table 3.4 Heterotic combinations identified among CIMMYT populations

Ecology	Population A	Population B	Grain color
Lowland tropical	Tuxpeño (P21)	ETO (P32)	White
	Mezcla Tropical Blanca (P22)	La Posta (P43)	White
	Amarillo Dentado (P28)	Cogollero (P36)	Yellow
	Amarillo Cristalino-1 (P23)	Blanco Dentado-2 (P49)	Yellow
Subtropical	Amarillo Subtropical (P33)	Amarillo Bajio (P45)	Yellow
	A.E. Dent-Tuxpeño (P44)	ETO Illinois (P42)	White
	SIW-HG88A (P501)	SIW-HT88B (P502)	White
Highland	(P902)	(P903)	White

CIMMYT population numbers are shown in parenthesis (Vasal et al. 1999)

3.4.1 Heterotic Patterns

Differences in heterosis among germplasm groups have led to the identification of general heterotic patterns and partners. The common heterotic pattern in temperate maize is stiff stalk (females) × non-stiff stalk (males), developed from the Reid × Lancaster pattern identified in the 1940s. The well-known hybrid B73 × Mo17 is an example of this pattern. In tropical maize the patterns are more diverse, though often not well defined. Studies of heterotic responses among CIMMYT populations over environments showed that the level of heterosis between populations rarely exceeded 15% (Vasal et al. 1992), but reinforced the importance of the Tuxpeño × ETO (coastal tropical flint) combination. Under subtropical conditions subtropical × tropical population crosses showed consistent heterosis. Table 3.4 shows population pairs improved by reciprocal recurrent selection aimed at increasing heterosis as well as yield per se.

In Kenya, Kitale Composite was a broad-based population developed mostly from a number of US white dent varieties adapted to the East African mid-altitudes. Hybrids became important with the introduction of Ecuador 573, which, when crossed with Kitale II, gave excellent heterosis and a pleasing plant type. These types of hybrids currently occupy about 70% of the Kenya highlands and are well disseminated in the highlands of Tanzania, Ethiopia, and Uganda.

In South and Southeast Asia, most of the maize area is grown during the monsoon season and requires a high level of foliar and ear disease resistance. Suwan 1 by harder dent Tuxpeños and Suwan 1 by other tropical flints are the most used heterotic partners.

Today, CIMMYT recognizes two main heterotic groups, A and B, where A is considered Tuxpeño types and B as non-Tuxpeños. Suwan 1 provides a third major heterotic group that combines with both A and B groups. However, because these groups are not well defined and were derived from broad-based populations, there is often as much heterosis among lines within A or B groups as there is between A and B lines (Reif et al. 2003) IITA initially developed populations with their own heterotic groupings but is gradually aligning these with CIMMYT's A × B heterotic pattern.

Duvick (2005) maintained that heterotic patterns are consolidated or even generated by selection, and Tracy and Chandler (2006) agreed with this assertion. Reciprocal recurrent selection has increased heterosis in some studies (Eberhart et al. 1995; Hallauer and Carena 2012). Molecular data shows genetic distance between stiff stalk and non-stiff stalk lines that has increased steadily since the 1960s in pioneer germplasm as each group was evaluated and improved by testers from the opposing group (Cooper et al. 2004). Remarkably, evaluations of temperate inbreds and their crosses over breeding eras have shown that heterosis per se has increased at only 22% of the rate of hybrid yield, while the rate of yield improvement of inbred parents was 80% that of the hybrid (Fig. 3.3). A similar response would be expected in tropical germplasm.

3.5 Breeding

3.5.1 The Genetic Gain Equation

This is most usefully written as $\Delta G = i\sigma_a h/t$, where ΔG is the yearly genetic gain; i, the standardized selection differential; h, the square root of narrow sense heritability (the ratio of additive genetic variance to phenotypic variance); σ_a, the square root of additive genetic variance; and t, the number of years per breeding cycle (Butruille et al. 2015). The variable i is related to the selection intensity expressed as percent selected individuals but is not the same. A decrease in selected individuals from 20% to 5% changes i from 1.40 to 2.06 and increases genetic gains by only 47%. In recurrent selection schemes, the selected fraction should not be reduced below five to ten individuals because of the risks of inbreeding during recombination and, in the early stages of population formation, the risk of recovering parental genotypes because of inadequate recombination. Typically, t in commercial pedigree breeding is 5–10 years, but can be as little as 6 months for half-sib recurrent selection and 1 year for full-sib recurrent selection. Annual gains therefore are greatest when heritability and additive genetic variance are large, the proportion of selected individuals is small, and time per cycle is short. Additive genetic effects are also termed the breeding value and are increased by allelic dosage or allelic substitution such as occur with marker-assisted selection (Moose and Mumm 2008). Where field trials are used to identify superior progenies, the presence of random variability in the trial caused by soil variability or pests results in a reduction of h (sometimes referred to as repeatability) and hence in ΔG.

Genetic gain equations can only be used as a guide. In most breeding situations, selection is for several key traits or for an index of traits, and genetic gain must take these traits into account. Finally, the proof of effectiveness lies not in the predictions but in realized gains under realistic field conditions.

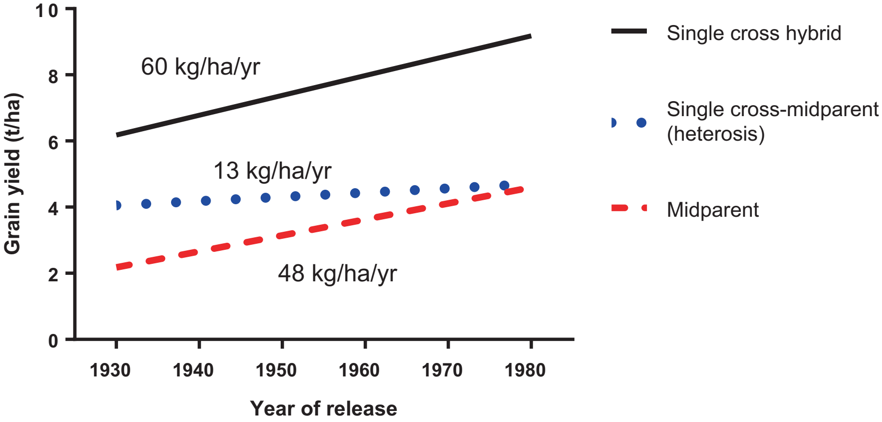

Fig. 3.3 Yields of single crosses, average yields of their parents, and the difference estimated as heterosis, as affected by the year of release of commercial hybrids. Data are from six hybrids and 12 lines from each 10-year period (Adapted from Duvick (2005))

3.5.2 Development of Useful Genetic Sources

Sources that include a high frequency of alleles affecting the expression of a specific trait have an increasing role in providing key genetic variation that can be introgressed into elite inbreds used in marker-assisted selection (MAS). CIMMYT has used two approaches in developing sources. The drought-tolerant population (DTP) is an example of the first approach. DTP was formed from 13 of the best putative sources of drought tolerance. Three hundred landraces and a number of diverse but improved sources of drought tolerance were compared with this base population, and 40 components were introgressed via a half-sib mixing phase followed by S1 recurrent selection using a progeny test under drought and heat. In C5, the population was split into yellow- and white-grained fractions and at C9 inbred lines were extracted (Monneveux et al. 2006) and tested for heterotic response and adaptation. A second approach used full-sib recurrent selection under managed drought stress within elite populations to increase the frequency of drought tolerance alleles in germplasm already adapted to the lowland tropics (e.g., Edmeades et al. 1999). Both approaches have generated lines that have become important sources of drought and heat tolerance, such as DTPYC9-F46-1-2-1-2 and La Posta Sequía C7F64-2-6-2-2 (Cairns et al. 2012). Thus, population formation and improvement have resulted in an increase in the frequency of drought-adaptive alleles and identification of superior sources of drought tolerance. The first approach is slow and should have been structured more strongly around heterotic groups and grain color. The second approach generates a useful product more rapidly and is the preferred route. It reinforces the assertion by Blum (1988) that stress tolerance alleles exist in low frequencies in most elite breeding populations, resulting in directly usable sources of tolerance to the key stresses of drought. Low *N*, heat, and acid soils, alone or in combination, are being identified among elite inbred lines. Trachsel et al. (2016b) identified a number of such lines, concluding that “it will be possible to develop hybrids tolerant to multiple abiotic stresses without incurring yield penalty under unstressed conditions using these lines.”

3.5.3 Population Improvement Methods

Although population improvement has become much less used over the past 20 years, it has had a useful role in building sources with good agronomic performance with relatively broad adaptation prior to extracting inbred lines. Population improvement methods have been extensively reviewed by Hallauer and Miranda (1988) and specifically for tropical germplasm by Pandey and Gardner (1992). The most effective methods involve ear-to-row family structures, where a progeny test, usually in the form of a yield trial, is followed by a recombination step of a superior fraction that generates the progenies for the next round of testing. This systematically increases the frequencies of favorable alleles. Population formation and improvement normally do not involve tracking and use of pedigrees and in CIMMYT’s case did not consider heterotic responses in its initial stages. The emphasis on compatibility of components used to form populations, however, provided some selection for general combining ability.

Population improvement methods are described by their family structures, and the most commonly use are half-sib, full-sib, and S_1 recurrent schemes (Paterniani 1990; Pandey and Gardner 1992). Most populations are structured around 200–1500 families, depending on objectives and resources:

- Half-sib recurrent schemes usually involve a series of females planted ear to row and detasseled, with a male pollinator comprised of a balanced bulk of all females or a subset of females. The male serves as a visual check against which female rows are selected, and three to five ears per selected female are chosen to use as the progenies for the next round of selection. The block must be isolated from other pollen sources by time or distance. It is usually unreplicated, though it can be repeated in other locations or planting dates. In tropical environments where irrigation is available, two cycles of half-sib selection can be completed per year. This same field layout can be used during pedigree breeding as an isolated crossing block where the male is the topcross parent and the females are inbreds selected as candidates for topcross testing.
- Full-sib recurrent schemes involve plant-to-plant crosses among 30–50 selected progenies, so both parents are known. Typically 200–300 of these crosses are grown ear to row in a replicated yield trial, though numbers of replications and plot size are limited by seed supply from single ears (or from two ears where reciprocal crosses are made). Selections are based on family performance at several sites. Remnant seed of the family is used during a single step of

recombination and progeny formation among the 30–50 selected families. In tropical locations one complete cycle of full-sib recurrent selection is possible per year, thus allowing progeny testing in the normal crop season and recombination during the dry season.

