Chapter 2
Phenotyping in Wheat Breeding

Govindan Velu and Ravi Prakash Singh

Abstract Approximately 25 % of global agricultural land is utilized for wheat cultivation, making wheat the largest food crop worldwide in terms of area. Wheat is the second most produced cereal crop after Maize with more than 650 million tons produced every year. Wheat productivity is increasing at less than 1 percent annually, while the annual productivity must increase at 2 % annually to meet the global demand. The potential of increasing arable land is limited; hence future increases in wheat production must be achieved by enhancing the productivity per unit area. Increasing grain yield, yield stability, resistance/tolerance to biotic and abiotic stresses, and end-use quality characteristics are among the most important wheat breeding goals.

The Green Revolution wheat varieties performed well in terms of responsiveness to fertilizer application and water-use efficiency. But now there is not a lot more water to spare, and fertilizer usage in some places has already passed saturation point, so a new Green Revolution will have to make even more efficient use of existing resources. Efficient phenotyping techniques are essential to develop new wheat varieties with higher yield potential, tolerate high temperatures and improved water-use efficiency or drought tolerance due to climate change and the dwindling supply of irrigation water. This book chapter describes various phenotyping techniques being used in national and international wheat breeding programs.

Keywords Wheat • Phenotyping • Grain yield potential • Biotic and abiotic stress tolerance • End-use quality • Biofortification

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2.1 Introduction

Global food demand is expected to be doubled by 2050, while production environments and natural resources are continuously shrinking and deteriorating (Barrett 2010). Sustainable food security in a world with a growing population and changing diets is a major challenge under climate change. Although estimates of food insecurity vary, the number of undernourished people already exceeds 1 billion and feeding this many people will require more than incremental changes (Fedoroff et al. 2010). Food production may need to increase by as much as 70 % by 2050 when the global population may likely reach 9 billion people (World Bank 2008). Food security depends not only on gross production of staples, but also on ability to provide income for farmers in developing countries, a diverse and balanced food basket, and the socio-economic factors that determine whether poor people, particularly women, are able to purchase, store, prepare and consume sufficient food.

The next 40 years will also have to deal with the potentially profound damage to farming from climate change, which in some parts of the world could reduce yields by one third. And disturbingly, for the first time since the Green Revolution, crop yields are growing more slowly than population. Between 1961 and 1990 wheat yields were rising at nearly 3 % a year (FAO 2008). During that period the world's population was growing by an average of 1.8 % per year. Between 1990 and 2007 population growth slowed down to 1.4 %, but the rise in annual wheat yields slackened to 0.5 % (Fig. 2.1). To be more precise, growth in population and demand for food have both slowed down, but crop yields have slowed at a higher rate.

Looking into the future, global wheat requirements are expected to increase from the current 685 million tons from 225 million hectares in 2009 with an average productivity of 3.04 t ha^{-1} (FAO 2011) to about 900 million tons in the year 2020 with an average productivity of 4 t ha^{-1} to meet demand (Ortiz et al. 2007). Thus, wheat not only has a key role to play in current food security, but also in future global food security and poverty reduction. Challenges for wheat breeders include increasing productivity, especially for agro-climatically marginal areas, and defending past yield gains against diseases and pests, of which rust may well be the most

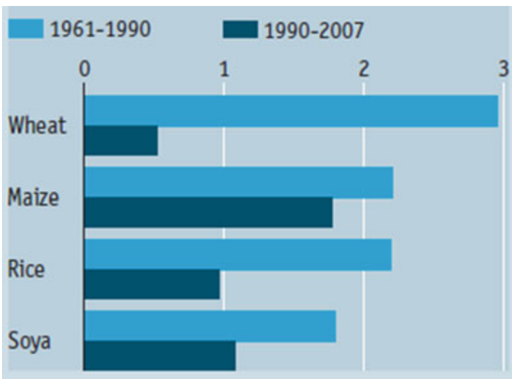

Fig. 2.1 Global annual yield growth of major staples (adapted from FAO, 2008)

important. In addition, water and nutrient use efficiency could increase productivity and profitability of resource poor farmers.

Phenotypic plant breeding will always be an important practice. This belief is due to: (1) it is progressively less expensive than marker assisted selection and marker assisted breeding, (2) visual selection for some traits remains a remarkably effective way of handling massive populations and experimental lines that are generated in a breeding program, (3) our understanding of the genome, while constantly increasing, may not fully explain the complexity of the phenotype. Complete QTL interactions will be difficult to identify, and hence more difficult to breed for successfully (Dudley 2008). Most importantly, all of the marker data, however obtained or used, must be associated with carefully measured phenotypes to establish the value of the marker(s), and (4) the environment in which we grow plants may change quite often, especially for biotic stresses. The first indication of these changes is the phenotype, hence plant breeders must always pay attention to the phenotype (e.g. the rise of Ug99 [TTKS] stem rust [*Puccinia graminis* f. sp. *tritici*]. Phenotyping wheat lines for performance in diverse environments and cropping systems, disease and insect resistance, and end-use quality is most important strategy for the development of widely adaptable, stable, and disease/pest resistant germplasm.

2.2 Shuttle Breeding Program for Higher Yield Potential and Resistance to Biotic Stresses

Development of broadly adapted, durable disease resistant, high yielding and stable wheat germplasm is the primary objective of any wheat breeding program across the world. To breed for wide adaptation and high yield potential, the International Maize and Wheat Improvement Center's (CIMMYT) wheat breeding program shuttles segregating materials between alternative sites within Mexico. The shuttle breeding methodology is unique to CIMMYT; it was proposed and implemented by Dr. Norman E. Borlaug (Borlaug 1968), initially accompanied by much criticism, but finally widely acclaimed. This methodology has been responsible for the production of photoperiod insensitive and widely adapted improved germplasm. In particular, the shuttle breeding process involving contrasting locations in regard to latitude, altitude and rainfall has proven a most efficient way to introduce and select genes for photoperiod insensitivity. Shuttle breeding in Mexico consists of growing segregating populations in two distinct environments, i.e., Ciudad Obregon (Sonora) and Toluca (State of Mexico).

The Ciudad Obregon location is irrigated, similar to the Nile Delta in Egypt and the Punjab in India, whereas Toluca is a high rainfall location (1,000 mm during the wheat growing season) situated 2,600 meters above sea level. Planting in Obregon occurs in November, and the maturation of plants coincides with increasing high temperatures in April and May. The harvested materials, after selection, are then transferred to Toluca where planting is undertaken in May and June and harvesting is completed in October and November, when temperatures are declining.

This shuttle methodology allows the harvest of two generations of segregating populations per year and cuts the length of the normal breeding cycle in half. The procedure has inherently allowed only photoperiod insensitive plants to flower at both locations and complete the maturity period within this very tight time limit.

Ciudad Obregon is located at 27.5°N, 40 masl, in the state of Sonora. It is a dry, irrigated, low-altitude site, located in a desert climate. Mean rainfall during the wheat crop cycle is about 50 mm. Irrigated yields in the region are high, in the order of 8–11 tons ha^{-1} in experimental plots and 6–8 tons ha^{-1} in farmers' fields. With a reduction in the number of irrigations, various types of drought stress can be created. This is one of the two most important breeding and screening sites for the CIMMYT wheat program. Inoculation of stem rust (*Puccinia graminis* f. sp. *tritici*) and leaf rust (*P. triticina*) by spray applications of susceptible border-mixtures ensures adequate infection of the entire targeted field. Rust inoculation is carried out in the latter part of January, with the spring wheat grown from November until May.

The alternate site for the CIMMYT shuttle breeding program is Toluca, which is located at 19°N, 2640 masl, and west of Mexico City in the State of Mexico. This temperate, high-rainfall, high-altitude site is the most important CIMMYT summer cycle location. This environment is conducive for disease expression, especially of stripe rust (*P. striiformis*), *Septoria tritici* and *Fusarium* head blight (FHB). Spray applications to susceptible border-mixtures provide stripe rust infection. Dispersal of infected straw at the tillering growth stage initiates epidemics of *S. tritici* and FHB. Spring wheat is grown from May until October. When planted in November, winter wheats are exposed to vernalizing temperatures during the winter that are low enough to initiate flowering.

