Chapter 22
Genomics of Wheat, the Basis of Our Daily Bread

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Abstract Wheat, being an important source of calories across the Americas, Europe, North Africa and Asia, is the most widely grown food crop in the world. Wheat yields have undergone a spectacular rise over the last half century, contributing to the Green Revolution in Asia. However, productivity increases appear to have reached a plateau in recent years and many consider that new advances in genomics will be essential to delivery the rates of productivity increases necessary to prevent hunger. New molecular tools will enhance on-going wheat breeding, offering the plant breeder considerable advantages in time, cost, and response to selection. Perhaps most importantly, it is believed that genomics tools will also facilitate much more efficient utilization of new sources of genetic variation for important agronomic traits from wild species. This chapter provides an overview of the botany and conventional breeding of wheat including a summary of past successes, the current primary breeding targets, and the major constraints to achieving those goals. We then focus on genomic advances in bread wheat and durum wheat during the past decade and the implications of these advances for increasing resilience, stability and productivity in tropical, sub-tropical and semi-arid production systems across the world. This includes the use of genomics to improve the search for, and the characterization of, new beneficial genetic variation and the identification of molecular markers to facilitate the efficient manipulation of that variation in breeding programs. Finally, we provide a list of the currently available trait markers and a perspective on likely future trends and challenges in wheat molecular breeding.

22.1 Introduction

Wheat is the most widely grown food crop in the world, occupying 216 million hectares (mha), producing 600 million tonnes (mt) of grain, compared to 153 mha of rice and 147 mha of maize (FAO 2006). It is one of the first domesticated food

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Fig. 22.1 The production in Afghanistan of wheat, the country's staple crop, has risen significantly in recent years, but average yields remain relatively low—on the order of 2.0–2.5 tons per hectare—with landholdings in many areas being small and not amenable to mechanization. (photo by CIMMYT, used with permission) (See color insert)

species and has been the major source of calories in Europe, West Asia, and North Africa since the inception of organized farming. It is widely grown across the temperate regions of Central Asia, Europe, and North America and is a major crop in many developing countries across the sub-tropical regions of the world including India (26 mha sown per annum-p.a.), Pakistan (8 mha p.a.), Iran (6 mha p.a.), Brazil (2 mha p.a.), Syria (2 mha p.a.), Egypt and Ethiopia (both over 1 mha p.a.) (see Fig. 22. 1). Wheat is an important source of calories across the world due to its wide agronomic adaptability, ease of grain storage, and the wide range of diverse food products that can be made from its flour. Bread wheat flour can be used for leavened bread due to the specific viscoelastic properties conferred by gluten, an elastic form of protein that traps the CO_2 emitted during fermentation, causing the dough to rise. Wheat flour can also be used to make flat bread which is more popular in South Asia, North Africa, and the Middle East, while it is used for making noodles in China and Japan, and for making biscuits across the world. In contrast, flour from durum wheat is used for pasta in the western world and for local products like couscous in North Africa. Dramatic increases in global wheat production have taken place during the last 50 years primarily due to increased productivity, rather than expansion of the cultivated area (Curtis 2002). Average global yields have risen from 1 t/ha in the 1950s to about 2.5 t/ha at the turn of the century (Curtis 2002). With world population projected to reach 7.9 billion by 2025 (US Census Bureau 1998), and assuming no changes in consumption patterns, significant increases in wheat production must be made to meet the expected demand for food. However, productivity increases appear to have reached a plateau in recent years and many consider that new

advances in genomics will be essential to delivery the necessary rates of productivity increases. New molecular tools will enhance ongoing wheat breeding programs, offering breeders considerable advantages in time, cost, response to selection, and opportunities to address new goals.

Wheat and its relatives comprise diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), and hexaploid (2n = 6x = 42) forms. Of the diploid wheats, einkorn wheat (*Triticum monococcum*), a possible donor of the A-genome, can still be found in limited cultivation with its wild form ssp. *aegilopoides* widely distributed across the Middle East. The tetraploid wheats, also known as emmer wheats, have two distinct forms; widely grown *T. turgidum* with genome AABB (2n = 4x = 28) and *T. timopheevii* with AAGG (2n = 4x = 28); both are found across the Fertile Crescent (Gill and Friebe 2002). The widely cultivated free-threshing, non-fragile, tetraploid form of *Triticum* spp., popularly known as durum or macaroni wheat, has the genome AABB. *T. dicoccum* (AABB) was most likely the first cultivated form of wheat. Cytological, archeological, and molecular genetic studies suggest that *T. dicoccoides* (AABB) arose by hybridization between the *T. urartu* (AA) and an unknown diploid with genome composition similar to the Sitopsis section of the genera *Aegilops* about 10,000 years ago (Zohary and Hopf 1993).

The hexaloid wheats are composed of two types, *Triticum aestivum,* also known as bread wheat or common wheat (AABBDD: 2n = 6x = 42), and *T. zhukovskyi* (AAAAGG: 2n = 6x = 42). The *Triticum aestivum* wheats arose from hybridization between tetraploid AABB species and *Aegiolops squarosa* (2n = 2x = 14; DD) (McFadden and Sears 1946). *T. zhukovskyi* possibly resulted from hybridization between *T. timopheevii* (AAGG) and *T. monococcum* (AA) (Upadhya and Swaminathan 1963). It is likely that these hexaploid forms arose during cultivation of the tetraploid progenitors in close proximity with diploid relatives, since there is no evidence of wild forms of hexaploid wheat. Despite the allopolyploid nature of bread wheat and durum wheat, both show disomic inheritance. Pairing between homoeologous chromosomes is mainly regulated by pairing homolog genes *Ph1* (Riley and Chapman 1958) and *Ph2* (Mello-Sampayo 1971). The allopolyploid nature of wheat allows it to withstand a variety of chromosome substitutions and additions, which has been widely exploited by researchers to develop cytogenetic stocks of most wheat chromosomes. These genetic stocks have been fundamental to many advances in wheat genetics and genomics during the last half century.