- S_1 recurrent selection schemes require three crop seasons to complete a selection cycle. Selfs are made in the first season and are phenotypically evaluated as S_1 families in the second season. In the third season, the superior families (20–40) are recombined by plant-to-plant crosses in all possible combinations, and a balanced bulk is prepared for selfing the following season. Superior S_1 families can immediately be advanced by selfing to form inbred lines. Since three cycles are not often possible in 1 year, one modification used when selecting for drought tolerance was to generate 1500 S_1 families and prescreen them in an unreplicated trial under heat and drought in NW Mexico in the summer. Numbers were then reduced to 200–250 for winter testing in replicated tests in the rain-free winter season in central Mexico, followed by recombination of the best 40 families from remnant seed (Edmeades et al. 1999). Seed per ear quickly becomes a limitation if more test locations are used.

Predicted gains under these three schemes indicate that ΔG is surprisingly similar from each, especially where genotype × environment interaction (GEI) is small. Reported gains for yield are around 3–10%/cycle for half-sib, 2–8%/cycle for full-sib, and 1–8% for S_1 selection (Johnston et al. 1986; Paterniani 1990; Pandey et al. 1991; Pandey and Gardner 1992; Bolaños and Edmeades 1993; Edmeades et al. 1999; Monneveux et al. 2006; Hallauer and Carena 2012). When progeny tests are conducted in several diverse environments, GEI decreases h, and gains can be less than expected, as in full-sib selection in CIMMYT's elite populations subject to international progeny testing (Pandey and Gardner 1992). Yield gains will decline when other traits such as disease resistance become priorities. Percent gains can also be high when the populations are relatively unimproved for the target trait or when mean yields are low. A better measure in such cases is gain in yield per unit area. As a rule of thumb, gains for drought tolerance from recurrent selection in germplasm previously unimproved for the trait have averaged 100 kg/ha/year (or 5% per year) when selection was conducted largely in a single dry environment (Bänziger et al. 2006). Because yield levels were low, these gains fall in the upper range of 2–5% gain/cycle expected from recurrent selection (Hallauer and Carena 2012). Gains in OPVs generated over the past 30 years in eastern and southern Africa in more advanced germplasm show sustained average annual gains under MSV infection (151 kg/ha), optimal conditions (95 kg/ha), and low N (69 kg/ha) but reduced gains under random drought stress (36 kg/ha) and no change under managed drought stress (Masuka et al. 2017b). These results suggest that gains under managed drought stress may decline as variability for ASI and barrenness become exhausted.

In summary, intrapopulation improvement has allowed rapid improvement in performance, provided a tool for testing effects of selection for a specific trait, and developed useful source germplasm. As the population has been improved, it has served as the source of an increasing proportion of elite inbred lines. Randomly extracted S_2 lines from three populations selected for drought tolerance vs. their conventionally selected equivalents, when topcrossed to a common tester, showed on average an improvement in drought tolerance that reflected the differences between their parent populations (Edmeades et al. 1997). The choice of a population in which to begin selfing is therefore a critically important decision that directly determines the probability of extracting high-performing inbreds (Hallauer and Miranda 1988).

A widely used interpopulation improvement scheme is reciprocal recurrent selection (RRS) (Paterniani 1990; Pandey and Gardner 1992; Eberhart et al. 1995). Here one population of a heterotic pair is used as the tester of the other (and vice versa) in a half- or full-sib mating system, with an emphasis on improving the performance of the cross between populations. Hallauer (1999) reported an average increase in midparent heterosis in four temperate population pairs from 9 in C_0 to 43% after an average of nine cycles of selection, essentially by increasing the divergence of allele frequencies. Improvement in the population yields per se, however, averaged only about 1%/cycle. Interpopulation schemes are efficient especially where dominance effects are large and increase the probability of extracting high-performing lines with an established heterotic response.

3.5.4 Pedigree Breeding

Pedigree breeding is by far the most common form of maize improvement and resembles RRS in that pedigrees that define ancestry and heterotic response are maintained and used to predict performance. Testers, usually inbred lines or single-cross hybrids, are selected from the opposing heterotic group to screen for general combining ability. The remaining lines are subject to specific combining ability tests with a small group of elite inbreds from the opposing group. Cooper et al. (2014) describe these steps and note that superior new inbreds are rapidly recycled to create new combinations within each heterotic group. Testing of topcross progenies is within the target population of environments (TPEs), using relatively few sites for GCA tests and many more locations for SCA testing. Field testing is followed by rapid data analysis and information extraction so lines can be advanced in off-season nurseries. Based on selection data, promising lines are advanced to the next level of multilocation testing.

The rate of gain from pedigree breeding has been evaluated mainly in temperate breeding programs and is summarized by Fischer et al. (2014). Gains in researcher-managed trials have averaged 100 kg/ha/year or around 0.8% annually. Given that the cycle length of most pedigree breeding programs is 5–10 years, this equates to a per cycle gain of 4–8% or 400–800 kg/ha. Correlated changes resulting from selection over time in temperate germplasm have been largely in traits that are associated with maintenance of leaf area through improved staygreen plus a very significant increase in ability to tolerate stresses, especially high plant density and drought. Resistance to lodging and to barrenness has increased significantly, and ASI and grain protein concentration have declined. Leaves have become more upright and tassel size has reduced, but there have been little or no changes in yield potential per plant (Egli 2015), plant height, leaf area, or time to flower (Tollenaar and Wu 1999; Tollenaar and Lee 2002; Duvick 2005; Barker et al. 2005; Campos et al. 2004, 2006). Regrettably, few assessments have been made of changes in biomass production and HI. Recent assessments of gains in conventional selection programs in eastern and southern Africa show gains in grain yield in CIMMYT-bred germplasm of 109, 33, 23, 21, and 141 kg/ha/year under optimal, managed drought stress, random drought stress, low *N* stress, and MSV infection, respectively (Masuka et al. 2017a). Relative gains in grain yield ranged from 0.6 (low *N*) to 2%/year (MSV), and ASI and barrenness decreased over time (Masuka et al. 2017a; Semagn et al. 2014). Correlations between inbred line and test cross performance, while normally low (c. 0.3) under optimal conditions, generally increase as abiotic stress levels increase to values around 0.5. This suggests a possible role for evaluations of inbred lines per se under severe drought stress conditions (Kebede et al. 2013).

3.5.5 Doubled Haploids

The process of developing inbred lines is time-consuming and, in many tropical maize breeding programs, is subject to error and loss through seed and pollen mixtures and inadequate field and seed storage facilities. The production of doubled haploid (DH) lines sharply reduces the time taken to develop homozygous lines with proven performance and reduces losses from seed identification errors. Doubled haploids have been in routine use commercially for the past decade to generate >0.5 million lines annually for each leading multinational seed company. Doubled haploids increase the rate of genetic gain by reducing t, the time to complete a breeding cycle, by 1–2 years, and produce lines whose uniformity makes them easier to phenotype and which are well suited to molecular marker applications.

The technology involves the use of a haploid inducer line (used as pollen parent) in crosses with desired source populations (as female parent). The inducer line carries phenotypic markers that enable differentiation of haploids from diploids (at the seed stage) in the induced progeny. Inducers show induction rates of 6.7–11.3% (Prigge et al. 2011), and the haploid induction rate is under polygenic control (Geiger and Gordillo 2009). The most widely used phenotypic marker is an *R1-nj* aleurone coloration visible in seed or a liguleless gene *lg2* that can be detected in seedlings (Melchinger et al. 2016). *R1-nj* anthocyanin marker inhibition is quite common in tropical maize germplasm and significantly reduces efficiency of haploid identification. Molecular markers that reliably differentiate germplasm carrying the anthocyanin color inhibitor have been identified by Chaikam et al. (2014). The *R1-nj* marker is also ineffective in germplasm with natural anthocyanin expression in pericarp tissue. Given these limitations, the CIMMYT team developed haploid inducer lines with triple anthocyanin color markers, including the expression of anthocyanin coloration in the seedling roots and leaf sheaths, in addition to the Navajo marker on the seed (Chaikam et al. 2016).

Tropically adapted inducers with high haploid induction rate are being developed by CIMMYT, in collaboration with the University of Hohenheim, Germany. CIMMYT, in partnership with Kenya Agricultural and Livestock Research Organization (KALRO), established a maize DH facility at Kiboko (Kenya) in 2013; the facility, developed through the financial support of Bill and Melinda Gates Foundation and the Syngenta Foundation for Sustainable Agriculture, offers DH development services to national programs and to small- and medium-enterprise seed companies in sub-Saharan Africa. The use of DH lines, along with marker-assisted selection (MAS), will increase rates of genetic gain from many tropical pedigree breeding programs.

3.5.6 Secondary Traits and Their Use in Selection

Secondary traits are often used in selection and frequently form part of a selection index along with grain yield. A secondary trait could give greater gains for the primary trait (grain yield) than selection for yield alone when $h_{\mathrm{GY}} < |r_G h_{\mathrm{ST}}|$ (Falconer and McKay 1996), where h_{GY} and h_{ST} are the square roots of heritability of grain yield and the secondary trait, and r_G is the genetic correlation between grain yield and the secondary trait. This condition is rarely met except when yield is low and the secondary trait is expressed best under stress. However, in most cases, secondary traits are added to a selection index along with the primary trait in the belief that the heritability of the index will exceed that of the primary trait and yield.

A useful secondary trait should be (1) genetically associated with grain yield under the target stress and be genetically variable and more heritable than yield; (2) cheap and fast to measure; (3) observed at or before flowering, so that undesirable parents are not crossed; and (4) not associated with yield loss under unstressed conditions. The value of a secondary trait can be assessed by analyses of correlation and heritability, by divergent selection for that trait, by modeling, or by statistical procedures based on selection index theory. Using this last approach, Bänziger and Lafitte (1997) determined that the use of secondary traits plus yield during selection for tolerance of maize to low soil *N* was about 20% more efficient than selection for yield alone, and this benefit increased as yield levels declined. Needless to say, very few secondary traits proposed mainly by nonbreeders have passed these tests!