The concept of photoperiod insensitivity was not known to science when the CIMMYT shuttle breeding program was initiated. It only became apparent when Dr. Borlaug began sending the breeding material to the Indian subcontinent and the Middle East in the early 1960s. The photoperiod insensitive genes, *Ppd1* and *Ppd2*, abound in CIMMYT's spring wheats, and along with the dwarfing genes, *Rht1* and *Rht2*, resulted in a new plant type, which was not only lodging tolerant, but dramatically higher yielding with high biomass due to pleiotropic effects or close linkage.

2.3 Historical Analysis of Yield Gains in the Cimmyt Wheat Breeding Program

There has been a continuous involvement of CIMMYT researchers in the evolution of plant types for different agro-ecological conditions. Since the mid-1950s, there has been a continuous rise in wheat yields in Mexico and elsewhere. The modern cultivars evolved from changes in plant type and structure (dwarfing genes), physiological aspects (photoperiod insensitivity genes), durable disease resistance (incorporation of rust resistance), robustness, delayed leaf senescence, and other changes in spike number, morphology, grain number, and size.

The yield potential of semi-dwarf wheat cultivars, regardless of their origin, has continued to increase at the rate of 1 % annually (Sayre et al. 1997). Yield potential of bread wheat in Mexico increased from 4.5 t ha^{-1} for the tall cultivar "Yaqui 50' to 6.5 t ha^{-1} for the first semi-dwarf mega-cultivars 'Siete Cerros 66' and 'Sonalika', which were widely grown Green Revolution cultivars. Increased yields in these cultivars were due to incorporation of dwarfing genes *Rht1* and *Rht2* that provided lodging tolerance, and responsiveness to fertilization and irrigation. Dwarfing genes were successfully transferred from short statured Japanese cultivar 'Norin 10'.

A better understanding of various genetic and non-genetic factors contributed to further yield increases and the development of superior cultivars like 'Baviocora 92' with a yield potential of about 9.0 t ha^{-1}, which represented a narrow genetic base or specific adaptation (Rajaram et al. 2002). However, 'Veery', a derivative of a Russian cultivar (winter wheat) and an Indian line of CIMMYT origin (spring wheat), and a CIMMYT advanced line 'Attila', represent a wider genetic base or wider adaptation and are known to be widely adapted and a stable performer in different parts of the world. This supports the hypothesis of Kronstad where he proposes the use of a wider genetic base in the breeding program (Kronstad 1996). Although thousands of crosses were made between winter and spring wheats, 'Veery' and 'Attila' were the successful spring types with the winter wheat parents 'Kavkaz' and 'Nord Desperez', respectively, and led to the development of mega-cultivars that were subsequently grown on millions of hectares around the world. These yield increases were often associated with the presence of the alien translocation commonly known as 1B.1R, where the short arm of chromosome 1B is replaced by the short arm of chromosome 1R of rye (*Secale cereale*) (Rajaram et al. 1995). Some of the new wheat genotypes developed in recent years have shown further increases in yield potential of 10–15 % over 'Attila' (Singh et al. 2007). Historical analysis of Elite Spring Wheat Yield Trial (ESWYT), tested over the past 15 years (1995–2009) across locations in many countries, showed mean yields of the five highest yielding entries with an annual gain of 0.66 % compared to 'Attila' (Sharma et al. 2012) and an another study using 30 years international data revealed about 0.7 % yield gain over the years in Mexico (Lopes et al. 2012) (Fig. 2.2). Results from this study demonstrate continuous genetic yield gains in the elite spring bread wheat lines developed and distributed by CIMMYT for the global irrigated and rainfed environments.

2.4 Grain Yield

The most significant objective of any wheat breeding program is to enhance the grain yield potential. Grain yield is a complexly inherited trait of low to moderate heritability and is strongly influenced by the environmental conditions. Higher grain yields are usually associated with delayed maturity, increased plant height and lower protein content (Heisey 2002). Yield enhancement is often achieved by not only selecting for greater yield potential but also by selecting for resistance to biotic

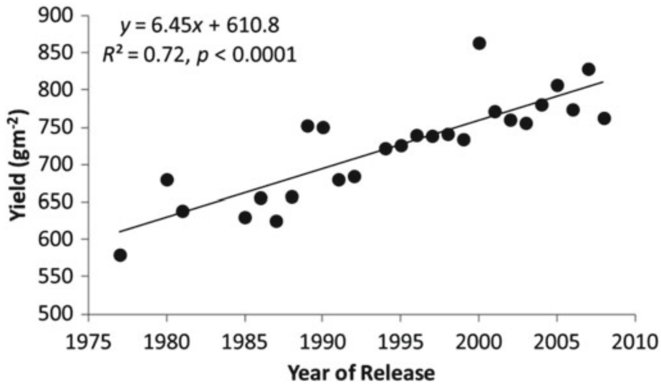

Fig. 2.2 Genetic yield gain over 30 years in a historic set of CIMMYT germplasm (adapted from Lopes et al. 2012)

and abiotic stresses that may limit the expression of cultivar's maximum yield potential. Breeding for enhanced yield depends on: (i) the genotypic variation for yield potential of parents used in the crossing program, (ii) selection intensity, and (iii) the degree to which genotypic differences in yield potential are expressed in the selection nursery. To achieve higher yield potential, plant breeders employ a range of crossing and selection methods. The role of wheat breeders includes: (1) introduce genetic variation, (2) inbreed and select among the variants, and (3) evaluate the selected lines in the diverse and varied environments where the lines may eventually be grown as a released cultivar (Baenziger and DePauw 2009).

The introduction of variation has historically been done by sexual crosses to make hybrids (usually single, three-way, double, or back crosses). Wheat breeding at CIMMYT until the early 1980s relied on simple, three-way (top) or four-way (double) crosses followed by the pedigree method of selection. Breeders realized that best advanced breeding lines were rarely derived from double crosses, with the possible reason being that the genetic variation generated by such crosses was large and the chances of recovering plants with desirable combinations of genes was low due to insufficient population sizes. With the globalization of CIMMYT's Bread Wheat Breeding Program in the 1980s, CIMMYT breeders relied on simple and three-way crosses and occasionally single backcrosses (Wang et al. 2003).

2.5 Crossing Block

Historically, many wheat breeding programs made relatively few crosses, often 60 or less. However, most wheat breeders would consider 250 crosses as the minimum and many programs make 1,000 or more crosses. The total number of crosses made will depend upon the number of parent lines available, the percentage used for cultivar development versus those made for parent development, as well as, how the resultant populations will be used.

The CIMMYT crossing block (CB) consists of collections of elite breeding source material from the ongoing breeding program and elite germplasm from different parts of the world. In order to facilitate crossing operations, each CB is sown on four different dates, about 7 days apart. The production of high yielding, widely adapted, stable and durable resistant spring wheat germplasm with acceptable quality is the primary consideration of the CIMMYT wheat program. For this reason, parental lines are being grouped and sub-grouped based on their country of origin or specific character expression (e.g. disease resistance, abiotic stress tolerance, industrial quality). Also, CB entries include: (1) major varieties released in different target countries, (2) elite CIMMYT and other germplasm identified from international and national testing, and (3) advanced lines exhibiting desirable expression of one specific trait or group of traits, including those made available by the pathology, wide cross, physiology and other sections within the wheat program.

Not all entries in a CB are involved in crossing. Before making crosses the male master (MM) and female master (FM) lists are first determined. The male master list represents the best entries in the CB based on field observations (agronomic type in the field from the past cycles and the current cycle) and relevant databases (yield, adaptation, disease resistance, end-user type, and protein content etc.,). These entries serve as male parents for simple crosses, but simple crosses are also made among these parents. The FM list includes some entries that still require specific improvements but carry certain traits of great interest, like high yield potential. These entries will only be used as female parents for crossing to the entries from the MM list.

F_1: about 1,000–1,200 crosses are made per cycle. Crosses are always directed toward targeted breeding, taking into account the relevant requirements such as, higher grain yield, Ug99 resistance, and heat-tolerance. The total number of F_1 seed from a cross will depend upon the cross type and the size of the population needed in the next generation to adequately represent the genotypic array. Mostly introduced materials are used as females in their original crosses, in order to possibly expand the genetic base of CIMMYT wheat cytoplasm, but also for practical/logistical reasons. As a rule, 3 spikes are emasculated and pollinated for simple crosses.