This chapter focuses on genomic advances in bread wheat and durum wheat during the past decade and their implications for increasing resilience, stability and productivity in tropical, sub-tropical, and semi-arid production systems across the world.

22.2 Progress in Conventional Breeding

Wheat productivity has undergone a spectacular rise over the last half century. Initial increases were due to the introduction and dissemination of high yielding, fertilizer responsive varieties with short stature, generally known as the Green Revolution varieties. The introduction of dwarfing genes and subsequent improvements

in harvest index increased grain yield, reduced crop lodging, and allowed farmers to apply higher rates of nitrogen fertilizer. Shuttle breeding, originally initiated by the International Maize and Wheat Improvement Center (CIMMYT) and based on growing alternate generations in two diverse environments in Mexico followed by international germplasm exchange and global testing networks, has made significant contributions to global advances in yield (Ortiz et al. 2006). The use of two growing seasons per year has facilitated rapid genetic gains and selection in these contrasting environments has led to the development of improved germplasm with wide adaptation, since the two locations differ in rainfall, temperature, photoperiod, and soil type (Rajaram et al. 2002). CIMMYT's shuttle breeding efforts resulted in the production of wheat lines with relative insensitivity to photoperiod, broad adaptability, and broad spectrum resistance to a number of important biotic stresses, primarily the rust diseases (Rajaram et al. 2002; Ortiz et al. 2007). These changes led to substantial improvements in wheat productivity; first in Asia and then in much of the rest of the developing world (Borlaug 1968; Trethowan et al. 2007). Consequently, many wheat breeding programs across the world have adopted multilocation testing in contrasting environments as an integral part of their breeding philosophy (Rajaram et al. 2002).

Since the Green Revolution, wheat breeders have maintained an average yield advance of 1% per annum (Byerlee and Moya 1993; Sayre et al. 1997), although improved crop management practices have also made significant contributions (Bell et al. 1995). Average national wheat yields have grown faster in developing countries than in the high income countries throughput the past 40 years. However, wheat yield increases have shown some leveling off in recent years (Reynolds et al. 1996).

22.2.1 Utilization of Genetic Variability from Wild Relatives

The primary gene pool of wheat includes the cultivated and landrace forms of hexaploid bread wheat and tetraploid durum wheat as well as the diploid A genome donor (*T. monococcum var. urartu*) and the diploid D genome donor (*Ae. squarossa*). The secondary gene pool includes polyploid relatives belonging to the genus *Triticum* and *Aegilops* that have at least one genome in common with wheat. The tertiary gene pool is composed of species with varying ploidy levels with genomes that are not homologous to those of cultivated wheat. Generally, crosses involving primary and secondary gene pools do not require special cytogenetic manipulation other than embryo rescue and culture to produce F_1 hybrids. Genomes of species in the tertiary gene pool often show homeologous relationships with the A, B, and D genomes of cultivated wheat. The primary gene pool of wheat represents a valuable source of genetic diversity that has been used in a number of wheat improvement programs. The landraces and wild species of the diploid A and D genomes also possess many novel genes and can be readily crossed with durum and bread wheat breeding lines. Accessions of *T. tauschii* ($2n = 2x = 14$; DD)

have been widely used in crosses with *T. durum* (2n = 4x = 28; AABB) for the "artificial" resynthesis of the hexaploid genomes of cultivated bread wheat (2n = 6x = 42; AABBDD) (Mujeeb Kazi and Rajaram 2002). The resultant F_1 hybrid embryos are then rescued and grown on culture media, followed by chromosome doubling using colchicine. CIMMYT has produced nearly 1,500 resynthesized hexaploid wheat lines. These have been extensively used, particularly in CIMMYT's rainfed wheat breeding program, to incorporate superior levels of drought tolerance in wheat lines targeted for marginal environments. However, these resynthesized synthetic hexaploid wheats have a number of undesirable agronomic traits such as shattering, tall stature, and late maturity, and not all durum wheat germplasm can be crossed with *T. tauchii* accessions due to hybrid necrosis. Fortunately, following backcrossing to agronomically elite breeding lines, the resultant "synthetic derivatives" have shown recovery of these deleterious traits and improved disease resistance and abiotic stress tolerance (Mujeeb-Kazi et al. 1998; Lage and Trethowan 2007). Direct introgression of D genome variation has also been accomplished by crossing hexaploid wheat directly with *Triticum tauschii* (Gill and Raupp 1987).

Wide ranges of whole chromosomal substitutions and partial chromosomal translocations have been made using species from the secondary and tertiary gene pools (reviewed by Sharma and Gill 1983; Jiang et al. 1994). Not all species in the tertiary gene pool can be successfully crossed with bread wheat, primarily due to the chromosomal differentiation. The wheat cultivar Chinese Spring has been used in many intergeneric crosses because it has recessive alleles at the "crossability" loci *kr1*, *kr2* and *kr3* (Falk and Kasha 1983). A number of disease resistance genes have been transferred to bread wheat through interspecific and intergeneric crosses (McIntosh et al. 2003). One particularly successful example involves the rye chromosome 1R. Wheat lines carrying the 1BL/1RS translocation are present in many high yielding wheat cultivars with wide adaptation (Rajaram et al. 1983).