3.5.7 Participatory Plant Breeding

Many large centralized breeding programs lack meaningful farmer contact during selection, despite advantages of scale, wide area testing, and access to germplasm (Morris and Bellon 2004). There is often inadequate farm-level testing, and frequently consumer response to disease reaction, grain texture and color, and ease of harvest and shelling are ignored. Differences in maturity, cooking quality, taste, and especially in stover quantity and quality can also be overlooked when breeding for grain yield (Witcombe 2006). Sometimes ear characteristics are more important than yield to farmer families (Louette and Smale 2000). Farmers have a unique comparative advantage at some stages in the selection process, and plant breeding without adoption of the product by the many small-scale farmers operating in target areas is one measure of failure.

The key breeding issue is the stage in product development when farmer input is most valuable. Farmer participation in goal setting and determining selection criteria may be adequate during the product development stage, but assessing the suitability of the finished variety as a preliminary to its release should also be a priority. During the selection process at researcher-managed locations, it is important to bear in mind the effect of the selection environment vs. farmers' fields. Gains in the selection environment must be related to gains in the target on-farm environments and rank similarly. Theory developed by Falconer and McKay (1996) shows that selection response in farmers' fields (R_{FF}) is greatest when genetic variance σ_{G}^2, selection intensity i, heritability in farmers' fields h^2_{FF}, and the genetic correlation r_G between selection environment and farmer's fields are all high, since $R_{\mathrm{FF}} = \sigma_G^2 i\, h^2_{\mathrm{FF}} r_G$ (Bänziger and Cooper 2001). Heritability and genetic variance for yield generally fall as yield levels decline relative to a high-yielding selection environment, since error variance relative to genetic variance rises, i.e., GEI between selection and target environments becomes important, and gains in farmers' fields will decline. If r_G is negative, gains in selection environments will lead to losses in yield in farmers' fields – but fortunately this is seldom the case. There is no substitute for on-farm and consumer testing of tropical maize varieties prior to their release, and increasingly variety release committees are demanding such data. Harvest field days, and ratings of varieties by farmers, both male and female, are an important part of product development and delivery (Bänziger et al. 2000). CIMMYT's maize product advancement process typically includes not only regional on-station trials of promising pre-commercial hybrids coming out of the breeding pipeline vis-à-vis internal genetic gain checks and commercial checks but also extensive regional on-farm varietal trials to ascertain the performance of the promising pre-commercial hybrids under farmer-managed conditions. This also provides opportunity for the socioeconomic team to assess farmers' own product as well as their trait preferences. The best entries coming out of this rigorous process are then allocated to public/private sector partners for varietal registration, scale-up, and delivery in the target geographies.

3.6 Traits

3.6.1 Determinants of Yield

The primary trait during selection is almost always grain yield, and much of the discussion in previous sections pertain directly to selecting for yield. The major drivers of grain yield are assimilates generated from radiation captured by a healthy and effective canopy and their efficient partitioning to grain. In the absence of any stress, grain yield in the tropics varies in proportion to radiation received (Jong et al. 1982) and is modulated by genetic potential. Increasing temperature speeds development and shortens crop duration, but has much less effect on net photosynthesis, so high temperatures will generally reduce yield by reducing the time available for assimilation (Muchow et al. 1990).

Grain yield (GY) can be defined by the following identity (Edmeades et al. 2000b):

$$\text{GY} = \text{RAD}^{*}\text{RI}^{*}\text{GLD}^{*}\text{RUE}^{*}\text{HI} \qquad (3.1)$$

where RAD is the incident radiation received per day; RI, fraction of radiation intercepted over the crop's life; GLD, green leaf duration; RUE, radiation use efficiency; and HI, harvest index. Using typical values, $\text{GY} = 23\text{MJ}/m^{2*}0.55^{*}103d^{*}1.7g/\text{MJ}^{*}0.45 = 10.0t/\text{ha}$.

Where water is the limiting production, Passioura (1977) proposed a parallel expression: $\text{GY} = W^{*}\text{WUE}^{*}\text{HI}$, where W, water transpired by the crop; WUE, water use efficiency; or biomass/unit water transpired (e.g., GY = 445 mm*50 kg biomass/mm × 0.45 = 10.0 t/ha). A similar identity can be used when N is the limiting growth: GY = N uptake × N use efficiency × HI (e.g., GY = 200 kg N uptake*111 kg biomass/kg/N^{*}0.45 = 10 t/ha).

From Eq. 3.1, breeders can increase grain yield through the last four variables – by ensuring that canopy closure is rapid, by boosting RUE and delaying leaf senescence through adequate nutrition and resistance to foliar disease and insects, and by selecting for traits like ASI that maintain a high HI. Grain yield under drought can be reduced because of direct effects on stand, leaf area, and RI, from accelerated senescence (reduced GLD) and from drought-induced barrenness which in turn reduce HI. Drought, and nitrogen deficiency, may also reduce RUE and possibly increase WUE, though effects on these are often less important than those on intercepted radiation and harvest index. If it occurs early in the crop life, then the leaf area is reduced and ear size declines. If later in the crop's life cycle, it will induce premature leaf senescence.

Grain yield can also be considered the product of its yield components:

$\text{GY} = \text{plants/m}^{2*}\text{EPP}^{*}\text{GPE}^{*}\text{WPG}$

where plants/m^{2}, established stand density; EPP, ears per plant; GPE, grains per ear; and WPG, weight per grain (g). Using typical values, GY = [5.5*1.1*500*0.33] = 10 t/ha. Although selection affects each component, they are normally negatively correlated. Increases in prolificacy are associated with a reduced ear size. Similarly, an increase in kernels per ear can be associated with reduced kernel weight (e.g., Uribelarrea et al. 2008) such that yield is little affected.

3.6.2 Increased Yield Potential

The steady increase in grain yield with selection in all classes of maize has been mainly associated with increases in kernels per plant and kernels/m^2 and not with changes in weight per kernel (Bolanos and Edmeades 1996; Chapman and Edmeades 1999; Duvick 2005). Changes that have occurred in grain yield with selection in temperate maize are summarized in Table 3.5.

Most of the changes in temperate maize were correlated with selection for high and stable yields through multilocation testing rather than direct responses to selection. Thus, when selecting for increased grain yield potential and stability, correlated increases occurred in leaf angle, staygreen (including foliar pathogen and insect pest resistance), vigorous silking with minimal delay under stress, and standability, all at high plant density. It is likely that tropical maize will ultimately mimic temperate maize in trait changes under selection, leading to increased partitioning to the ear, accelerated by selection for reduced leaf and tassel size, increased kernel number/m^2, tolerance of high plant density, and staygreen. These traits lead to general stress tolerance, i.e., an ability to withstand any stress that acts primarily by reducing photosynthesis per plant.

Evidence suggests that prolificacy is not a trait that imparts stress tolerance per se, and Echarte and Andrade (2003) concluded that HI was higher in non-prolific vs. prolific hybrids. Prolificacy is an opportunistic trait and may have a role in exploiting good growing conditions when maize at low plant density is intercropped with lower-growing crops. It is also a useful trait when maize is planted at low densities in the expectation of severe drought stress, as in semiarid parts of South Africa. Husk cover remains a high priority as ear size increases to ensure that infestation by diseases and insects and damage from birds are minimized, and rapid dry down minimizes risks of pest infections or mold building up prior to harvest or in storage.

3.6.3 Selecting for Abiotic Stress Tolerance

Drought and low *N* are the most common of these stresses and fortunately the responses of the plant to moderate levels of each are similar. In most production environments, drought, unlike *N* stress, occurs unpredictably throughout the season, and its variability will likely worsen with global climate change. Spatial variability caused by changes in soil texture means that a typical variety will be exposed to varying levels of drought or *N* deficit within the same field. Variation in grain yield under both is strongly associated with kernel number per plant, and this is directly

Table 3.5 Rates of change in specific morphological and stress-related traits under selection in temperate maize, their approximate heritabilities in tropical maize, and relative selection priorities (L, low; M, medium; **H**, high)

Trait	Temperate			Tropical			
	Δ/year	Comment	Ref[a]	h^2	Priority	Comment	Ref[a]
Morphology and phenology							
Leaf angle score[b]	0.1	Leaves more erect	2	H	M	Erect leaves for intercropping, high density tolerance	1
Tassel weight (g)	−0.05	Smaller tassels	2	H	**H**	Smaller tassels give stress tolerance, reduce shading	7, 25
Plant height (cm)	ns	Maintained	2	H	**H**	Maintain height below 2.5 m to reduce lodging	8
Time to 50% anthesis	ns	Maturity maintained	2,5	H	L	Fitted to suit cropping system	11
Grain fill duration	na	Increased	1	?	M	Increase; raises yield potential and HI	22
Husk cover	na			H	**H**	Increase tip cover to prevent pathogen/pest invasion	23
Grain dry down rate	na			?	M	Increase; reduces drying time in field and pest risks	22
Leaf area	na	Less gives density tolerance	6	M	M	Reduce leaf size to increase optimum plant density	7
Productivity of grain and biomass							
GY at opt density (kg/ha)	90	Yield increased	1	M	H	Yield potential and density tolerance increased	7,8
Staygreen score[b]	0.12	Senescence is delayed	4	L	H	Increase; need smaller leaves that live longer	11
Kernel weight (mg)	0.7	Increased	2	M-H	L	Maintained or increased	11,21
Prolificacy	0.002	Barrenness decreased	2	M	L	Increase for intercropping systems and yield stability	15, 16

(continued)

Table 3.5 (continued)