F_1Top or backcross: A majority of F_1's are top crossed to one or more third parents, or a single (limited) backcross is made back to the adapted parent. About 15–20 spikes are used for top or backcrosses in order to get 400–500 seeds to capture maximum variation from the simple cross F_1's. Backcrosses are carried out to stabilize variability, as the genetic distance between parents becomes greater, and are proving very effective in expanding adaptation and performance while introgressing new genetic diversity. The single backcross approach was initially aimed at incorporating resistance to rust diseases based on multiple, additive, minor genes (Singh and Huerta-Espino 2004). However, it soon became apparent that the single backcross approach also favored selection of genotypes with increased yield potential. The underlying reason was that single backcrosses shift the progeny mean towards the higher side of the curve and favors the retention of most of the desired additive genes from the recurrent parent while simultaneously allowing the incorporation and selection of additional useful small-effect genes from the donor parent.

Per cycle, about 500–750 top and/or backcrosses are made. An epidemic is created of the prevalent diseases, either in Ciudad Obregon or Toluca. In the first segregating generation of such crosses, the best plant types with good agronomy and resistance to diseases will be selected. Individual spikes from selected plants are then bulk threshed and advanced to F_2 stage.

2.6 F_1 and Segregating Populations

Once the variation has been introduced, the wheat breeder must decide how best to select and inbreed. In every selection protocol there is a hierarchy in which the breeder must choose in what order the traits will be selected. Simple selection techniques can be used to eliminate undesirable phenotypes from the F_2 population. Some examples of simple selection techniques include inoculating segregating populations with a particular disease so resistant types can be selected (Pozniak and Hucl 2004), and planting populations in a given environment so the winter or spring growth habit segregants will be winterkilled or not vernalized (Dowell et al. 2006). These selection protocols can quickly eliminate obvious undesirable types at relatively low cost and with high efficiency. The resulting population is smaller but contains valuable traits at a higher frequency than an unselected population. Therefore, the population size becomes more manageable.

Once a cross has been made and classified, its segregating progenies are selected in a shuttle breeding fashion between Toluca and Ciudad Obregon. All the elite CIMMYT advanced materials go through this shuttle breeding program, and it allows the breeders to get improved germplasm with wider adaptability, disease resistance and higher yield potential.

The CIMMYT wheat breeding program was using the modified-bulk selection scheme, where individual plants were harvested in the F_2 generation to grow as an F_3 generation, bulk selection was then practiced in the F_3–F_5 generations. Individual plants or spikes were once again harvested in the F_5 or F_6 generation (Rajaram et al. 2002). Following the study by Singh and coworkers which showed that selection schemes had little or no effect on the performance of progeny lines, but it was the choice of parents that determined the progeny response, a 'selected-bulk breeding scheme' was introduced in the bread wheat improvement program (Singh et al. 1998). Under this scheme, in all segregating generations until F_5 or F_6, one spike from each of the selected plants is harvested as a bulk and a sub-sample of seed used to grow the next generation. Individual plants or spikes are then harvested in the F_5 or F_6 generation. This scheme allows the retention of a larger sample of selected plants without increasing the cost and was found to be highly efficient in terms of operational costs. Moreover, retaining a large sample of plants in segregating populations increases the frequency of rare segregants carrying the most desirable genes.

Below, detailed information on advancement of each of the segregating and advanced generation materials under the selected-bulk breeding scheme for the bread wheat improvement program is included.

F_2: Each of the F_2 populations consists of about 1,500–2,000 plants per cross, which are space-planted at 10–15 cm between plants. This includes simple, top and (limited) backcrossed F_2 populations. An epidemic is created of the prevalent diseases. The poorest F_2 populations are completely discarded. Within the better F_2 populations, the best plants are selected by the breeders based on good agronomic type, appropriate height, synchronous tillering, desired spike type, large spike, good fertility, durable disease resistance, and desired maturity. Individual spikes from selected plants are bulk threshed and advanced to the F_3 generation.

F_3 to F_5: The bulk seed (20 g) from selected F_2 plants is planted in two beds of 8–10 m in length to achieve about 400 plants/population. An epidemic is created of the prevalent diseases. The best plants are selected by the breeders based on agronomic type, fertility, lodging tolerance, durable disease resistance, and expected yielding ability, plus somewhat for phenotypic uniformity. Subsequently, in the F_5 generation selected plants are harvested and threshed on an individual basis and the seed is visually observed for grain filling characteristics, boldness, lack of diseases, yellow berry, other markings, and color. About 30–50 % of the plants are thus discarded.

F_6: Selected individual plants or spikes from the F_5 generation are planted in small plots of 0.7 to 1.0 m in length in a paired row. This system of planting allows thousands of entries to be planted in a small area. Again, an epidemic is created of the prevalent diseases. The best and most uniform lines are visually selected and harvested in bulk. The plump and bold grain lines retained after visual seed selection. Best entries with desirable agronomic features and good grain characteristics are promoted to yield trials (YT).

YT: Selected best entries from the F_6 generation are promoted to the first-year YTs. YTs are planted in an Alpha-Lattice-Latinized design with 2–3 replications, 2 checks and 28 entries in each trial. Checks are normally high-yielding commercial cultivars or the highest yielding cultivar identified from the breeding program. YTs are conducted in Ciudad Obregon (Fig. 2.3). Trial entries are planted in 2 beds of 3 rows each of 2 m length. Plots are harvested after physiological maturity using plot combine harvesters, and then weighed manually for grain yield. High-yielding entries are selected on relative yield over checks, agronomic type, disease resistance, and additional industrial quality tests, including alveograms (using Ciudad Obregon seed), and the best lines are promoted to the Elite Yield Trial (EYT) or second year yield testing.

PCs:(*Parcela chica*- Spanish acronym for small plots) are planted separately at the same time as the yield trials, with exactly the same entries as in the YTs, in an area where relevant diseases are artificially inoculated. The PCs provide disease resistance data. In addition, the PCs form small seed multiplication plots, where rouging can be carried out to provide clean, pure seed for subsequent cycles.

EYT: EYTs are conducted using the best yielding entries from the YTs. The EYTs are grown under representative and relevant environmental conditions. Latinized alpha-lattice design with 2–3 replications is used. Again the EYTs are conducted in

Fig. 2.3 Standard yield trial plots at Cuidad Obregon, Mexico

Ciudad Obregon, under five different environmental conditions, where the targeted conditions can be simulated: for example, Mega-environment 1 (ME1) by applying 5–6 irrigations; ME4 by supplying limited water under drip-irrigation, and by restricting the number of irrigations (one irrigation during pre-sowing and booting stages), thus creating very stringent drought conditions; ME5 by planting late (in February) resulting in considerable heat stress at the time of flowering and grain filling. Thus each target environment (e.g. ME1: irrigated, ME4: drought, ME5: heat) is somewhat represented or simulated during the yield trial phase. Each entry is planted in 2 beds, each 80 cm in width, 3–4 m long with 3 rows per bed. Also, another environment created for a planting method called *melgas* (*melga* means "irrigation basin" in Spanish) with 30 plots/*melga* basin. In *melgas* every entry is sown as an 8-row plot, 3.8 m long.

EPCs: EPCs (Elite *Parcela Chica*) are planted separately at the same time and with exactly the same entries as in the EYTs, in an area where relevant diseases are artificially inoculated. The EPCs provide disease resistance data. In addition the EPCs provide clean, pure seed for subsequent cycles.

2.7 IBWSN: (International Bread Wheat Screening Nursery)

After considering grain yield, stress (biotic/abiotic) tolerance and end-use industrial quality characteristics, and data from across the five environments, the best lines are selected as candidates for the International Bread Wheat Screening Nursery (IBWSN) for distribution to collaborators across the world. The lines that enter into the IBWSN are top yielders in the EYTs, but also showed good resistance and performance in the EPCs which are always planted at the same time under disease stress, where artificial inoculation with virulent races (rust) takes place. These advanced lines are expected to be good for diseases since they have been screened for resistance since the F_1 generation onwards. Industrial quality is also taken into account, but a very high-yielding line that has low industrial quality may still be included in the IBWSN, since many countries still value quantity over quality. The lines also should not have lodged excessively in the EYTs, and must be very uniform.