22.2.2 Advances in Genetics and Breeding of Agronomic Traits

Wheat is cultivated from the tropics to the fringes of the Arctic and from sea level to over 3,000 m elevations on the Andean plateau. This wide range of adaptation has been made possible by the presence of genes controlling vernalization, photoperiod response, and early maturity, thus enabling wheat breeders to tailor cultivars to different agro-ecological regions. Vernalization response is conditioned by a homoelogous set of genes designated as *Vrn-A1* (*Vrn1*), *Vrn-B1* (*Vrn2*), and *Vrn-D1* (*Vrn3*) located on the short arms of homoelogous chromosomes 5A, 5B, and 5D, respectively (Worland, 1996). Response to day length is primarily conditioned by another homoelogous set of genes, *Ppd-D1* (*Ppd1*), *Ppd-B1* (*Ppd2*), and *Ppd-A1* (*Ppd3*) located on the short arms of chromosomes 2D, 2B, and 2A, respectively. However, other genes located on other chromosomes may also play a role in vernalization and photoperiod response (Law et al. 1998). The genetic control of early

maturity, considered to be conditioned by genes conferring earliness per se, is less well documented.

Recent increases in wheat yields have been associated with dramatic reductions in plant height resulting in significant increases in harvest index. Although a tall plant can compete with weeds more effectively, a plant with shorter stature is more efficient in partitioning assimilates to the grain and tends to be more lodging tolerant. Twenty-one genes controlling plant height have been described in wheat (McIntosh et al. 2003). The two most important height-controlling genes are *Rht-B1* and *Rht-D1*. located on chromosomes 4BL and 4DL, respectively (Gale et al. 1975; McVittie et al. 1978), with most semi-dwarf wheat germplasm possessing alleles *Rht-B1b* or *Rht-D1b* which are mutants insensitive to gibberllic acid (Peng et al. 1999). These two genes acting alone can reduce plant height by an average of 18 cm while at the same time significantly increasing spikelet fertility in high input environments (Flintham et al. 1997).

More recently, doubled haploids have been used to improve the speed and precision of wheat breeding (Aung et al. 1995; Tuvesson et al. 2003). Doubled haploid systems allow rapid generation of homozygous lines which improves breeding efficiency by decreasing the amount of time required to develop fixed lines. They also allow the breeder to select among fixed lines at the maximum level of genetic variability, viz. at the first generation after crossing. Wheat doubled haploids can be generated through anther or microspore culture (Konzak and Zhou 1991) or by using a maize pollen induction system (Laurie and Bennett 1986). In European breeding programs, thousands or even tens of thousands of doubled haloids are produced as part of the annual breeding process (Dayteg et al. 2007). Doubled haploid systems also enable easy integration of molecular markers in breeding programs as well as facilitating mapping and genetic studies within breeding populations (Dayteg et al. 2007; Howes et al. 1998).

22.3 Structural Genomics

22.3.1 Molecular Cytogenetics and Physical Mapping

In situ hybridization techniques, developed in the late 1960s, allow the detection of DNA sequences directly in cytological preparations on glass slides. Although originally developed using radioactive probes, these techniques were later optimized for utilization with non-radioactive labels such as biotin-dUTP and digoxiginin (reviewed in Jiang and Gill 1994). More recently, fluorochromes have been used for fluorescent in situ hybridization (FISH) with increased sensitivity and precision while facilitating the detection of multiple targets on the same chromosome preparation (Mukai et al. 1993; Oliver et al. 2006). Modified forms of traditional chromosome banding techniques (C-banding and N-banding) coupled with in situ hybridization procedures have also been used to detect and characterize

alien translocations and multi-copy DNA sequences (Jiang and Gill 1993). These techniques can also be used in phylogenetic and evolutionary studies (Badaeva et al. 2002). Recent advances in molecular cytogenetics procedures have been reviewed by Jiang and Gill (2006).

Endo and Gill (1996) have developed a set of deletion stocks of specific wheat chromosomes. These deletion stocks can be used to physically locate genes and expressed sequence tags (EST) on specific chromosomal regions, such as the control of homologous pairing gene *Ph1* on chromosome 5BL (Gill et al. 1993), vernalization response gene *Vrn A-1* on chromosome 5AL (Sarma et al. 1998), and the grain hardness locus, *Ha,* on chromosome 5DS (Sarma et al. 2000). Establishment of the relationship between genetic and physical maps by mapping a series of microsatellite markers on to deletion bins has also been accomplished (Sourdille et al. 2004).

22.3.2 Molecular Markers as Tools

The allohexaploid nature of bread wheat, with three distinct genomes makes it the largest of the cultivated cereals. The haploid complement of bread wheat has approximately 40 times more DNA (16×10^9 bp) than rice (4×10^8 bp). Genetic characterization studies have established that about 95%–99% of the hexaploid wheat genome is not transcribed (Sandhu and Gill 2002a). Most of the transcribed genes in wheat seem to exist in clusters spanning physically small chromosomal regions that are designated as gene rich regions (Sandhu and Gill 2002b).