Trait	Temperate			Tropical			
	Δ/year	Comment	Ref[a]	h^2	Priority	Comment	Ref[a]
HI, unstressed (%)	0.1	Slight increase	2	M-H	H	Increase except where value of stover is high	8,17,19
Photosynthesis, no stress	ns	Unchanged	3	?	L	Increase and stabilize across stresses	20
Stress tolerance – abiotic							
GY, irrigated control	196	High-yield environment	5	M	M	Maintain or increase yield potential	9,10
GY, flowering drought (kg/ha)	120	60% of irrigated gain	5	M-L	H	Increase; decrease aborted ears and kernels	9,10
GY, grain filling drought, kg/ha	50	25% of irrigated gain	5	L	M-H	Increase; concurrent with improved staygreen	9,10
ASI, drought (°Cd)	−2.6	Synchrony improved	5	M	H	Decrease; improve floral synchrony, kernel set	11, 25
Ears per plant	0.002	Less barrenness	2	M-L	H	Increase; reduce barrenness under stress	11, 25
Kernels per ear	1.6	More under drought	5	M	M	Increase; reduce kernel abortion under stress	11, 25
Lodging (%)	−0.9	Stands better	2	L	H	Increase stalk and root strength	8, 24
Leaf rolling score[b]	0.035	Rolls more readily	4	H	L	Limited importance under drought; sheds radiation	11
Heat tolerance		Improved	1,3	?	H	Increase at flowering	12,14
Grain protein (%)	−0.03	Less protein, more starch	2	?	M	Maintain or increase under low soil *N*	18

See footnotes for sources. Modified from Fischer et al. (2014)

[a] Sources: 1, Duvick (2005); 2, Duvick (1997); 3, Tollenaar and Lee (2011); 4, Barker et al. (2005); 5, Campos et al. (2006); 6, Lambert et al. (2014); 7, Fischer et al. (1987); 8, Johnson et al. (1986); 9, Edmeades et al. (1999); 10, Bolaños and Edmeades (1993); 11, Bolaños and Edmeades (1996); 12, Cairns et al. (2012); 13, Zaidi and Singh (2005); 14, Cairns et al. (2013); 15, Motto and Moll (1983); 16, de Leon and Coors (2002); 17, Worku and Zelleke (2007); 18, Lafitte et al. (1997); 19, Echarte and Andrade (2003); 20, Echarte et al. (2008); 21, Campos et al. (2004); 22, Cross (1975); 23, Demissie et al. (2008); 24, Pandey and Gardner (1992); and 25, Chapman and Edmeades (1999)

[b] Scores are from 1 (least desirable) to 9 (most desirable)

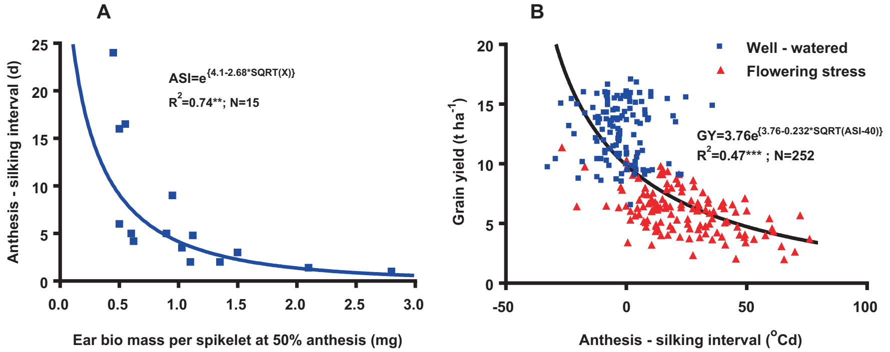

Fig. 3.4 (**a**) ASI vs. ear biomass per spikelet at 50% anthesis in a tropical population grown at high plant density in Mexico; (**b**) grain yield vs. ASI in 126 elite temperate hybrids under either severe drought at flowering or no stress, Woodland, CA (Edmeades et al. 1993; Edmeades 2008)

affected by stress that occurs 10–14 days either side of anthesis. ASI is a morpho-physiological trait that can be used to predict kernel set, barrenness, and grain yield under stresses (Chapman and Edmeades 1999), including those associated with high plant density (Dow et al. 1984). The correlation between ASI and grain yield (GY) under stress at flowering is often −0.4 to −0.7 (Bolaños and Edmeades 1996). This association of grain yield with ASI has been observed in landraces, populations, lines, and even elite Corn Belt hybrids and reflects rate of growth of ovules and hence of silks (Fig. 3.4).

Having ears and tassels develop synchronously has stabilized kernel set under stress and lifted HI (Chapman and Edmeades 1999). A reduction in the size of tropical tassels would reduce shading and competition during ear growth, but how far can it be taken? In three tropical populations, recurrent selection reduced tassel branch number by 7–9% per cycle, and over six cycles tassel biomass declined from 7.1% to 5.5% of shoot biomass at 50% silking. Tassel weight fell from 9.3% to 5.4 g/plant for the population Tuxpeño, and concomitantly optimum density increased by 16% and yield by 13% (Fischer et al. 1987). Although comparisons of tassel weight are complicated by pollen shed and senescence, tassel weight in temperate maize declined from 5 g/plant in 1933 to 2 g/plant in 1993 as a result of selection for higher yields (Duvick and Cassman 1999). Reduced tassel size, if accompanied by decreased ASI, should not jeopardize kernel set because of reduced pollen supply in modern stress-tolerant tropical hybrids. Selection for reduced tassel size continues to be a useful route to higher yields and improved density tolerance in tropical maize.

Unlike grain yield, secondary traits such as ASI, barrenness, and staygreen scores have stable or even increasing heritabilities as drought stress at flowering intensifies, and when combined with yield in a selection index, they improve the heritability of that index. Indices such as these were applied to several maize populations as proof of concept and to a single population selected under low *N* (Lafitte and Edmeades 1994). Selection outcomes for drought in tropical populations (Table 3.6)

Table 3.6 Selection gains in six tropical maize populations

		Yield			ASI SS	Ears/plant
		SS	WW	Low *N*	SS	SS
Population	Cycles selected	kg/ha/cycle			d/cycle	no./cycle
La Posta Sequía	3	229**	53 ns	233**	−1.2**	0.07**
Pool 26 Sequía	3	288**	177**	207**	−1.5**	0.08**
Tuxpeño Sequía	8	80**	38**	86**	−0.4**	0.02**
Pool 18 Sequía	2	146**	126**	190**	−2.1**	0.05**
DTP1	6	160*	80 ns	210*	−0.6**	0.03**
DTP2	9	80*	120 ns	60 ns	−0.3**	0.01*
Mean gain		164	99	164	−1.0	0.04

Sources: Edmeades (2012), Monneveux et al. (2006)

Four were evaluated at three to eight drought sites and two low *N* sites, and two (DTP1, DTP2) were evaluated at one low *N*, one severe stress, or one well-watered location. Yields relative to unstressed levels were 30% under drought stress (SS) and 59% under low *N*

Symbols *, **, and ns signify significant rate of change per selection cycle at $P < 0.01$, $P < 0.05$, or $P > 0.05$, respectively

indicate significant gains also under low *N*, first reported by Bänziger et al. (1999). Tolerances to these two stresses are related – through the common mechanisms of partitioning to the ear under stress and through staygreen. It is not until *N* deficiency becomes very severe with yields reduced by >65% that *N*-specific traits become important, and the genetic correlation between yield under drought and low *N* decreases to non-significance. As previously noted, gene action for drought tolerance is generally additive, while tolerance to low *N* has a greater dominance component (Betrán et al. 2003a; Makumbi et al. 2011). Inbred line performance is therefore a better guide to hybrid performance under low *N* than under drought (Makumbi et al. 2011) with the line/hybrid correlation under low *N* sometimes reaching 0.65 (Zaidi et al. 2003b).

A useful manual (Bänziger et al. 2000) outlined practical steps to manage stress levels and improve precision of trials conducted under drought or low *N*. These methodologies have been refined and updated recently for several abiotic stresses (e.g., for drought tolerance, see Zaman-Allah et al. 2016). Improved phenotyping methods have been augmented with modern molecular selection tools (Semagn et al. 2014; Beyene et al. 2015, 2016) and with more precise phenotyping methods that rely on uniform field conditions and remote sensing (Lu et al. 2011; Masuka et al. 2012; Araus and Cairns 2013; Trachsel et al. 2016).

Temperatures during the main cropping season are predicted to increase in most tropical maize-growing areas (Jones and Thornton 2003). The change is predicted to be greatest in night temperatures and accompanied by increased vapor pressure deficits. The development of fertile pollen and silks, and therefore kernel set, is threatened by temperatures >38–40 °C (Schoper et al. 1987; Westgate and Bassetti 1990; Cicchino et al. 2010). Heat tolerance is often associated with drought tolerance, but recent reports indicate they are largely independent traits (Cairns et al. 2012). Edreira et al. (2011) observed that temperate hybrids were more susceptible than tropical hybrids to heat stress at flowering and noted that this stress delayed anthesis and in so doing shortened ASI. If selection environments include hot dry summers typical of Mediterranean climates, then the two traits can be improved simultaneously. Global warming predictions indicate both traits will be increasingly important in the future (Lobell et al. 2011a). Conversely, cold tolerance may be required as maize continues its progress as a winter crop in the Indian subcontinent (Zaidi et al. 2010a), as well as heat tolerance to cope with very high pre-monsoon temperatures after flowering. Although considerable genetic variation for cold tolerance exists in highland germplasm (Eagles and Lothrop 1994), it is not a global priority trait for tropical maize.

Acid soils are relatively common in the tropics, usually where rainfall is high and soils are weathered and leached, or they are associated with specific parent material. Recent assessments of progress are very promising (Pandey et al. 2007). Under pH 4.7, and Al^{3+} saturation of 60%, yields of OPVs in 1993 were around 3 t/ha but had increased to 9 t/ha in hybrids by 2008.

Waterlogging tolerance is of greatest value in low-lying and poorly drained areas such as the Indo-Gangetic Plain of India and where maize is cultivated in rice paddies in Southeast Asia and Brazil. Here heavy monsoon rains or typhoons frequently leave summer crops with their roots underwater – something that is often fatal for maize. Waterlogged plants wilt as if droughted, and symptoms are exacerbated under bright sunlight. The ability to maintain chlorophyll and a short ASI and the formation of aerenchyma and brace roots under anaerobic soil conditions are adaptive responses associated with grain yield in waterlogged conditions. Gene action is mainly additive and good progress can be expected from recurrent selection (Zaidi et al. 2010b).