2.8 ESWYT (Elite Spring Wheat Yield Trial)

The best entries from the IBWSN enter into the Elite Spring Wheat Yield Trial (ESWYT). This trial consists of 50 entries of 2 replications arranged in an alpha-lattice design. Each ESWYT has 45 new bread wheat lines, four CIMMYT checks (not necessarily the same each year) and one local check that presumably is the best locally adapted commercial variety at individual sites. To maintain genetic diversity, representative entries from diverse genetic backgrounds are included in the trial. Individual experimental plots grown by the cooperators usually vary in size and are adapted to the local yield trial planting practices used by the cooperators. A different randomization is used for each site. The trial management practices are based on standard crop husbandry practices for specific sites. A field book with instructions for trial management and data recording are provided to each collaborator along with seed shipment. Seed packages are prepared and dispatched to collaborators in different countries by CIMMYT's Seed Information and Distribution Unit in Mexico. The CIMMYT wheat program distributes several yield trials annually that are targeted to specific wheat growing environments and management conditions in many developing and developed countries through its collaborative international wheat improvement network. The ESWYT is targeted at irrigated environments with higher production; this trial is often grown in other environments also, as materials adapted to other environments are also identified by the cooperators. There are more than 100 sites worldwide where CIMMYT's yield trials are grown annually; however, data recovery is about 50 %. The sites are representative of different mega-environments based on their classification by CIMMYT (Rajaram et al. 1995). The diverse global wheat testing locations where ESWYT trials are grown encompass a great deal of contrasting environmental conditions that might be expected in the future due to climate change.

2.8.1 *Variety Release*

The final stage before cultivar release is the extensive evaluation phase. At this stage, there is often little that one can do, other than extensively test, to build a database that ensures that accurate information has been obtained to make the right decision. This must be done over time and locations and should target environments where the cultivar will most likely be recommended to be grown, but also surrounding environments that will test its robustness. However, these trials still need to be undertaken in the most efficient manner (generally considered to be using incomplete block designs or nearest neighbor analyses (Stroup et al. 1994), grown in the most representative locations, and correctly interpreted (Roozeboom et al. 2008). As the lines continue to be advanced, the complexity and expense of the selections assays will also increase. While considerable information is available on how to analyze genotype × environment interaction, there are two aspects that need to be considered in detail. The first is ensuring that the locations are representative of critical regions within the target environments. The second aspect is that given the correct testing locations, it is important to learn from those locations how to interpret the data. In this case, every testing site in every year tells a story. The successful breeders will be able to understand why one line did well or poorly at a site based upon the line's and site's history.

2.9 Biotic Stresses

2.9.1 Resistance to Diseases

The major fungal diseases of wheat caused by biotrophs, include the three rusts (stem, leaf and yellow), powdery mildew, bunts and smuts; whereas, those caused by hemibiotrophs include *Septoria tritici* blotch, *Septoria nodorum* blotch, spot blotch, tan spot and *Fusarium* head blight (FHB). The biotrophs are highly specialized and significant variation exists in the pathogen population for virulence to specific resistance genes. Evolution of new virulence through migration, mutation, recombination of existing virulence genes and their selection is more frequent in rust fungi. Therefore, breeding for resistance to these diseases needs a strategic approach to enhance the durability of resistance.

The three rust diseases, stem (or black), leaf (or brown) and stripe (or yellow), caused by fungi *Puccinia graminis* f. sp. *tritici*, *P. triticina* and *P. striiformis* f. sp. *tritici*, respectively, continue to cause losses, often major, in various parts of the world and hence receive high attention in breeding. The phenomenon of the erosion of race-specific resistance genes, or their combinations, has led scientists to look for alternative approaches to resistance management. Van der Plank was the first epidemiologist to clearly define the theoretical basis of the concepts of resistance (Van der plank 1963). This approach was widely recommended for breeding leaf rust resistance by Caldwell (1968), stem rust resistance by Borlaug (1972), and yellow rust resistance by Johnson (1988). The wide application of such a concept in breeding for leaf rust resistance, commonly known as slow rusting, has dominated in CIMMYT's bread wheat improvement program for almost 40 years with major impacts (Marasas et al. 2003). Recently, we have begun to understand better the genetic basis of race-nonspecific or durable resistance to rust diseases and this knowledge is being routinely applied in breeding. The development and deployment of wheat cultivars with such resistance will provide a long-term genetic solution to rust control.

2.9.2 Breeding for Resistance to Rusts

In the late 1960s and 1970s, there was a revival of the concept of general (race-nonspecific) resistance and its application in crop improvement (Caldwell 1968). In the early 1990s, once the genetic basis and diversity of slow rusting resistance became more clear, high yielding lines that combined four or five additive, minor genes for both leaf and yellow rusts and with near-immune levels of resistance were developed through 3- and 4-way crosses involving lines carrying different minor genes (Singh et al. 2000).

Recently, simple and three-way crosses have been commonly used in the CIMMYT breeding program, with one or more parents carrying adult plant resistance (APR), and these are being used to breed new high yielding, near-immune wheat materials resistant to all three rusts. To transfer minor gene based resistance into a susceptible adapted cultivar or any other selected genotype, a ‘single backcross selected bulk’ scheme is being used in CIMMYT wheat breeding program. In this scheme high yielding lines are crossed with a resistance donor parent; 20 spikes from F_1 plants from each cross are then back-crossed to the improved lines to obtain 400–500 BC1 seeds. Rust resistant and agronomically desirable plants are selected from large segregating populations grown under artificially created rust epidemics, where the pathogen race that had virulence for race-specific resistance genes present in the populations are used to create the epidemics. Selection is practiced from the BC_1 generation onward for resistance and other agronomic features under high rust pressure. Because additive genes are partially dominant, BC_1 plants carrying most of the genes show intermediate resistance and can be selected visually. About 1,500–2,000 plants are space sown in the F_2, whereas about 600–800 plants are maintained in the F_3–F_5 populations. Plants with desirable agronomic features, low to moderate terminal disease severity in early generations (BC_1, F_2 and F_3) and with low terminal severity in later generations (F_4 and F_5) are retained. Under the selected bulk scheme, one spike from each selected plant is harvested as a bulk until the F_4 generation, and plants are harvested individually in the F_5. Bulking of selected plants poses no restriction on the number of plants that can be selected in each generation as the harvesting and threshing are quick and inexpensive, and the next generation is derived from a sample of the bulked seed. Because high resistance levels require the presence of four or five additive genes, the level of homozygosity from the F_4 generation onward is usually sufficient to identify plants that combine adequate resistance with good agronomic features. Moreover, selecting plants with low terminal disease severity under high disease pressure means that more additive genes may be present in those plants. Selection for seed characteristics is carried out on seeds obtained from individually harvested F_5 plants. Small plots of the F_6 lines are then evaluated for agronomic features, homozygosity of resistance etc., before conducting yield trials.

2.9.3 Targeted Breeding for Resistance to UG99 Group of Races of Stem Rust Pathogen

Characterization of existing spring wheat breeding materials for resistance to Ug99 and its derivative races of stem rust pathogen in field trials in Kenya and as seedlings in greenhouses at USDA-ARS Cereal Disease Laboratory (St. Paul, MN, USA) during 2005–2009 resulted in the identification of several wheat lines with varying levels of adult plant resistance (APR). Information on the resistance was made available on www.globalrust.org and also summarized by Njau et al. (2010). Wheat lines 'Kingbird', 'Kiritati', 'Pavon 76', 'Muu', 'Parula' and a few others were identified as carrying a high level of APR.

In the absence of molecular markers for APR genes and the absence of the Ug99 race in Mexico, a shuttle breeding scheme between Mexican field sites (Ciudad Obregon in northwestern Mexico during winter, and Toluca or El Batán in the highlands near Mexico City during summer) and Njoro, Kenya, was initiated in 2006 to screen and select breeding materials resistant to the Ug99 race of stem rust fungus in Njoro, near Nairobi, Kenya. The Ug99 race of stem rust was recognized as a major threat to wheat production and food security as more than 80 % of the world wheat's is susceptible to this race.