There are a number of marker technologies available for genetic characterization in wheat and each system has its advantages and disadvantages (Langridge et al. 2001). Restriction fragment length polymorphism (RFLP) markers are valuable in comparative genetic analysis and synteny mapping, but are not suitable for routine marker-assisted selection (MAS). Random amplified polymorphic DNA (RAPD) markers are also no longer commonly used in wheat due to lack of reliability and robustness, although some RAPD markers linked to important genes of interest have been converted to more robust sequence tagged site (STS) markers (http://maswheat.ucdavis.edu). More recently, microsatellite markers (also known as simple sequence repeats; SSR) have become popular due to their robustness as an assay system, plus their highly polymorphic and co-dominant nature of inheritance (Somers et al. 2004). Diversity array technology (DArT) is a microarray-based hybridization technique that allows simultaneous genotyping of several hundred polymorphic loci distributed across the genome (Jaccoud et al. 2001). The large number of loci that can be genotyped simultaneously makes DArT technology an efficient method of low cost, high-throughput genotype fingerprinting and map construction (Akbari et al. 2006; Semagn et al. 2006). However, its potential as a tool in marker-assisted selection is still not clear. More recently, single nucleotide polymorphism (SNP)-based markers are beginning to be developed in wheat (Ravel

et al. 2006; Somers et al. 2003). SNPs are highly abundant in all genomes, and SNP markers are highly amenable to automation offering dramatic increases in throughput potential and unit cost efficiency. However, the frequency of SNP polymorphisms in wheat breeding populations is surprisingly low (Ravel et al. 2006). Therefore, at the present time, SSR markers remain the assay of choice for marker-assisted selection in wheat. ESTs have also become valuable in SNP discovery and for developing SSR markers. Since ESTs are derived from expressed gene sequences, they provide an efficient route for the development of candidate gene-based markers (see section 22.4.1).

The development of linkage maps in bread wheat and durum wheat has been generally slow compared to other important crops such as rice, maize, barley, and soybean. This is partly due to the large genome size of wheat and the resulting large number of linkage groups that require molecular characterization (21 linkage groups in bread wheat as opposed to 10 in rice, 12 in maize, and 7 in barley). In addition, wheat has a low level of detectable polymorphism with most marker systems. A number of linkage maps are available in hexaploid bread wheat (e.g. Roder et al. 1998; Somers et al. 2004; Semagn et al. 2006; Akbari et al. 2006) and on a lesser scale for durum wheat (Blanco et al. 1998; Elouafi and Nachit 2004). The International Triticeae Mapping Initiative (ITMI) generated the most comprehensive publicly available linkage map in wheat based on a single seed descent-derived population originating from a cross between the cultivar Opata85 and a resynthesized hexaploid wheat (W7984) developed at CIMMYT (http://wheat.pw.usda.gov/). Attempts have been made to develop consensus linkage maps in wheat, the latest having over 4,000 loci (Appels 2003). Somers et al. (2004) developed a high density consensus linkage map by using common SSR markers on each chromosome in four different mapping populations. However, even the most comprehensive consensus wheat linkage map lacks uniform marker coverage across all chromosomes, particularly the D genome.

22.3.3 Genome Diversity Analysis

The assessment of genetic diversity among cultivars is indispensable for plant breeding purposes since it provides a means for analyzing variation available in germplasm collections. Measures of genetic diversity were initially based on co-ancestry and pedigree records (Van Beuningen 1997; Kim and Ward 1997). Pedigree records are relatively abundant in wheat; however, they often lack detail, especially when large numbers of breeding lines or cultivars are being assessed. Furthermore, the underlying assumptions of co-ancestry are rarely met as selection ensures that gene frequency is not random, thus coefficients of parentage remain a theoretical estimate of the identity by descent (Cox et al. 1985; Graner et al. 1994). Molecular markers have enabled the estimation of genetic variation at the molecular level. Molecular marker profiles can be used to follow the effects of selection and genetic drift (which take place over breeding cycles), leading to more accurate estimates of

the relationships among genotypes. Among the different marker systems currently available, SSRs are most commonly used for genetic diversity analysis. However, new platforms based on DArT and SNP markers have the potential for simultaneous screening of whole genome haplotypes and will make detailed analysis of genetic diversity relatively straightforward and cost effective (Jaccoud et al. 2001; Rafalski 2002).

A popular opinion is that the intensive selection practiced by modern plant breeders over the last decades has dramatically reduced the genetic diversity among cultivars, narrowing the germplasm base and limiting future advances from breeding (Tanksley and McCouch 1997). Extensive cultivation of germplasm with a narrow genetic base creates a significant genetic vulnerability risk because mutations in disease or insect populations or changes in environmental conditions may result in drastic crop losses. This risk has been highlighted by the outbreak of a new virulent strain of stem rust resistance (*Puccinia graminis,* Ug99) in southwest Uganda (http://www.globalrust.org/).