3.6.4 Biotic Stresses

An assessment of the major diseases and insect pests of tropical maize (Table 3.7) shows that priority diseases are maize lethal necrosis (MLN), maize streak virus (MSV), turcicum leaf blight (TLB), gray leaf spot (GLS), and ear rots in Africa; post-flowering stalk rots, downy mildew, banded leaf and sheath blight (BLSB), and *Fusarium* and *Diplodia* ear rots in Asia; and tar spot complex, TLB, and GLS in Latin America. The customary approach to breeding for resistance is to expose segregating progenies or inbred lines to each disease in "hotspots" where they occur naturally and repeatedly at a high level. Here we focus on MLN because it is relatively new to sub-Saharan Africa and seriously threatens maize production and productivity in the continent.

Maize lethal necrosis (MLN) occurs when maize is coinfected with two viruses, maize cholorotic mottle virus (MCMV) and a potyvirus, most frequently the sugarcane mosaic virus (SCMV). SCMV has been present in Africa for perhaps 50 years, but MCMV is much more recent and is more dangerous of the two (Mahuku et al. 2015). The MLN disease is spread by seed contamination and was first reported in

Table 3.7 Major tropical maize diseases and insect pests, their areas of importance, and their breeding priority (L, low; M, medium; *H*, high)

Scientific name	Common name	Area of importance in tropics[a]	Priority	Ref[b]
Diseases				
Exserohilum turcicum	Turcicum leaf blight	ST and HL globally	**H**	1,3,4
Bipolaris maydis	Maydis leaf blight	LT and ST globally	M	1,8
Puccinia sorghi	Common rust	ST and HL globally	M	1,3
Cercospora zeae-maydis	Gray leaf spot	ST of E, S. Africa	**H**	1,3,4
Sphacelotheca reiliana	Head smut	ST in hot dry areas	M	3
Phaeosphaeria maydis	*Phaeosphaeria*	ST of the Americas	M	1,3
Maize streak virus	Streak	LT, ST of SS Africa	**H**	2,3
Maize lethal necrosis	MLN	ST of East Africa	**H**	2,9
Peronosclerospora spp.	Downy mildew spp.	LT Asia and Africa	**H**	1
Phyllachora maydis and Monographella maydis	Tar spot complex	LT Central America	M	1,4
Rhizoctonia solani f. sp. *sasakii*	Banded leaf and sheath blight (BLSB)	LT, ST of South Asia	**H**	1
Fusarium moniliforme	Ear and stalk rots	LT and ST globally	**H**	1,3,4
Stenocarpella maydis	*Diplodia* ear and stalk rot	ST and cool areas	M	1,3
Aspergillus flavus	Ear rot, aflatoxins	ST and dry areas	**H**	1,4
Insects				
Spodoptera frugiperda	Fall armyworm	LT, ST in Americas	M	7,12
Diatraea saccharalis	Sugarcane borer	LT, ST in Americas	M	7,12
Ostrinia furnacalis	Asian maize borer	LT, ST of Asia	M	7,12
Chilo partellus	Spotted stem borer	LT, ST Africa, S. Asia	**H**	1
Busseola fusca	African maize stem borer	ST of S. and E. Africa	**H**	7,12
Sesamia calamistis	African pink borer	LT of Africa	M	1,12
Heliothis zea	Corn earworm	ST, HL globally	M	7,12
Sitophilus zeamais	Grain weevil	Global	M	6,10
Prostephanus truncatus	Larger grain borer (LGB)	C. America, Africa	**H**	5
Parasites				
Striga hermonthica	*Striga*	Sub-Saharan Africa	**H**	11, 13

See footnotes for sources

[a] *LT* lowland tropics, *ST* subtropical areas, *HL* highland tropics

[b] 1, Zaidi and Singh (2005); 2, Semagn et al. (2014); 3, Vivek et al. (2010); 4, Cairns et al. (2012); 5, Kumar (2002); 6, Abebe et al. (2009); 7, Ortega (1987); 8, CIMMYT (2004); 9, Mahuku et al. (2015); 10, Demissie et al. (2008); 11, Makumbi et al. (2015); 12, Mihm (1997); and 13, Ejeta and Gressel (2007)

the Rift Valley of Kenya in 2011 (Wangai et al. 2012) and since then has caused considerable losses to maize production in several countries in eastern Africa, including Kenya, Ethiopia, Rwanda, Tanzania, and Uganda. Though thrips and leaf beetles (MCMV) and aphids (SCMV and other potyviruses) are the principal vectors, infection via soil contamination, especially with MCMV in debris in the soil, also appears possible.

An early infection of MLN at the seedling stage results in necrosis and death and zero grain yield. Infection later in crop development also results in leaf death but some grain may form. Based on survey data, de Groote (2016) estimated annual yield losses to MLN in Kenya of up to 90% in infected areas and grain losses countrywide at 500,000 tons worth US $180 million. Incidence varies markedly by season and location with more than 50% of farmers affected in Western Kenya. With external donor support, CIMMYT together with KALRO established an MLN screening facility at Naivasha, Kenya, in 2013. The facility enables screening of the elite lines and hybrids from African public and private institutions under artificial infection. All older commercial cultivars are susceptible to varying degrees. Through intensive screening of >75,000 germplasm entries, CIMMYT identified sources of tolerance/resistance to MLN as well as to MCMV. Five MLN-tolerant hybrids have been released, and seed is being scaled-up or commercialized by seed companies in Kenya, Tanzania, and Uganda. A further 22 MLN-tolerant/MLN-resistant hybrids were tested in National Performance Trials (NPTs) in East Africa in 2016 (Prasanna 2016). CIMMYT has also made significant progress in identification, validation, and deployment of molecular markers for resistance to MLN, though genetic studies have shown that resistance to MLN is controlled by multiple loci with relatively small effects (Gowda et al. 2015). Pyramiding sources of MLN resistance using molecular markers to minimize linkage drag is a high priority, and around 50 widely used CIMMYT lines are presently being converted to MLN-tolerant versions using marker-assisted backcrossing. The current outbreak of MLN may cost 5 years of genetic progress for yield and other traits, while sources of MLN tolerance/resistance are identified and introgressed – a price worth paying to avoid a pandemic.

CIMMYT is presently focusing on developing breeder-ready markers for improving specific disease resistance traits. A recent example is MSV, a major disease affecting maize productivity in several African countries. Nair et al. (2015) fine-mapped, identified, and validated a set of SNP markers for a major QTL for MSV resistance (*msv1*). The validated MSV resistance haplotype is now used in forward breeding imparting MSV resistance to elite inbred lines with tolerance to drought, heat, or MLN and for deriving improved biotic and abiotic stress-tolerant lines. Production of SNP markers is also currently being developed by CIMMYT for resistance to other key diseases, especially TSC, TLB, and GLS and for improved nutritional characteristics.

Insects Maize insect pests are ubiquitous and cause considerable losses. The most serious (Table 3.7) are the stem borers of Asia and Africa that often consume foliage and damage ears as well as cause extensive stalk breakage. The larger grain borer of Central America and more recently of Africa continues to cause extensive loss to inadequately stored grain. Since natural infection is spatially variable, the infestation with artificially reared larvae of the target insect is an important step to developing host plant resistance (see Mihm 1997). While there is genetic variation in resistance to all of these insects, there is no naturally occurring immunity. Commercial maize transgenic events have been successfully deployed to control lepidopteran pests in maize grown in South Africa, Argentina, Paraguay, Brazil, and the Philippines. There is, however, no equivalent transgene that can effectively control storage insects.

Striga The parasitic weed *Striga hermonthica* represents a special type of biotic stress. It produces about 40,000 tiny seeds per plant, and each year a fraction germinate and infect roots of hosts such as maize and sorghum. Symptoms of infection include stunting, wilting, loss of chlorophyll, and in some cases complete loss of developing ears, especially where soil *N* levels are low. It affects much of the savanna zone of West Africa and the middle altitudes of East Africa (Ejita and Gressel 2007). No immune sources of maize have been identified. Lane et al. (1997) reported that some plants in a wild progenitor of maize, *Zea diploperennis*, restricted parasite penetration of its roots and impaired the development and survival of *Striga*. IITA scientists developed *Striga*-tolerant inbred lines and hybrids using this natural genetic resistance from teosinte (Amusan et al. 2008) and have deployed it through commercial varieties that combine drought and *Striga* tolerance in West Africa (Badu-Apraku 2010; Badu-Apraku and Fakorede 2013). The deployment of imidazolinone-resistant maize hybrids whose seed is coated with imazapyr herbicide prior to planting provides good protection from *Striga* (Kanampiu et al. 2007). This naturally occurring gene has been incorporated into commercial varieties/hybrids in East Africa (Makumbi et al. 2015).

3.6.5 Grain Nutritional Quality

3.6.5.1 Provitamin A-Enriched Maize

Several countries in sub-Saharan Africa and Central America grow white maize, a consumption of which could result in a relatively high level of vitamin A deficiency, especially among those who treat it as a staple food. Also, most yellow maize grown and consumed throughout the world has only 2 µg/g or less of provitamin A carotenoids. CIMMYT has been successful in developing an array of provitamin A-enriched maize germplasm. Under the HarvestPlus-Maize Program, where the primary target is improving provitamin A concentration in the endosperm, considerable progress has been achieved to date at CIMMYT and IITA on developing provitamin A-enriched maize germplasm, in active collaboration with several institutions/universities worldwide (Prasanna et al. 2014). The first-generation provitamin A-enriched hybrids developed by CIMMYT have about 6–9 µg/g of provitamin A; three of such hybrids have been released in 2012 in Zambia. Eight second-generation provitamin A-enriched maize hybrids (with >10 μg/g of provitamin A) have been released in 2015 in Zambia, Zimbabwe, and Nigeria. A clinical trial conducted on 140 children in Zambia demonstrated that high provitamin A (17–24 μg/g) orange maize grain was as effective as vitamin A supplementation in correcting deficiency (Gannon et al. 2014).