The 'single-backcross, selected-bulk' breeding approach (Singh and Trethowan 2007) is being applied for transferring multiple minor genes to adapted backgrounds. The BC_1 plants are selected for desired agronomic features and resistance to leaf and stripe rusts, and harvested as bulk in Mexico. F_2 plants derived from the BC_1, simple, and top crosses with desirable agronomic features and resistance to leaf and stripe rusts are selected for agronomic traits and resistance to other diseases at Ciudad Obregon or Toluca and harvested as bulks (Table 2.1). If the F_2 populations were grown at Ciudad Obregon, where the quarantine disease Karnal bunt may occur, the F_3 populations are grown at Toluca for another round of selection. About 1,000 seeds of each F_3 and F_4 population obtained from the Toluca harvest are grown at Njoro, Kenya for selection under high stem rust pressure during the off-season. Populations not carrying sufficiently resistant plants are discarded. Selection of plants with high to adequate resistance is carried out, selected plants are bulk-harvested and plump grains are selected for establishing F_4 and F_5 populations of about 1,000 plants during the main season at Njoro under high stem rust pressure. Because stem rust affects grain filling, we expect plants with insufficient resistance to have shriveled grains. Selection in main season is carried out in the same manner as off-season and about 400 plump seeds harvested from the selected plants are returned to Mexico and grown at Ciudad Obregon under high leaf rust pressure for

Table 2.1 Flow of breeding materials in the Mexico–Kenya shuttle breeding scheme

Year	Locations	Activities
1	Cd. Obregon	New crosses made
	El Batán	F_1 grown, BC_1 and F_1-Top made on selected F_1
2	Cd. Obregon	BC_1 and F_1-Top (350 plants), F_2 (1,000 plants from simple crosses) grown and selected for agronomic traits and leaf rust resistance. Spikes from selected plants are harvested as a bulk and plump grains are retained
	Toluca	F_2 (1,000 plants from BC_1 and F_1-Top) and F_3 (350 plants from F_2 simple) grown and selected for agronomic traits, resistance to stripe rust, and *Septoria tritici*. Spikes from selected plants are harvested as a bulk and plump grain are retained
3	Njoro	F_3 and F_4 (800 plants) grown under stem and stripe rust pressures. Plants with high to adequate resistance are tagged and harvested as a bulk. Plump grains are retained
	Njoro	F_4 and F_5 (800 plants) grown, spikes from short plants resistant to stem and stripe rust are selected and harvested as a bulk. Plump grains are retained
4	Cd. Obregon	F_5 and F_6 (350 plants) grown and selected for agronomic traits and resistance to leaf rust. Plants are harvested individually and those with plump grains are retained
	El Batán and Toluca	Advanced lines grown as small plots, selected for agronomic traits and resistance to stripe rust and *Septoria tritici* blotch at Toluca and leaf rust at El Batán. Best lines are harvested in El Batán and those with plump grains are promoted to yield trials
5	Cd. Obregon, Njoro and Santa Catalina	Advanced lines grown as replicated yield trials at Cd. Obregon and as small plots at all three sites. They are phenotyped for leaf rust, stem rust and stripe rust at Cd. Obregon, Njoro and Santa Catalina, respectively, and the best lines are retained
	El Batán, Toluca, and Njoro	Seed of International Nurseries Candidates multiplied at El Batán. Lines are also grown at all sites and phenotyped for leaf rust, stripe rust, stem rust, *Septoria tritici* blotch, *Fusarium* head blight, etc. Quality analysis is conducted using Cd. Obregon grain
6	Cd. Obregon, Mexicali and Njoro	2nd year yield trials conducted in 5 environments at Obregon, seed multiplication for international distribution at Mexicali and phenotyped for stem rust resistance at Njoro
	El Batán	International Yield Trials and Screening Nurseries prepared and distributed
7	International	Countries with wheat seasons between April–December
8	International	Countries with wheat seasons between October–June

Source: Singh et al. (2006, 2010)

final selection as individual plants in the F_5 and F_6 generations. Small plots of advanced lines obtained by selecting individual plants in Ciudad Obregon are grown at the El Batán and Toluca field sites to select for agronomic characteristics and resistance to leaf and stripe rusts (Singh et al. 2006; 2010).

2.9.4 Rust Screening Methodology and Selection Environment

The probability of identifying resistant parents and resistant progenies is increased by the availability of a reliable screening methodology and a favorable environment for disease development. Depending on the disease and choice of the type of resistance, the methodology may require simple tests in the greenhouse on seedlings or adult plants and replicated field tests. Protocols for screening for resistance to most diseases are well established and can be employed in breeding for resistance. Inclusion of check cultivars for resistance and susceptibility is important to assess the disease pressure and degree of resistance. Choice of field sites with reliable environmental conditions is crucial for progress when selection is to be carried out in field conditions.

To study the genetics of wheat rusts, and to breed for resistance, it is essential to have efficient and reproducible methods of producing infection under controlled and field conditions. When breeding for resistance to rusts, it is usually sufficient to produce infection on all of the plants in a test. The test must be adequate to accurately determine the infection types on host genotypes.

The CIMMYT wheat breeding program handles a large amount of breeding material, so rust testing is usually done under field conditions. Exposing the breeding materials under field conditions has several advantages. In most cases, except in a limited number of locations, where natural epidemics occurred for rusts, artificial inoculation is required. Good conditions should be provided for plant growth and the spread of the rust. To obtain a uniform rust pressure the spreaders are sown as borders around the entire experimental field and as small hills at one end of each plot. Most nurseries include rust susceptible spreaders at intervals of every 25 to 50 entries. The spreaders are used for inoculation and insure that there is ample spore production and spread of the rust. The more frequent the spreader rows, the more uniform the epidemic is expected to be. The spreader can be planted as a single susceptible cultivar but it is often useful to mix two or three susceptible cultivars of different maturities in order to extend the epidemic over a longer period. Check lines with known rust reactions are commonly planted at regular intervals to provide a check for rust reaction and for agronomic features. Segregating generations such as F_2–F_5 are usually planted in long rows, but the advanced generations are planted in short rows. Under both of these conditions, spreader rows are good enough to supply ample spores for disease epidemic.

Periodically, the disease pressure should be monitored in the spreader rows to ensure that there is adequate disease development for the supply of ample spores. Sometimes there may be problems with an uneven rust distribution. In this case the rust infection will be heavier in areas nearest to the spreaders, particularly early in the development of the epidemic. Uneven spore loads can be a problem when selecting for some types of resistance such as slow rusting. The resistance can also be overwhelmed by heavy spore loads and the differences between plants or lines become obscured. In special cases, it may not be desirable to have spreader rows but instead it may be beneficial to inoculate the whole nursery uniformly.

Use of one or a mixture of races depends on the purpose of the test. One race should be used if the purpose is to test for the presence of a specific gene for resistance or to test for resistance to a particularly important race, such as a highly virulent race like Ug99. A mixture should be used if the purpose is to simulate a natural field epidemic or to select for resistance against all of the races that are normally present in the field.

2.9.5 Field Inoculation Techniques

Several techniques have been used to inoculate field nurseries with urediospores, including injection, dusting, spraying and transplanting infected plants. In the CIMMYT rust nurseries, spraying of urediniospores suspended in a light mineral oil (Soltrol 170) is practiced. This oil has proven to be a satisfactory carrier of the spores, it has low phytotoxicity and the spores are readily wetted and suspended in oil, which is its major advantage over water. A low concentration of spores (0.5 mg per ml) is sufficient to give good results. Sprayers that produce a very fine mist are used and for larger areas, several types of hand or power-operated low volume sprayers are available. Rust infection is initiated approximately 6–8 weeks after planting.