Characterization of CIMMYT bread wheat breeding lines from 1950–2003 showed a significant decrease of genetic diversity in the improved CIMMYT lines of the 1980s. However, this was followed by an increase in genetic diversity in lines from the 1990s through to 2003, largely due to substantial increases in the use of landraces and synthetic derivatives in breeding nurseries during this period (Reif et al. 2005; Warburton et al. 2006). CIMMYT breeders have been using landraces and synthetic derivatives as new sources of resistance to diseases and tolerance of abiotic stresses. This trait-driven approach has clearly also had positive effects on the overall levels of genetic diversity in breeding material without causing detrimental effects on progress in yield improvement. However, other molecular marker studies analyzing individual regional breeding programs over time have provided conflicting conclusions on the effect of selection on overall genetic diversity (Donini et al. 2000; Christiansen et al. 2002; Roussel et al. 2004, 2005; Khan et al. 2005; Fu et al. 2005, 2006). This is likely to be due to differences in size and structure of the populations studied, differences in the type of marker and statistical analysis applied, and differences in breeding strategies and goals. Nevertheless, maintaining a high level of genetic diversity in CIMMYT's global breeding programs is considered important to ensure good progress in the adaptive breeding by end-user national and regional programs while minimizing the chance of homogeneity effects across large wheat breeding areas creating unacceptable levels of risk of large scale disease epidemics. Thus, for CIMMYT wheat breeding programs, the emphasis on introduction of novel sources of variation for important agronomic traits has an important spillover on overall genetic diversity which should be of benefit to most other breeding programs and target cropping systems.

Significant screening of old and unimproved germplasm as well as materials from the primary and secondary gene pools maintained at gene banks has also been conducted at the molecular level. Examining wheat landraces has revealed high levels of genetic diversity and major genetic differences between landraces and improved materials demonstrating that selective pressure from evolution and modern plant

breeding has formed two independent gene pools (Hao et al. 2006, Reif et al. 2005; Dreisigacker et al. 2005; Zhang et al. 2005b, 2006). The characterization of species from the primary and secondary gene pools allows the discovery of additional genetic variability. The level of variation available within the species of the bread wheat progenitors such as *T. dicoccum* and *T. tauschii* etc. has been shown to be extensive and considerably higher than in the AB and D genome of wheat, respectively (Lage et al. 2003; Li et al. 2003). Results are generally closely related to the eco-geographical origin of the examined accessions, indicating that genetic diversity is highly correlated to geographic distribution. This may mean that geographical information systems (GIS) data could be sufficient for coarse level stratification of wheat genetic resources within some species. However, for some species such as *T. dicoccoides,* where there is a substantial amount of variation within populations, this approach is less likely to be effective. Novel alleles observed in germplasm collections can be introduced into cultivated wheat via marker-assisted intergeneric hybridization followed by introgression or by genetic transformation (Rajaram and van Ginkel et al. 2001).

22.3.4 Association Genetics

Association analyses in plants detect quantitative trait loci (QTL) based on the strength of the correlation between variation in a trait phenotype and a marker genotype (Zondervan and Cardon 2004). Association mapping offers greater precision in determining QTL location than family-based linkage analysis and should lead to more efficient marker-assisted selection tools and gene discovery programs. Association analysis also promises to help connect sequence diversity with heritable phenotypic differences. Unlike family-based linkage analysis, association analyses do not require family or pedigree information and can be applied to a range of experimental and non-experimental populations (Kraakman et al. 2004). Collections of homozygous wheat cultivars are particularly suitable for association analyses as multiple tests over years and environments can be used to generate high quality phenotype data for a wide range of traits (Morgante and Salamini 2003).

Various methods of association analyses have been developed (reviewed by Mackay and Powell, 2007). For association analyses to be possible, LD must be present in the population under study. LD can simply be defined as the “non-random association of alleles at different loci”. It is the correlation between genetic polymorphisms (detected by SSRs or SNPs, etc.) that are the consequence of a shared history of mutation and recombination. In addition, population structure including several factors such as genetic drift, selection, and admixture can also cause LD between markers and traits (Flint-Garcia et al. 2003). Thus, association analyses must take care to remove these circumstantial correlations that cause false positive results.

Knowledge of the extent of LD in plants is limited (Flint-Garcia et al. 2003). LD in the out-crossing species maize decays within a few hundred base pairs in diverse samples (Tenallion et al. 2001), though the extent of LD increases when narrower selections of germplasm or products of artificial selection are analyzed (Jung et al. 2004; Remington et al. 2001). In self-pollinating species, such as wheat, levels of long-range LD are expected because the rate of effective recombination is reduced by the breeding system. A recent genome-wide study in Arabidopsis has shown that LD at most loci decays within 250 kb (Nordborg et al. 2002). High LD at distances up to 10 cM was found among AFLP loci in barley cultivars (Kraakman et al. 2004). In wheat, LD should be equally extensive, as it is predominantly self-pollinating and has undergone severe bottlenecks in its evolution and strong selection pressures throughout its breeding history. Within subgroups of 134 durum wheat accessions characterized with 70 SSRs, high levels of LD were reported for tightly to moderately linked locus pairs (<20 cM), but LD levels were greatly reduced for loosely linked (more than 50 cM) and independent locus pairs (Maccaferri et al. 2005). In a population of 149 soft winter wheat cultivars, Breseghello and Sorrells (2005) determined LD in chromosome 2D and part of 5A with 62 SSRs. Consistent LD on chromosome 2D was < 1 cM, whereas in the centromeric region of 5A, LD extended for $\sim$5 cM. In the same study significant associations between kernel traits and SSR markers were found in agreement with previous QTL studies and alleles potentially useful for selection were identified.

Large-scale EST sequencing projects (section 22.4.1) allow direct analyses of DNA sequence polymorphisms and the identification of haplotypes representing several linked SNPs (Caldwell et al. 2004; Gu et al. 2004; Giles et al. 2006). Furthermore, detection of SNP polymorphisms resulting in a dramatic change of phenotype can be crucial if new alleles are to be rapidly and easily identified (Ravel et al. 2006). The development of high-throughput SNP and DArT genotyping platforms will allow cost effective genome-wide association analysis, thereby enabling more efficient allele mining.