3.6.6 Trait × Management Interactions

The history of temperate maize improvement is characterized by positive trait × crop management interactions that have led to steady increases in productivity at the farm level (Fischer et al. 2014). Increasing tolerance to high plant densities was accompanied by a doubling of planting density over the past 70 years in the USA and a concomitant rise in the optimum density for grain yield. Improved drought tolerance in temperate maize hybrids allowed them to withstand drought far better in the 2012 US drought than during a drought of comparable severity in 1988 (Boyer et al. 2013). Similar interactions have been exploited in tropical maize, but to a lesser degree. Excellent progress has been made in improving drought tolerance in tropical germplasm (Edmeades et al. 1999; Bänziger et al. 2006; Semagn et al. 2014). More than 230 drought-tolerant varieties and hybrids have been released in sub-Saharan Africa over the last 10 years and in 2016 are being grown by more than five million farm families on more than two million hectares. These new cultivars have stabilized and increased yields in some countries, such as Ethiopia (Abate et al. 2015). However, increased tolerance to high plant densities has not been fully exploited in tropical germplasm to date since most cultivars are being developed and evaluated under densities of 40–70,000 plants/ha – a density that has not changed in the last 40 years. A notable exception is in the northwest of Mexico where maize is planted up to 120,000 plants/ha in irrigated areas. While lower densities can be justified because intercropping is practiced in some maize fields in the tropical world, plant density should be considered a selection tool for increasing abiotic stress tolerance and improving resistance to barrenness and lodging.

Harvest index remains stubbornly low in tropical hybrids at around 0.42 (Zaidi et al. 2003a). In some areas such as the Indian subcontinent and East Africa, the value of stover may approach that of grain, and for these areas, a lower HI may result in better retention of nutrients in stover, thereby increasing its market value. There is considerable genetic variation for stover quality and production in modern tropical genotypes, and it is possible to combine high grain yield with excellent in vitro digestibility of stover (Zaidi et al. 2013). However, low HI may also reflect reduced sink strength by the ear, which may be associated with reduced biomass production. There is still considerable room for improvement in HI in tropical maize germplasm.

3.7 Field Trials and Phenotyping

3.7.1 Multilocation Testing vs. Managed Stress Environments

Multilocation testing (MLT) of progenies and especially of advanced selections has a proven track record in maize, as attested by the steady improvements in yield and in stress tolerance in temperate germplasm developed using this testing method (Duvick 2005; Cooper et al. 2014). Because successful varieties and hybrids rely on an integrated array of traits giving rise to stable and high yield, MLTs will continue to play a critically important role in their identification. Evaluation in a randomly selected subsample of the target population of environments (TPEs) has gradually increased allele frequencies for stress tolerance and identified a number of genotypes with tolerance to drought (Campos et al. 2006), high density, and unidentified stresses that have led to increased plant-to-plant uniformity (Edmeades 2012). TPEs within megaenvironments defined by G × E interaction (GEI) patterns are often identified by geography (e.g., Löffler et al. 2005; Cairns et al. 2012), but can also be identified by yield level within a geography. Weber et al. (2012) and Windhausen et al. (2012) noted that GEI for yield could be reduced by subdividing test sites in eastern and southern Africa into low- (<3 t/ha) vs. high-yielding environments, rather than by dividing them geographically into eastern vs. southern Africa. Data from test sites do not need to be treated equally – the incidence of known stresses at specific sites within the MLT system can be used to weight results from those locations more heavily than those from other test sites (Löffler et al. 2005).

The success of the MLT approach is unchallenged, but the use of randomly selected test locations is a costly approach, in part because common stresses such as drought are spatially variable and stochastic in nature. The use of well-characterized managed stress environments (MSEs) for traits such as drought allows the stress intensity and timing to be repeatable from year to year and easily measured. In addition, the development of MSEs for acid soils, low *N*, and *Striga* increases the spatial uniformity within screening trials. For example, most tropical maize breeders now opt for a proportion of MSEs that represent the intensity and timing of an important type of drought stress in that TPE. These are usually rain-free, irrigated locations that allow stringent control of the nature, timing, and intensity of water stress. Low *N* MSEs where the soil *N* level is reduced by cutting and removal of crop residues now play an important part in regional testing networks in sub-Saharan Africa and South Asia. It is important that MSEs maintain a significant positive genetic correlation for yield with the TPE (Cooper et al. 2014), something that low *N* sites used during the normal crop season in Africa that appear to do better than drought screens conducted in the dry season (Weber et al. 2012).

Theory for assessing gains in the target environment from MSEs conducted in a winter dry environment or in a managed "hotspot" indicates that gains are greatest when genetic variance σ_G^2, heritability in the MSE, and the genetic correlation between the MSE and the TPE are all high. Managed stress normally ensures that heritability and genetic variance for the trait are maximized, and data from MSEs can be weighted more heavily during selection than data from randomly stressed sites. Comparisons of a drought MSE vs. the TPE for a tropical population (Byrne et al. 1995) suggest that about 80% of gains in yield under drought observed in the MSE were also observed in the TPE. The combination of screening in MSEs with testing via MLTs in the target environment provides an important insurance against bias arising from excessive dependence on MSEs that are consistently conducted out of season. The judicious use of this combination of test sites has reduced testing costs and resulted in improved performance under drought (Bänziger et al. 2006).

3.7.2 Designs and Plot Management

As stress levels rise in field experiments, underlying spatial variability in soil texture and depth that affects plant-available water or *N* becomes apparent. This produces visible spatial variation in stress symptoms and a pattern of correlations among plot residuals. During screening, numbers of genotypes under test often range from 150 to 1000, so block sizes (replications) are large. Obvious trends in soil texture, historical fertility, and weed populations should be anticipated when trials are laid out. Replications properly located across such gradients can partially remove the effects of trends during analysis. While there is no complete substitute for soil of uniform depth and texture or uniform incidence of the pest of interest, row-column designs and suitable incomplete block designs (e.g., alpha (0,1) lattice designs) help to block and account for variation that occurs within large replications (Barker et al. 2005; Brown et al. 2014; Cooper et al. 2014). Bordering requirements when testing genotypes of different height also imply that single-row plots should be avoided, though seed and land supplies often dictate that the larger numbers of entries that can be screened in single-row plots can result in an overall increase in gain. Smaller plots may also result in less exposure of replicates to large-scale soil variation and therefore increase the precision of experiments conducted under severe stress (Bänziger et al. 1995).

A good plot practice is essential so that heritabilities are maximized (Bänziger et al. 2000). Great care should be exercised when planting trials under abiotic stress to avoid missing hills and plots, since the absence of complete competition increases the availability of radiation, water, and *N* to adjacent plots as well. For the same reasons, bordering increases in importance as stress levels rise, and end plants near wide alleys should be discarded from each plot because increased access to inputs may render them virtually unstressed. Mechanization of planting, side-dressing, and harvesting generally reduces error and contributes to increased heritabilities, while allowing a significant expansion in the numbers of genotypes than can be screened or tested. Mechanical shelling in the field also allows indirect pressure for increased shelling percentage as a component of increased HI. When screening for drought, it is important to stratify entries by flowering date where possible, since water stress increases with time at this sensitive growth stage, and differentially penalizes later flowering entries. Heritabilities are higher in trials of homozygous vs. segregating genotypes, but inbred lines are more difficult to manage (Bolaños and Edmeades 1996). Finally, the severity of stress imposed is important. If grain yields fall to <15% of unstressed levels, the heritability for yield falls and secondary traits become erratic. On the other hand, if stress levels are insufficient, then the genetic correlations between stress and unstressed are high, and little new information is obtained. In general, the target yield reduction should be around 50% of potential, and two stress levels are often used to bracket this goal and ensure that the appropriate level of stress is obtained in at least one of them. It is always wise to include an unstressed repetition of the trial so that changes in yield potential can be monitored during selection. The use of drip irrigation techniques and the judicious use of N additions or lime can be used to modify the severity and uniformity of specific stresses more accurately (Bänziger et al. 2000). A very effective tool to enhance the quality of data gathered under drought or any other source of stress is the use of mixed models to analyze data, since they represent a cost-effective way to reduce the impact of soil and experimental heterogeneity on field data and to increase the genetic gain to environmental noise ratio, in other words heritability, and therefore expected genetic gains.

3.7.3 High-Throughput Phenotyping

Advances in genotyping have led to dramatic reductions in cost per data point, but this has not been matched by a corresponding decline in the cost of phenotypic data until recently when remote sensing of traits has been deployed. The traditional emphasis on collection of quality data from field trials remains as important as ever for traits such as grain yield and yield components, dates of 50% silking and anthesis, biomass, plant height, staygreen and leaf rolling scores, and disease and insect scores. To reduce error rate from transcription errors, plots should be identified by weather proof bar-coded tags, and data should be collected directly on a handheld tablet that reads the bar code to identify the plot. This electronic data record should allow for notes and comments against plot numbers as well as numerical data and scores, but if not then these should be recorded in a field book that includes maps, instructions, randomizations, etc.

Remote-sensed data is increasingly important when phenotyping, is nondestructive, and can be repeated as often as necessary. In its simplest form, it involves the use of handheld sensors such as infrared thermometers, digital cameras, and GreenSeeker® sensors (Cairns et al. 2012). In recent years, there has been a sharp increase in the use of unmanned aerial vehicles such as tethered balloons, regular aircraft, and recently miniature helicopter and fixed-wing drones fitted with multispectral cameras (Araus and Cairns 2013). These new methods of hyperspectral analyses are both fast and cheap to use and take readings over a short interval under stable atmospheric and crop conditions (Zaman-Allah et al. 2015), and repeated passes 5–15 days apart provide an understanding of how traits change over time.