2.9.6 Measuring Rust Severity

Breeders need to decide the degree of resistance that is acceptable and select only plants with at least that level of resistance. A common procedure is to record the percentage of the leaf or stem that is covered with uredia. The leaves or stems are compared with diagrams on which various percentages of the area have been covered with spots of various sizes and represent pustules. Based on the size of pustules and the associated necrosis or chlorosis, infection responses are classified into four discrete categories: R= resistant, MR= moderately resistant, MS= moderately susceptible and S= susceptible (Roelfs et al. 1992). Infection responses overlapping between any particular two categories are denoted using a dash. For instance, 'MR-MS' denotes an infection response class that overlaps the MR and MS categories. Rust severity can also be assessed using 0–100 % following the modified Cobb Scale (Peterson et al. 1948). Entries are evaluated for rust severity two to three times between heading and plant maturity. The area under the disease progress curve (AUDPC) can be determined following the formula described in Roelfs et al. (1992).

2.9.7 Greenhouse or Growth Chamber Tests

Greenhouse or growth chamber tests can be used for limited amounts of material when it is particularly important to test with a specific race or to speed up the breeding process. All greenhouse studies in CIMMYT are conducted at El Batán, in Mexico, where a collection of rust races is preserved. In the greenhouse, plants are inoculated with a specific race by spraying with urediniospores suspended in Soltrol oil using an atomizer. After inoculation, plants are transferred to a dew-chamber overnight to ensure germination and infection of the pathogen. Greenhouse evaluation of rust infection type responses follow the 0–4 Scale described in Roelfs et al. (1992). The slow rusting components; latent period, receptivity, and uredinium size etc., are scored on flag leaves in repeated greenhouse experiments according to the method described by Das et al. (1993), Lee and Shaner (1985), and Singh and Huerta-Espino (2003).

Host response at seedling stage is normally scored as susceptible or resistant depending on the infection type produced against a particular race of the pathogen. Testing for major or specific resistance is normally done on the primary leaf under controlled conditions. Screening involves use of a single isolate in each test and includes susceptible check lines and differential lines that possess designated genes for resistance. Inoculation is performed 7 days after planting and disease observations are taken based on infection type reaction 10–14 days after inoculation.

2.9.8 Resistance to Pests

More than 100 insect species have been identified as pests in wheat. A limited number of insect pests are systemically important to wheat worldwide, including the greenbug, hessian fly, Russian wheat aphid and stem sawfly. The insects and mites that have a negative impact on wheat production have complex biology, varied reproductive behaviors, diverse food and survival habits, and powers of dispersal. This makes screening for pest resistance very challenging.

2.10 Tolerance to Abiotic Stresses

Climate change-induced temperature increases (heat stress) and drought are estimated to reduce wheat production in developing countries by 20–30 % (Lobell et al. 2008; Rosegrant and Agcaoili 2010). Heat and drought tolerance of crops varies greatly and wheat is among the most sensitive of the major staples. Wheat breeders have successfully developed cultivars better adapted to heat and moisture stress conditions resulting in a 0.5 to 1.3 % grain yield increase per annum in many drier wheat producing areas (Byerlee and Traxler 1995; Manes et al. 2012). Considerable success has been achieved in breeding for lodging resistance by developing semidwarf cultivars, resulting in the Green Revolution. The improved lodging resistance conferred by reducing culm length and increasing harvest index has further allowed exploitation of yield promoting factors like response to irrigation and fertilization. In recent years major emphasis has been given to tolerance to heat and drought as these stresses limit productivity in many parts of the world.

2.10.1 Canopy Temperature (CT)

Under drought, selection for cooler CT permits genetic gains for grain yield and genotypes with cooler canopies have been shown to extract more water from deeper soil profiles (Reynolds et al. 2007). Canopy temperature depression (CTD) is usually expressed as canopy temperature (T_c) minus air temperature (T_a), and it is positive when the canopy is cooler than the air. It has been used as a selection criterion in wheat breeding in terms of heat and drought stress tolerance (Balota et al. 2007). According to Munjal and Rana (2003), a cooler canopy and high stomatal conductance during the grain filling period is assumed to be the basic morpho-physiological criteria for higher grain yield under heat stressed conditions. Furthermore, Balota et al. (2008) reported that wheat cultivars with a high CTD showed a trend of higher yield under heat and drought stress.

Canopy temperature depression can be measured almost instantaneously using an infrared (IR) thermometer in a breeding plot. Since the measurement integrates the temperature of several plants at once, the error normally associated with traits measured on individual plants is reduced. Investigations into this methodology in warm environments (Amani et al. 1996) have shown that CTD was best associated with performance when measured at higher vapor pressure deficit (i.e., warm, sunny conditions and during grain-filling). Irrigation status was not a confounding factor within the normal frequencies of water application. Under drought, studies found that optimum time for CTD measurements are in the morning and afternoon between full ground cover and late booting, and during grain filling. Line performance seems to be better predicted when CTD is measured in the morning during grain filling or prior to heading.

2.10.2 Rapid Screening in Breeding Populations

CTD is routinely used by CIMMYT's wheat breeding program for rain-fed environments to enrich for alleles associated with dehydration resistance. All F_3 and F_4 bulks (1,000 per cycle) are screened for CT under drought; a larger number of plants, which are expressing favorable agronomic traits but with warmer CT (compared to checks) are discarded.

When CTD was compared with other potential selection traits (grain number, biomass, phenological data, and yield) measured in the selection environment, none of the other traits showed a greater association with performance in the target environment than CTD. In addition to yield, breeding objectives must take into account multiple factors, such as disease tolerance and phenology. Therefore, it would be logical when incorporating CTD into a selection protocol, to select for relatively genetically simple traits such as agronomic type and disease resistance in the early generations (e.g., F_2–F_3). Selection for complex traits, such as CTD, could be employed in subsequent generations, when more loci are homozygous, perhaps in preliminary yield trials.

CTD readings are normally measured using the infrared thermometer (Model IRTS-P, Apogee Instrument, Inc., Logan, UT, USA) with a 4° field of view, which is equipped with an extendible thermistor to read air temperature. The data for each plot are the mean of 4–5 readings taken from the same side of each plot, at an angle of approximately 30° to the horizontal, in a range of directions that cover different regions of the plot and integrate many leaves. Measurements are normally made in mid-afternoon because of low wind, as a high wind velocity may disturb the temperature in and around the canopy.

Canopy temperature is largely a function of stomatal conductance (Amani et al. 1996) that can also be measured rapidly using a viscous flow porometer (Condon et al. 2008). An evaluation of the effectiveness of integrating CT into other criteria used by breeders showed that selecting for cooler plots, in addition to visual selection for plant type, improved the ability to identify the very highest yielding lines (van Ginkel et al. 2008). Economic analysis supported the idea that incorporating selection for Stomatal Aperture Traits (SAT) into a breeding program is likely to result in increased efficiency associated with the ability to cull more lines, thereby, reducing the size of yield trials (Brennan et al. 2007).

The development of relatively easy to use spectral radiometers offers another high throughput screening approach for comparing spectral reflectance indices (SRIs) of genotypes. The composition of light reflected by canopies is a function of many physiological factors including light interception, hydration status of tissues and pigment content and composition of photosynthetic tissue (Araus et al. 2001). A number of SRIs have been shown to be correlated with the yield of genotypes (Montes et al. 2007).

2.11 Grain Quality

Enhancing wheat quality improves processing efficiencies, makes more desirable and more diverse consumer products and ensures the competitiveness of farmers, grain merchandisers, millers and end processors. Wheat quality criteria vary drastically depending on the end-use. Similarly, wheat cultivars may show large differences in their processing and end-use quality attributes. Therefore, while setting breeding priorities and strategies, one must determine: the cultivar's intended end-uses and/or the demands of the targeted market, specific quality traits to breed for, and genotype × environment × management interactions that may influence the quality of the resulting cultivar.

Once the parental lines are characterized and the crossing plan defined, the probability of selecting desirable lines depends on the intensity and effectiveness of the quality-selection pressure applied; the best results are obtained by breeding for the targeted environment (warm, dry, wet and erratic) and screening F_3–F_5 lines for desirable genes and allelic variations controlling grain-compositional traits (Arbelbide and Bernardo 2006), complemented by rapid, high-throughput conventional small scale tests such as flour sedimentation and Near Infrared Reflectance Spectroscopy (NIRS), which are related to end-use processing quality (Peña et al. 2002). Conventional small scale quality tests explain end-use quality only partially; in advanced breeding stages (F_6–F_8), quality screening should be based on more specific food processing (dough viscoelasticity and mixing properties, starch pasting properties, baking performance) and end-product quality attributes (Peña et al. 2002). Finally, multi-location yield trials exposing advanced elite lines to environmental variation and farmer's crop management practices are necessary to identify the few genotypes combining stable yield and quality attributes across locations and years.