22.3.5 Genetic Characterization of Traits

Dense linkage maps with markers well distributed across the genome and associated information on sequence variation are invaluable resources for determining the expression of large numbers of genes in synteny mapping and gene characterization. Characterization of a range of simply inherited qualitative traits as well as dissection of complex traits in to Mendelian components have been reported for a range of traits such as yield, vernalization, photoperiod response, tolerance to abiotic stresses, maturity, and agronomic parameters associated with quality (see Hoisington et al. 2002 for a review). However, the precision of field phenotype data and the size and appropriateness of mapping populations, continue to be the most

rate limiting factors for successful marker identification and subsequent applications in wheat breeding. Bulked segregant analysis (BSA: Michelmore et al. 1991), using pools of the extreme genotypes from the phenotypic distribution of the target trait, has also been used in wheat to characterize simply inherited traits (Eastwood et al. 1994) and to identity quantitatively inherited genes of large effect (William et al. 2003; Shen et al. 2003). The success of this approach, although considerably less expensive compared to linkage map construction, is highly dependent upon the quality of the phenotype data. One disadvantages of this approach is a reduced probability of identifying markers for QTLs of small effect. However, these are increasingly seen as of minimal importance for subsequent practical application in molecular breeding, as it is currently difficult to devise efficient breeding systems for pyramiding large numbers of small effect QTLs for an individual trait. Markers identified through BSA must still be mapped to establish their genomic location. Nevertheless, BSA offers a rapid and cost effective process for identifying a small number of the most important markers which can then be screened across the entire population for precision mapping. Public databases such as Graingenes (http://wheat.pw.usda.gov/cgi-bin/graingenes) provide frequently updated information on mapped traits in wheat (see Table 22.1 for a current overview).

In addition to use of traditional marker-based approaches in genetic characterization of traits of importance, comparative genomics tools enable researchers to make cross-genome comparisons of structure and function at the molecular level among different species. The information derived from these studies makes it possible to transfer genetic information from model species, where a wealth of genomic information is available, to other species which are more complex at the molecular level and have less genomic characterization (Gale and Devos 1998; Feuillet and Keller 1999; Freeling 2001). Successful application of comparative genomics can facilitate the identification and characterization of genes conditioning target traits in the species of interest. For example, rice with an extensively studied small genome is the model species for cereal crops. Although extensive macrosynteny has been observed between rice and wheat, there are numerous discontinuities in microsynteny due to evolutionary events. This often complicates the transfer of information between species (Sorrels et al. 2003). Thus, for complex agronomic traits, comparative genomics may not identify all the important loci in the target species. Nevertheless, synteny mapping involving species such as rice, barley, and *Triticum monococcum,* and map-based cloning, have been used successfully to clone wheat *Vrn-A1* gene (Kato et al. 1999; Yan et al. 2003). Similarly, synteny mapping involving Arabidopsis, rice, maize, and wheat has enabled the successful isolation of important alleles of major dwarfing genes *Rht-B1b* and *Rht-D1b* (Peng et al. 1999); perfect markers were subsequently developed for the *Rht* genes by Ellis et al. (2002). Another successful application of synteny mapping was the identification of the wheat grain protein locus *Gpc-6B1* on chromosome 6B, which was found to be highly co-linear with a 350 kb region on rice chromosome 2; candidate genes identified in rice were used to saturate the wheat linkage group *Gpc-6B1*. These efforts led to the development of a codominant PCR marker for this trait (Distelfeld et al. 2006). Other recent examples of positional cloning based on comparative

Table 22.1 Markers reported to be associated with genes in wheat (updated from Hoisigton et al. 1998)