Remote sensing methods can be divided into those depending on spectral reflectance (e.g., SPAD, NDVI), digital imagery (e.g., leaf color and leaf area), and thermal imagery (e.g., canopy temperature) (Masuka et al. 2012; Zaman-Allah et al. 2015; Vergara-Diaz et al. 2016). Good relationships between grain yield, biomass, and NDVI have been reported for maize (Lu et al. 2011; Cairns et al. 2012; Trachsel et al. 2016). Other traits include ear imagery to estimate yield components and silk number per ear and measurements of plant height and flowering date. The rapid rise in the number, utility, and cost-effectiveness of drones equipped with standard digital cameras will certainly multiply options for using red/green/blue (RGB) wavelength ratios in the near future (e.g., Vergara-Diaz et al. 2016). Although the rate of data collection is very high using remote sensing, images are typically data dense. The processing of these in real time is computationally demanding but is needed to generate a representative value that differentiates among genotypes and can be used for selection. There is an extensive effort underway to determine what additional vegetation indices can be obtained from remote sensing of segregating progenies in small plots and what these indices can contribute to selection. Near-infrared (NIR) analysis can also be used to detect concentrations of specific metabolites in tissues, in some cases nondestructively (Araus and Cairns 2013).

3.7.4 Data Management

Data processing can become a bottleneck in breeding. For many maize breeders from smaller institutions, the easiest software to access is the Breeding Management System (BMS), formerly known as the Integrated Breeding Platform (https://www.integratedbreeding.net/breeding-management-system). The IBP was developed by the Generation Challenge Program of the CGIAR for a diverse array of tropical crops. It has now evolved into the BMS supported by the Bill and Melinda Gates Foundation as a stand-alone software suite that currently can be downloaded free of charge. BMS is automatically linked to the GENSTAT® suite of analysis programs and has dedicated cloud computing or stand-alone options for database management. BMS is being adopted by a number of leading public sector breeding programs. The suite of programs comprising this package is well suited to MLT data analysis and MAS as well. The BMS offers to many national breeders the real prospect that MAS (including marker-assisted backcrossing) can be implemented in real time in NARS' breeding programs. It also includes information on how to outsource genotyping, something that makes real-time MAS possible for many programs.

It is important to emphasize the importance of database management and archival of annotated results in a machine-readable and easily accessible form in maize breeding programs that are committed to long-term crop improvement. Such databases facilitate any study of genetic gain vs. time, allow head-to-head comparisons of hybrids over time and space, and allow meta-analysis of large, diverse, and often unbalanced datasets.

3.8 Applied Biotechnology

The broad goals of biotechnology in crop improvement relate to discovery and location of new and useful genetic variation and to an accelerated rate of genetic gain. For a general overview of DNA technologies and molecular marker types, see Brown et al. (2014).

3.8.1 Association Mapping

A common means of gene discovery is through genome-wide association study (GWAS). GWAS or association mapping through analysis of linkage disequilibrium is a powerful tool for dissecting complex traits and identification of potential favorable alleles that can contribute to the enhancement of target traits. Association mapping can establish marker-trait associations in panels of inbreds. Typically, it is applied to association mapping panels that consist of several hundred diverse homozygous lines. Random association between alleles is reduced by genetic linkage, creating disequilibrium. Heavily selected temperate inbreds show a high level of LD (many alleles linked in blocks of ~100 kb and moving as a unit during selection), whereas tropical maize shows a rapid decay of LD (2–5 kb) because of its long history of recombination as OPVs and relatively recent history of selection. Lines are generally genotyped with several thousand SNP markers, though increasingly >500,000 polymorphic SNPs are being generated through genotyping-by-sequencing methods and utilized in association and selection studies. GWAS generally has low statistical power for associating rare alleles with phenotypic differences (Yan et al. 2011). The outcome from GWAS is a series of precisely defined genomic regions associated with the trait of interest that can be linked to candidate genes identified through resequencing those regions. This can be used to develop simple PCR gene-based markers for marker-assisted selection (MAS). While GWAS studies have been useful at identifying regions of interest across diverse genetic backgrounds, outcomes of these studies have produced relatively few useful candidate genes or regions for subsequent use in MAS. Bernardo (2008) observed that GWAS involving poorly adapted genotypes has provided relatively little useful information for breeders. This is in part because of false positives arising from existing relationships among lines in the study and because of the very considerable challenges of accurately phenotyping a diverse set of lines in any single environment. Yan et al. (2011) noted that increasing the numbers of genotypes has a much greater effect on the efficiency of GWAS than increases in marker density. GWAS leads are usually validated by analysis of biparental mapping populations.

3.8.2 Accelerating Genetic Gain Through Marker-Assisted Selection

Gene-phenotype associations form the basis of MAS. These are normally established through careful phenotyping and genotyping with molecular markers of a segregating $F_{2:3}$ population or a set of recombinant inbred lines (RILs) from a biparental cross. Relatively few QTLs have been used in MAS, in part because their phenotypic effects are dependent on the genetic background of the lines in the study and may interact with the environment (e.g., Jiang et al. 1999). Furthermore, some are associated with minor genetic effects, and the cost of the technology exceeds the benefits from added genetic gain. A problem occasionally still arises from false positives because too few genotypes were involved in the study (Beavis 1994). A further logistical issue has been a failure in some programs to have genotypic data available at the time selection decisions are made. If selection decisions are delayed by one crop cycle, most of the benefits in MAS are lost (Bernardo 2008).

Despite these challenges, a number of tropical maize programs such as CIMMYT's are using production SNP markers to ensure that specific regions associated with disease resistance or grain quality are present in selected lines, as previously noted (Prasanna et al. 2014). Marker-assisted backcrossing (MABC) is also widely used in the transfer of these traits and is routine in commercial companies in the transfer of key QTL and transgenes to elite inbred lines. QTLs are increasingly being identified that function in a range of genetic backgrounds. Recently a meta-QTL analysis across three tropical maize biparental populations (RILs) showed six constitutive genomic regions associated with drought tolerance (Almeida et al. 2014) and identified an 8 Mb region delimited in 3.06 that harbored most of the morphophysiological traits associated with improved performance under drought.

Marker-assisted recurrent selection (MARS) has been used on a wider scale to accelerate breeding (see Sect. 7.5) in large commercial breeding programs in the USA (Crosbie et al. 2006; Edgerton 2009). In brief, MARS functions by establishing gene-phenotype associations among F_3 progeny topcrosses of a biparental cross and uses these to guide selections for three additional selection cycles. It is a scheme that is heavy on phenotyping and functions by identifying those QTLs with significant effects on the trait of interest. Although gains from MARS can be double those of conventional selection (Eathington et al. 2007; Beyene et al. 2015, 2016), in practice it has been a resource-intensive process involving progenies of biparental, but in some cases multiparental, crosses, which in retrospect, were not always the best choices. For these reasons, and the development of genotyping-by-sequencing methods producing up to a million SNP-based markers, MARS has been largely subsumed into genomic selection (GS) (Chap. 2).

The comparative effectiveness of MARS and GS was tested by CIMMYT in a study of gains under drought in East Africa. Gains were evaluated from MARS in ten biparental tropical crosses, using 148–184 F2:3 progenies crossed to a single tester. Lines were genotyped with 190–225 SNP markers, and a selection index based on phenotypic and marker data was applied in order to select families for recombination in C0. In each successive cycle, the selection index was applied to plants that had been genotyped with 55–87 SNPs (Beyene et al. 2015, 2016). Gains under optimal conditions were 93 kg/ha/year and under water stress were 46 kg/ha/year, vs. 50 and 15 kg/ha/year, respectively, from conventional selection under similar conditions (Semagn et al. 2014). A comparison between conventional selection and GS was undertaken in 13 biparental populations using 191–326 SNPs (i.e., relatively few for GS), and the best 10% based on GEBVs were intermated. Overall genetic gain from GS under drought was 52 kg/ha/year vs. 16 kg/ha/year using conventional selection. MARS and GS therefore appear able to increase genetic gain by a factor of two to three times that of conventional selection under drought stress.

Building on its effectiveness in temperate maize breeding, GS would also become a key component of the toolbox tropical maize breeders have at their disposal. Its potential would be fully exploited when, GS is also used to predict parental combinations and therefore to increase heterosis.

3.8.3 Transgenics

Transgenic technologies have received tremendous attention by the commercial seed sector since the first transgenic hybrid became commercial in 1996. The majority are herbicide-resistant (mainly glyphosate (RoundUp Ready®, RR)) or insect-resistant (*Bt*) cultivars. Recently, a transgenic event encoding a cold shock protein from the bacteria *Bacillus subtilis* and providing drought tolerance in maize, MON87460, has been approved for release in South Africa, and breeders are seeking approval for its release in Kenya and Uganda. By 2015 transgenic cultivars of all crops were planted on 180 M ha annually, of which 53% were in developing countries and 29% were maize – mainly Brazil, Argentina, Paraguay, South Africa, Uruguay, Bolivia, and the Philippines (James 2015). The adoption of transgenic varieties has been a sound investment for the vast majority of farmers, with farmers in developing countries receiving $4.22 for every dollar they invested in transgenic seed in 2013 (Brookes and Barfoot 2015). The release of transgenic crops is subject to extensive regulation, and they cannot be tested or grown without a legal and functional regulatory system in place. Transgenic maize imported from producing countries to the developed world is also subject to stringent safety testing. These are indications of public wariness of this technology and concerns that it is owned by a few large multinational seed companies. Yet 20 years after the launch of the first commercial Bt hybrids, there have been no validated cases of health-related problems among animals and humans.

A major challenge for breeders is stewardship of the transgene to ensure that it remains in its designated genetic background. This means that transgenic and conventional germplasm must be separated during seed processing and preparation and especially in the field where stray pollen can lead to adventitious presence of the transgene in conventional grain. A further issue with *Bt* genes is preventing the buildup of resistance to the *Bt* toxin in local lepidopteran insect populations, and this requires the planting of 20% of the cropped area to non-*Bt* hybrids. Enforcement of this refugia requirement is essential but challenging.

One example of public breeder access to transgenes is provided through the Gates Foundation-funded project Water Efficient Maize for Africa (WEMA). Here Monsanto's drought tolerance transgenic event MON87460 (Castiglioni et al. 2008) and their widely used *Bt* gene MON810 are available for royalty-free use under license by local maize seed companies in Kenya, Uganda, Tanzania, and Mozambique, once the transgenes have been deregulated in those countries. In South Africa where the transgenic event conferring tolerance to some lepidopteran insects MON810 was released 18 years ago, target insects have developed a significant level of tolerance to this *Bt* toxin (van Rensberg 2007) necessitating its replacement by the MON89034 transgenic event. CIMMYT is also involved in the extensive field testing of conventionally improved drought-tolerant hybrids, since transgenic and conventional improvements are thought to be additive in effectiveness. WEMA is being executed by the African Agricultural Technology Foundation (AATF) based in Nairobi, Kenya. It can be expected that MON87460 and MON810 will be detected in local maize varieties quite rapidly after their open release, simply because of gene flow via pollen. This may have implications for transboundary movement of produce and seed to countries unwilling to import genetically modified maize. Nonetheless, the prospect of future use of these transgenes in sub-Saharan Africa is a significant and exciting development.