2.11.1 Grain Hardness

Grain hardness is a grain quality trait associated with the milling properties of wheat (Miller et al. 1982) and with the baking quality of the resulting milling products. Milling times, milling energy requirements and the level of starch damage produced in the milled flour are all influenced by grain hardness. Hard wheats require longer milling times and more milling energy, and produce a larger amount of damaged starch. Rapid small-scale methods (based on grinding time, grinding volume, or particle size distribution) used to determine grain hardness make it relatively easy to screen for hardness as early as the F_3 generation. Near infrared reflectance and transmittance (NIR, NIT) analysis of the particle size distribution of whole grain flour or analysis of the intact grain samples are particularly fast and useful in early generation screening.

2.11.2 Starch

Native starch, which is the main component of the wheat grain (70–75 % dry weight), shows little influence on the functional properties of wheat flours used in bread, cookie and cake making. However, damaged starch (mechanically damaged during flour milling), by exposing its components (amylose and amylopectin) to interact with other constituents of the baking formula, influences importantly the water absorption and fermentation time requirements of bread-making dough, as well as the staling and crumb textural properties of bread. Some small amount of damaged starch is desirable in bread-making flours but highly undesirable in cookie- and cake-making flours, as it may reduce considerably the expansion capacity of the cookie dough (Miller and Hoseney 1997). This is the reason that the cookie and cake industries use soft wheat flour, which has a minimum amount, if any, of mechanically damaged starch and, consequently, low flour water absorption.

The Amylograph/Viscograph and more recently the Rapid Visco Analyser (RVA) are used to obtain a complete profile of starch pasting properties. While the first requires a large sample size and a considerably long testing time, the RVA requires a 3 to 4 g sample and only a few minutes to reveal the pasting profile of the tested material. Therefore, the RVA is now considered a rapid test suitable for the early selection of wheat lines possessing desirable starch pasting viscosity for noodle making (Bhattacharya and Corke 1996; Panozzo and McCormick 1993).

2.11.3 Proteins

Protein content is a key specification for wheat since it is related to many processing properties, such as water absorption and gluten strength. Protein content also can be related to finished-product attributes, such as texture and appearance. Bakers use protein content results to anticipate water absorption and dough development time for processes and products, because higher protein content usually requires more water and a longer mixing time to achieve optimum dough consistency. Grain protein content (GPC) in wheat varies between 8 % and 17 %, depending on genetic make-up and on external factors associated with the crop. A unique property of wheat flour is that when in contact with water its insoluble protein forms; a visco-elastic protein mass known as gluten. Gluten, comprising roughly 78 to 85 % of total wheat endosperm protein, is a very large complex composed mainly of polymeric (multiple polypeptide chains linked by disulphide bonds) and monomeric (single chain polypeptides) proteins known as glutenins and gliadins, respectively (MacRitchie 1994). Glutenins confer elasticity, while gliadins confer mainly viscous flow and extensibility to the gluten complex. Thus, gluten is responsible for most of the viscoelastic properties of wheat flour doughs and is the main factor dictating the use of a wheat variety in bread and pasta making. Gluten viscoelasticity, for end-use purposes, is commonly known as flour or dough strength.

The sodium dodecyl sulphate (SDS) sedimentation tests (Axford et al. 1979) can be used to obtain a semi-quantitative estimation of the amount of glutenin (or indirectly, of general gluten strength). These tests, which are based on the expansion of mainly glutenins (also known as gel proteins) in lactic acid or SDS/lactic acid solution, are currently the most rapid and reliable single small-scale tests (Weegels et al. 1996). These tests are widely used to screen early generation wheat lines in relation to their general gluten strength type (strong to weak).

SDS-PAGE of whole protein extracts can be used in breeding programs as an early generation technique to select lines possessing desirable high molecular weight glutenin (HMWG) subunit composition and in advanced stages to define desirable HMWG combinations in progeny of new crosses. Low molecular weight glutenin (LMWG) subunits are also important in determining gluten viscoelasticity (Weegels et al. 1996).

Some of the key quality tests conducted in CIMMYT wheat breeding programs are elucidated below.

2.11.4 Falling Number

The level of enzyme activity can be measured by the falling number test. Yeast in bread dough, for example, requires sugars to develop properly and therefore needs some level of enzyme activity in the dough. Too much enzyme activity, however, means that too much sugar and too little starch are present. Since starch provides the supporting structure of bread, too much activity results in sticky dough during processing and poor texture in the finished product. If the falling number is too high, enzymes can be added to the flour in various ways to compensate. If the falling number is too low, enzymes cannot be removed, which results in a serious problem that makes the flour unusable.

The falling number instrument analyzes viscosity by measuring the resistance of a flour-and-water paste to a falling stirrer. Falling number results are recorded as an index of enzyme activity in a flour sample and the results are expressed in time as seconds. A high falling number (for example, above 300 s) indicates minimal enzyme activity and sound quality wheat flour. A low falling number (for example, below 250 s) indicates substantial enzyme activity and sprout damaged wheat flour.

2.11.5 Sedimentation Test

The sedimentation test provides information on the protein quantity and the quality of flour samples. Positive correlations were observed between sedimentation volume and gluten strength or loaf volume attributes. The sedimentation test is used as a screening tool in wheat breeding as well as in milling applications. The sedimentation test is conducted by holding the flour sample in an acid solution. During the sedimentation test gluten proteins swell and precipitate as a sediment. Sedimentation values can be in the range of 20 or less for low-protein wheat with weak gluten to as high as 70 or more for high-protein wheat with strong gluten.

2.11.6 Farinograph

The farinograph results are used as parameters in formulation to estimate the amount of water required to make dough, to evaluate the effects of ingredients on mixing properties, to evaluate flour blending requirements, and to check flour uniformity. The results are also used to predict processing effects, including mixing requirements for dough development, tolerance to over-mixing, and dough consistency during production. Farinograph results are also useful for predicting finished product texture characteristics. For example, strong dough mixing properties are related to firm product texture. The farinograph determines dough and gluten properties of a flour sample by measuring the resistance of dough against the mixing action of paddles (blades). Farinograph results include absorption, arrival time, stability time, peak time, departure time, and mixing tolerance index.

2.11.7 Alveograph

The alveograph determines the gluten strength of dough by measuring the force required to blow and break a bubble of dough. The results include P Value, L Value, and W Value. Stronger dough requires more force to blow and break the bubble (a higher P value). A bigger bubble means the dough can stretch to a very thin membrane before breaking and indicates the dough has higher extensibility; that is, its ability to stretch before breaking (L value). A bigger bubble requires more force and will have a greater area under the curve (W value).

The alveograph test provides results that are common specifications used by flour millers and processors to ensure a more consistent process and product. The alveograph is well suited for measuring the dough characteristics of weak gluten wheats. Weak gluten flour with low P value (strength of gluten) and long L value (extensibility) is preferred for cakes and other confectionery products. Strong gluten flour will have high P values and is preferred for breads.

2.11.8 Mixograph

The mixograph test quickly analyzes small quantities of flour for dough gluten strength. Wheat breeders use mixograph results to screen early generation lines for dough gluten strength. Flour water absorption measured by the mixograph often serves as bake absorption in bread baking tests. The mixograph determines dough and gluten properties by measuring the resistance of dough against the mixing action of pins. Mixograph results include water absorption, peak time, and mixing tolerance. The mixograph curve indicates gluten strength, optimum dough development time, mixing tolerance (tolerance to over-mixing), and other dough characteristics. The amount of water added (absorption) affects the position of the curve on the graph paper. Less water increases dough consistency and moves the curve upward. The mixograph test measures and records the resistance of dough to mixing with pins. Peak Time is the dough development time, beginning the moment the mixer and the recorder are started and continuing until the dough reaches maximum consistency. This indicates optimum mixing time and is expressed in minutes. Mixing tolerance is the resistance of the dough to breakdown during continued mixing and affects the shape of the curve. This indicates tolerance to over mixing and is expressed as a numerical score based on comparison to a control. Weak gluten flour has a shorter peak time and less mixing tolerance than strong gluten flour.