Trait	Locus	Source	Marker type	Chr.	Reference
Fungal Disease Resistance					
Leaf rust	*Lr1*	*T. aestivum*	RFLP/STS	5DL	Feuillet et al. 1995
	Lr9	*Ae. umbellulata*	RAPD/STS RFLP	6BL	Schachermayr et al. 1994; Autrique et al. 1995
	Lr10	*T. aestivum*	RFLP/STS	1AS	Schachermayr et al. 1997
	Lr13	*T. aestivum*	RFLP	2BS	Seyfarth et al. 1998
	Lr19	*Ag. Elongatum*	STS	7DL	Prins et al. 2001
	Lr20	*T. aestivum*	RFLP	7AL	Neu et al. 2002.
	Lr21	*T. tauschii*	RFLP	1DS	Huang and Gill 2001
	Lr23	*T. turgidum*	RFLP	2BS	Nelson et al. 1997
	Lr24	*Ag. elongatum*	RFLP RAPD/STS RAPD/SCAR	3DL	Autrique et al. 1995; Schachermayr et al. 1995; Dedryver et al. 1996
	Lr25	*S. cereale*	RAPD	4BL	Procunier et al. 1995
	Lr27	*T. aestivum*	RFLP	3BS	Nelson et al. 1997
	Lr29	*Ag. elongatum*	RAPD	7DS	Procunier et al. 1995
	Lr31	*T. aestivum*	RFLP	4BL	Nelson et al. 1997
	Lr32	*T. tauschii*	RFLP	3DS	Autrique et al. 1995
	Lr35	*Ae. Speltoides*	SCAR	2B	Gold et al. 1999
	Lr37	*Ae. Ventricosa*	STS/CAPS	2A	Helguera et al. 2003
	Lr39	*T. Tauschii*	SSR	2DS	Raupp et al. 2001
	Lr47	*T.speltoides*	CAPS	7A	Helguera et al. 2000
	Lr50	*T. timopheevii*	SSR		Brown-Guedira et al. 2003
	Lr51	*T. speltoides*	STS		Helguera et al. 2005
Stem rust	*Sr2*	*T. turgidum*	STS	3BS	Hayden et al. 2004
	Sr22	*T. monococcum*	RFLP	7AL	Paull et al. 1995
	Sr24	*Ag. elongatum*	STS	3DL	Mago et al. 2005
	Sr26	*Ag. elongatum*	STS	6A	Mago et al. 2005
	Sr38	*Ae. Ventricosa*	STS/CAPS	2A	Helguera et al. 2003
	Sr39	*Ae. speltoides*	STS	2B	http://maswheat.ucdavis.edu
	Sr R	*Secale cereale*	STS	1B/1D	Mago et al. 2002
Stripe rust	*Yr5*	*T. spelta*	STS	2BL	Yan et al. 2003; Chen et al. 2003
	Yr10	*T. aestivum*	SSR	1BS	Wang et al. 2002
	Yr15	*T. dicoccoides*	SSR	1B	Peng et al. 2000
	Yr17	*Ae. Ventricosa*	STS/CAPS	2A	Helguera et al. 2003
	Yr26	*H. Villosa*	SSR	6A	Ma et al. 2001
	Yr28	*T. aestivum*	RFLP	4DS	Sing et al. 2000
	YrH52	*T. dicoccoides*	SSR	1B	Peng et al. 2000
Powdery mildew	*Pm1*		RFLP	7AS	Ma et al. 1994
	Pm2		RFLP	5D	Ma et al. 1994
	Pm3		RFLP	1A	Ma et al. 1994,
	Pm4a		RAPD		Li et al. 1995
	Pm4b		AFLP		Hartl et al. 1998

Table 22.1 (continued)

Trait	Locus	Source	Marker type	Chr.	Reference
	Pm12	*Ae. speltoides*	RFLP	6B/6S	Jia et al. 1994
	Pm13	*Ae. longissima*	STS	3S	Cenci et al. 1999
	Pm18		RFLP	7AL	Hartl et al. 1995
	Pm21	*Haynaldia villosa*	SCAR	6VS, 6AL	Liu et al. 1999
	Pm25	*T. monococcum*	RAPD	1A	Shi et al. 1998
	Pm26	*T. turgidum*	RFLP	2BS	Rong et al. 2000
	H9		RAPD		Dweikat et al. 1994
	H21	*Secale cereale*	RAPD	2RL	Seo et al. 1997
	H23, H24	*T. tauschii*	RFLP	6D, 3DL	Ma et al. 1993
	H25	*Secale cereale*	SSR	4A	http://maswheat.ucdavis.edu/protocols/
	H31	*T. turgidum*	STS	5B	http://maswheat.ucdavis.edu/protocols/
Pest Resistance					
Russian	*Dn2*		SSR		Miller et al. 2001
Wheat	*Dn4*		SSR		Liu et al. 2002
Aphid	*Dn6*		SSR		Liu et al. 2002
Quality traits					
Kernel hardness	*Ha*	*T.aestivum*	STS	5B/5D	Giroux and Morris 1997
High protein	Gpc-B1	*T. dicoccoides*	ASA	6B	Distelfeld et al. 2006
LMW glutenins		*T. turgidum*		1B	D'Ovidio and Porceddu 1996
HMW glutenins	*Glu -D1 -1*	*T. aestivum*	ASA	1DL	D'Ovidio and Anderson 1994
Other Traits					
Heterodera avenae reistance	*Cre1*	*T. aestivum*	STS	2BL	Ogbonnaya et al. 2001
	Cre3	*T.tauschii*	STS	2DL	Ogbonnaya et al. 2001
Stature	*Rht-B1b*	*T. aestivum*	STS	4B	Ellis et al. 2002
	Rht-D1b	*T. aestivum*	STS	4D	Ellis et al. 2002
	Rht8	*T. aestivum*	SSR	2B	Korzun et al. 1998
Virus	*Bdv2*	*Ag. intermedium*	STS	7DL	Stoutjesdijk et al., 2001
Cadmium uptake		*T. turgidum*	RAPD		Penner et al. 1995
Meiotic pairing	*ph1b* deletion		STS	5BL	Qu et al. 1998
Vernalization	*Vrn-A1*	*T. aestivum*	STS	5A	Sherman et al. 2004

genomics include the identification of candidate genes associated with a QTL for Fusarium Head Blight resistance (Shen et al. 2006) and with a locus conferring sensitivity to Tan Spot toxin (Lu et al. 2006). Several web-based genomic resources that can be used in comparative genetics and synteny mapping are also available (e.g. http://www.gramene.org).