3.9 Seed Production and Marketing

Plant breeding is never an end by itself: its return on investment occurs in farmers' fields. Too often maize breeders have declared "mission accomplished" at product release and have failed to engage in ensuring that seed of their improved varieties reaches intended users. This process starts with insisting that hybrids and varieties are tested in farmers' fields during development so that possible reasons for non-adoption are recognized before a variety is released. The second step, well beyond breeders' control, is the development of the seed industry needed to deliver to end users the efforts of plant breeders.

A national seed industry typically passes through various stages of maturation. Government-sponsored seed production usually gives way to many small start-up seed companies that use publicly available germplasm from CIMMYT, IITA, or NARS and a few multinationals who often do not breed in-country but import seed for testing. Competition narrows the field to a few successful national companies and multinationals that may purchase smaller companies to increase sales volume or enter into research agreements with them to access germplasm and technologies. Sub-Saharan Africa is currently characterized by many small start-up seed companies that struggle to maintain sales volumes and seed quality and cannot afford an agronomist/breeder on staff or a large demonstration program for new products (MacRobert 2009; Langyintuo et al. 2010).

Although the hybrid seed business has been important in some countries like Brazil and Argentina since the 1940s, only in the early 1970s did it begin to spread among other Latin American countries. Pioneer Hi-Bred Int. established its first non-USA breeding station in Jamaica in 1964. In tropical Southeast Asia, the seed business became more competitive in the early/mid-1980s when several large seed companies established hybrid breeding research. Counting on Suwan 1 as a good source of adaptation, three-way hybrids and then single crosses started to compete with this excellent OPV and occupy significant area, particularly in Thailand, Indonesia, and the Philippines. The single-cross hybrid DK888 was released by DeKalb in Thailand in 1993 and became a widely planted cultivar for several decades. The spread of hybrid seed businesses in Thailand, the Philippines, and Indonesia sparked a similar response in India, Vietnam, Myanmar, and other Asian countries. Today, the hybrid maize seed business in these countries constitutes an area of rapid growth.

3.9.1 Product Types

In tropical areas, particularly in marginal production environments or where few inputs are used and agronomy is poor, the choice of OPVs vs. hybrids is still strongly debated. The analysis hinges around relative seed prices vs. yield differences, since OPV seed price may be that of commercial grain if farmers retain their own seed. Pixley (2006) concluded that the best hybrids in southern Africa outyielded the best OPVs by around 18% across a range of yield levels and that hybrids deliver greater value to farmers than OPVs when farmer yield is consistently over 2 t/ha. This figure is a guide only and depends on prices of grain and seed, the yield advantage of hybrids over OPVs under stress, tolerance of farmers to risk, and the availability of quality hybrid seed. Nonetheless improved OPVs are still the most reliable option for farmers that cannot reach the 2 t/ha yield level, while for hybrids their advantage became clear at yields >3 t/ha. Pixley's analysis showed that recycling hybrid seed (sowing F_2 seed) was least profitable at all yield levels because it yielded an average of 32% less than F_1 seed. Unfortunately the practice of recycling hybrid seed can reach 50% in drought-prone areas of East Africa (de Groote, 2013, personal communication). However, a viable seed industry depends ultimately on the annual sales of hybrid seed, and the use of hybrid seed has been shown to contribute to increased farmer welfare (Mathenge et al. 2014). Furthermore, the rate of turnover of varieties is likely to be greater when hybrid seed is grown, and this brings benefits to seed companies and farmers alike (Gaffney et al. 2016). Most successful companies in the tropics market a small amount of improved OPV seed as a service and make their main income from hybrids. There is room for both products, and a mature seed industry operating in a risky production environment like sub-Saharan Africa should be encouraged to focus on both.

What sort of hybrid is most appropriate? The hybrid seed industry began with double crosses (i.e., a cross between two single crosses) but noticed that three-way crosses were around 6% and single crosses around 19% higher yielding (Pixley 2006). There are benefits in three-way crosses. The single-cross seed parent is high yielding and stress tolerant, so costs per unit of seed are low, and the male inbred pollinator can be destroyed after flowering, thus making theft of inbred lines difficult. However, the increasing yields possible on inbreds designated as females, improved security based on DNA fingerprinting, and increasing skills in the seed sector make single crosses increasingly attractive. There is little doubt that single crosses will ultimately replace three-way crosses in all but the toughest seed production environments, provided the cost of seed of single crosses can remain within reach of resource-poor farmers.

3.10 Future Prospects and Outlook

The future of tropical maize breeding looks very promising, though the challenges of the changing physical environment will be a major headwind against future yield increases (Thornton et al. 2011). Important for increasing and stabilizing maize yields in the tropics will be traits related to tolerance of high temperature and drought, along with horizontal resistance to changes in virulence of pests as temperatures rise. Many of the developments that will lead to increased and stable yields may occur in the emerging private sector, and in temperate areas private sector investments have driven a steady increase in maize yields over the past 25 years that significantly exceeds that of rice and wheat (Fischer et al. 2014). There continues to be a need for balance in terms of the roles of public sector international research-for-development efforts on maize for smallholders in low-yielding environments with that of private sector technological edge for increasing and stabilizing yields of tropical maize.

The following appear to be major opportunities in tropical maize:

- Density tolerance is needed. The key developments of increased tolerance to high plant density and improved stalk strength in temperate maize (Fischer et al. 2014) have yet to occur in the majority of tropical germplasm. A systematic planting of all experimental plots at 30–50% higher density than farmer's fields would be a good starting point, even if lodging and barrenness are occasionally severe. Tollenaar and Lee (2011) also conclude that increased stress tolerance is the key to further gains in yield and yield stability in temperate maize.
- Adoption and rate of turnover of tropical varieties need to increase. The rapid replacement of existing varieties with new stress-tolerant hybrids is perhaps the best way of ensuring that genetic changes in varieties can keep up with climate change (G. Atlin, 2014, personal communication).
- Yield potential needs to increase. This can be from obvious changes in partitioning such as reductions in tassel size and leafiness and increases in HI and shelling percentage. The size of individual ears and yield per plant at low densities have changed little with selection in temperate maize (Duvick 2005; Egli 2015), so

increased yields have come from a steady rise in ears per unit area. However, increases in kernel number per ear in tropical maize growing in optimal environments could come from increased emphasis on synchrony of pollination and kernel development (Cárcova and Otegui 2007). Yield potential increases might also arise from longer-term changes in key enzymes such as rubisco (Parry et al. 2003) and its activator, rubisco activase (Salvucci et al. 2008) whose temperature responses and kinetics may well be altered through gene editing procedures in the future. Such changes would enhance fitness in hot dry environments that are predicted to become the norm.

- Phenotyping is growing in importance and is an essential component of future breeding activities. Tropical soils are generally more variable than temperate soils, so physical and statistical techniques that can be used to minimize these effects should always be used (Barker et al. 2005). The basics of uniform stands and adequate bordering are still being neglected in some tropical breeding programs, and continued investments in high-quality field research facilities, mechanization to plant and shell, and managed stress environments are needed in increasing numbers. Remote sensing for estimating traits that will increase the heritability of a selection index should be energetically explored and embraced (Lu et al. 2011; Cairns et al. 2012; Araus et al. 2012; Araus and Cairns 2013; Zaman-Allah et al. 2015; Vergara-Diaz et al. 2016). The routine use of remote-sensed traits such as plant height is sharply reducing labor requirements in the field, and anthesis and perhaps silking date observations are currently under evaluation.
- Molecular breeding will play an increasingly important part in tropical maize improvement. The most promising emerging technologies today are genomic selection linked with doubled haploid production. The availability of doubled haploid facilities, high-density genotyping as well as high-throughput and low-cost genotyping capacities, marker resources, and analytical capability through the offices of CIMMYT and the Breeding Management System put these methods increasingly within reach of medium-sized tropical maize breeding programs. Transgenics will have an increased role as trust in the technology increases (Edgerton 2009), and there is ample scope in the tropics for more extensive use of herbicide and insect resistance technologies, as well as those relating to disease and abiotic stress tolerance. Gene editing will undoubtedly have significant impact, though this may be 5–10 years away in tropical maize.
- The selection cycle will continue to shorten. Doubled haploids are clearly here to stay. Prediction of performance will help narrow the numbers of genotypes evaluated in the field. Genomic selection and the use of production markers that identify specific haplotypes and provide a framework for prediction shorten the breeding cycle by reducing phenotyping needs for several generations. In fact, genotypes can be assessed from seed chips of doubled haploids and known susceptible segregants can be eliminated before a seed is sown.
- Identification of new and useful genetic variability within the species is a likely outcome of investments in screening maize landraces in initiatives such as the

Seeds of Discovery (SeeD) project, and these will likely find future uses as defensive traits (Tester and Langridge 2010; Prasanna 2013).

- Tools for data management and information extraction in real time will become increasingly necessary as both phenotyping and genotyping move into the millions of data points per genotype. There will be a growing need for efficient algorithms that reduce this *tsunami* of data to an index used to rank genotypes. The development and use of a mixed model framework for the analysis of multilocation and managed stress datasets will allow information to be mined from older as well as current datasets (Cooper et al. 2014).
- Training of a new generation of field-oriented breeders is essential. Firsthand knowledge of germplasm, environments, and their interactions remains at the heart of successful tropical maize breeding despite the sometimes cosmetic appeal of new technologies and tools. There is no substitute for trained staff observing genotype and consumer reactions in the field, especially in farmers' fields. The current shortage of young graduates in field plant breeding, particularly in tropical crops such as maize, represents a significant threat that must be addressed and overcome in order to maintain, and ideally increase, the current rates of genetic gain in this fascinating crop.

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