2.11.9 Quality Criteria Used in the CIMMYT Wheat Breeding Program

Wheat quality improvement is an important component in the CIMMYT wheat breeding program. Therefore, wheat progenitors and lines derived from the crosses are assessed for diverse quality attributes. Besides relevant quality data, wheat breeders receive an end-use quality classification, which is intended to aid the breeder in identifying the distribution of wheat end-use quality types (Peña 2009). Table 2.2 shows wheat quality classes related to grain quality attributes. Based on wheat quality attributes, an end-use quality type is classified.

2.11.10 Breeding for Improved Human Nutrition

Micronutrient malnutrition arising from dietary deficiency of bio-available minerals and vitamins affects more than half of the world's population, especially women and preschool children. In particular, zinc (Zn) and iron (Fe) deficiencies are a growing public health concern, especially in the developing world. A new public health approach to alleviate deficiencies of these mineral nutrients in developing countries is through biofortification of staple food crops. Biofortification involves development of micronutrient-dense staple crops using the best traditional plant breeding approach. The HarvestPlus (www.harvestplus.org) initiative of the Consultative Group on International Agricultural Research (CGIAR) has aimed to develop and distribute biofortified varieties of major staple crops, including bread wheat, that have high concentrations of these essential micronutrients. CIMMYT is leading the efforts in development and dissemination of high-yielding, disease-resistant wheat varieties with significantly increased Zn and Fe concentrations.

Genotypic variation for grain Zn and Fe concentration in wheat has been demonstrated (Graham et al. 1999). A genetic study of a bread wheat mapping population showed continuous variation for both Zn and Fe suggesting that it is a quantitative trait controlled by several genes (Shi et al. 2008). As our understanding of the underlying genetic control of Zn concentration is poor, breeding has focused on crossing materials of unrelated parentage and intermediate micronutrient status with the aim of identifying transgressive segregants. Provided sufficiently large F_2 and F_3 population sizes are maintained and genetic drift minimized, the F_4 and later generations are screened for Zn concentration once a higher level of homozygosity has been achieved. The most promising sources for grain Zn concentration are wild relatives, primitive wheats, and landraces. Current breeding efforts at CIMMYT have focused on transferring genes governing increased Zn from *T. spelta* and *T. dicoccon* based synthetics, landraces, and other reported high Zn sources to high yielding elite wheat backgrounds.

Table 2.2 Bread wheat quality classification

Hardness class and grain color	Gluten type	Abbreviated description[a]	End-use type[b]
		Acceptable Quality Types[c]	
Hard Wheat			
Hard-white (HW) and hard-red (HR)	*Strong (S)*	HW-S, HR-S	**1a, 1b** (Pan type breads, mechanized industry)
HW, HR	*Medium strong (MS)*	HW-MS, HR-MS	**2a** (Leavened breads in general, baguette, etc., semi-mechanized industry)
			2a (Flat breads: two-layer breads, baladi, etc.)
			2a (Dry and fresh noodles: alkaline, white, instant)
			2b (Steamed bread, North-China style)
HW	*MS*	HW-MS	**2b** (Flat breads: single-layer, chapati, etc.)
HW, HR	*Weak (W)*	HW-W, HR-W	**3a, 3b** (Dense breads: Moroccan, etc., flour tortilla)
Soft Wheat			
Soft-white (SW) and soft-red (SR)	*S (and MS)*	SW-S(MS), SR-S(MS)	**4a** (Steamed bread, South China style)
SW	*MS*	SW-MS	**4a** (White-salted noodles, China, Japan, and Korea)
SW, SR	*W*	SW-W, SR-W	**4b** (Biscuits, cakes, and steamed bread of SE Asia)
Quality types unacceptable for mechanized and semi-mechanized bread making[d]*. These can be considered as feed (or utility) wheat*			
H	*Tenacious (T)*	HW-T, HR-T	**5** (Feed or utility wheat)
H	*Weak-Tenacious (WT)*	HW-WT, HR-WT	**5** (Feed or utility wheat)
S	*T*	SW-T, SR-T	**5** (Feed or utility wheat)
S	*WT*	SW-WT, SR-WT	**5** (Feed or utility wheat)

Source: Peña (2009)

[a]Criteria to determine grain hardness class-gluten type abbreviated code: Grain hardness (Hard, H or Soft, S), followed by grain color (White, W or Red, R), followed by a hyphen, and then by gluten type (Strong, S; Medium-strong, MS; Tenacious, T; Weak, W; and Weak-tenacious, WT). Example: HW-S, hard and white with strong gluten; SR-T, soft and red with tenacious (short) gluten

[b]End-use type number followed by letter **"a"** has higher protein content than the same followed by the letter **"b"**: **Type 1a** should have grain protein above 12.5 % (12.5 % M. B.); **Types 2a and 3a** should have grain protein above 11.5 % (12.5 % M. B.); **Type 4a** should have grain protein above 11.0 % (12.5 % M. B.); **Type 5** has no differentiation regarding protein content

[c]All wheat lines/varieties falling within the quality type 1a to 3b must possess moderate to high gluten extensibility

[d]Quality types 4a, 4b and 5 are characterized for having slightly- to non-extensible gluten character, which is generally undesirable for making bread or flat bread.

New primary hexaploid synthetic wheats and landraces with significantly higher Zn concentrations are used as donor parents. Limited backcross populations of between 400 to 800 plants with elite materials and subsequent F_2 (1,200–2,400 plants) and F_3–F_4 (400–800 plants) are grown, and plants with desired agronomic features selected. BC_1-F_4s and BC_2-F_3s are grown in small plots for selection of agronomic traits and leaf and yellow rust resistance. Agronomically superior, rust resistant F_4/F_5 lines are then measured for Zn concentration. Selected advanced lines with higher Zn and desirable agronomic traits are tested for grain yield potential and grain Zn concentration in replicated yield trials (Velu et al. 2010). Best leads with high yield potential along with considerable Zn concentration (above the target Zn concentration of 33 mg/kg) are then deployed to the national partners as a HarvestPlus Yield Trial (HPYT). The first set of advanced lines derived from crosses of high yielding wheats with genetic resources possessing high Zn and Fe such as *Triticum spelta*, landraces and synthetic wheat based on *T. dicoccon* were tested at nine locations in South Asia and Mexico for Zn and Fe concentration, grain yield and other traits. Although G × E interaction was significant, high heritabilities were observed for Zn and Fe concentrations at individual sites and across environments, reflecting non-crossover type of interaction (Velu et al. 2012). This trend was confirmed by the high genetic correlations between locations that showed similar ranking of entries across locations, indicating that it is possible to select the best adapted entries with high Zn concentration. Pooled data across locations showed increments of 28 % and 25 % over the checks for Zn and Fe. A considerable number of entries exceeded intermediate to full breeding target Zn concentrations, indicating that it is possible to develop Zn-biofortified varieties with competitive yields and other farmer preferred agronomic traits.

This breeding method relies on the development of very large populations and significant investment in Inductively Coupled Plasma (ICP) analysis for micronutrient status. A major shortage is that selection pressure for Zn cannot be applied in early generations as the evaluation of single plants does not give an accurate measure of micronutrient status. Soil analyses as well as grain analysis of systematic checks at CIMMYT's research station in Ciudad Obregon showed that soil Zn concentration may have been much more heterogeneous than soil Fe concentration. Large variation in soil Zn can confound or mask genetic differences among lines, thereby preventing the identification of lines with genetically superior concentrations of grain Zn. One strategy to reduce this problem is to use a systematic check, alpha lattice designs, and spatial analyses of segregating and advanced populations. Another potential strategy that needs further study is the use of Zn-containing fertilizer (foliar or soil applied) to homogenize soil Zn concentration (Oury et al. 2006). The development of cheaper and more rapid screening assays for Zn, such as X-ray florescence (XRF) screening techniques, allows wheat breeders to apply greater selection pressure in early generations, thereby minimizing the effect of "misclassified" lines on eventual outcomes.

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