22.4 Functional Genomics

22.4.1 EST Development

EST development in wheat and other members of the Triticeae was lagging well behind many other plant species during the 1990s. Consequently, the global wheat research community, through the International Triticeae Mapping Initiative (ITMI), launched a collaborative effort to improve genomic resources for wheat, barley, rye, and wild relatives. As a first stage the International Triticeae EST Cooperative was established (http://wheat.pw.usda.gov/genome/). This group encourages laboratories each to contribute 1,000 or more ESTs; over 25,000 ESTs were accumulated within the first six months, and now wheat has the largest public EST database (over 850,000) of any plant species. Table 22.2 provides an overview of the number of ESTs publicly available for various members of the Triticeae at the time of writing. Key to the utilization of these EST resources is the availability of suitable database structures that facilitate the retrieval of relevant EST and related information. GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) has been the most widely used database for wheat and barley genetic and genomic information for many years (Matthews et al. 2003) and it continues to provide access to mapped and annotated ESTs. A comprehensive wheat EST database with annotations can be downloaded from http://harvest.ucr.edu/. More specific databases were assembled to support the development of the Affymetrix wheat gene chip and to provide information on EST assemblies. BarleyBase (http://www.barleybase.org/) has been one of the most important of these (Shen et al. 2005). There are also databases that link the requirements of crop scientists with EST resources and provide some valuable tools for wheat researchers such as CR-EST (Kunne et al. 2005). These extensive wheat EST resources have proven highly valuable in analyzing the expression of wheat genes and provide a tool for rapid gene expression profiling. A clear description of this application was recently provided by Mochida et al. (2006) based on ESTs derived from a set of 21 cDNA libraries. A more extensive, but less well structured set of libraries, was used by Chao et al. (2006) to provide an expression profiling resource. More specifically, Ciaffi et al. (2005) used EST resources to study spikelet development to identify possible candidates for more detailed analysis, while Ogihara et al.

Table 22.2 Number of Triticeae EST available in the public databases (1st Sept 2006)

Species	Number of ESTs
Triticum aestivum (wheat)	854,015
Hordeum vulgare subsp. vulgare (barley)	437,321
Hordeum vulgare subsp. spontaneum	24,150
Triticum monococcum	11,190
Secale cereale	9,195
Triticum turgidum subsp. durum	8,924
Aegilops speltoides	4,315
Triticum turgidum	1,938

(2003) used the expression profiles to group functional genes. Expression analysis in polyploid wheat has led to some surprising results. The analysis of expression patterns of homoeologous genes in wheat, based around the use of ESTs generated from diverse tissues, has confirmed that homoeologous genes can be expressed in just one genome and silent in one or both of the remaining genomes (Mochida et al. 2004). Further, the tissue specificity of homoeologous genes was also found to vary. It was particularly surprising to find that 72% of the homoeoloci studied showed genome-specific expression.

The EST collections are also proving valuable resources in supporting positional cloning projects in wheat. The large scale mapping of wheat ESTs carried out through a large NSF-funded project in the USA has provided a resource that is being used by wheat researchers around the world (http://wheat.pw.usda.gov/NSF/). The USA study used 7,104 EST in Southern hybridizations against wheat aneuploid stocks and a deletion line series to assign ESTs to specific chromosome bins. Each EST detected an average of 4.8 restriction fragments and 2.8 loci. The resultant map placed over 16,000 loci into their respective chromosome bins (Qi et al. 2004). The bin maps not only place a large number of genes onto the wheat physical and genetic maps but also provide a means for comparative genomics across the cereals. Resources such as these are valuable tools in comparative studies (for example, Hattori et al. 2005).

The large size of the wheat EST collections has provided opportunities for the development of several important resources. A clear application has been the development of microarray platforms. One of the earliest was a cDNA-based array (Wilson et al. 2004). However, oligo arrays have also been produced. The most widely used is the Affymetrix wheat Genechip (http://www.affymetrix.com/products/arrays/specific/wheat.affx) which represents over 55,000 transcripts. It is anticipated that transcript profiling datasets based on this array and other systems will be publicly available for wheat researchers in the near future, similar to those already available for barley (http://www.barleybase.org/). A reference dataset for wheat based on the Affymetrix GeneChip is currently under development and is likely to be released soon. This dataset will match a tissue series already developed for barley (Druka et al. 2006).

The wheat EST databases have also been used to develop SSR and SNP markers (reviewed by Varshney et al. 2005). There are several reports describing the development and mapping of such markers and comparing them to SSRs derived from other techniques (for example, Gadaleta et al. 2006; Yu et al. 2004). The collection of EST-derived SSRs is now extensive and they have proven useful in linking wheat genetic maps to maps from other cereals based on orthology to the genes from which the SSRs were derived (Tang et al. 2006; Zhang et al. 2005a). The EST-derived SSRs appear to be more readily transferable between species than previously developed SSRs, although the number of alleles detected and the level of variation tends to be lower. Nevertheless, EST-derived SSRs have proved useful for diversity studies (Zhang et al. 2006) and are suitable for determining variation and mapping in the wild relatives of wheat (Mullen et al. 2005). The development of SNPs from EST resources has been slower than EST-SSR discovery.

However, a large-scale effort is underway through an NSF-funded project in the USA (http://wheat.pw.usda.gov/NSF/). A database of primers, SNPs, and the status of the program can be found at http://rye.pw.usda.gov/snpworld/Search).

22.4.2 TILLING

Mutagenesis has been widely used in crop improvement since the 1950s and many modern cultivars carry mutations induced by chemical mutagenesis or ionizing radiation. The recent discovery of enzymes capable of cleaving single base mismatches provides a tool for high throughput screening of single base differences in mutant populations and allows mutant alleles to be found in a target gene. The technique, referred to as targeting induced local lesions in genomes (TILLING), has revitalized mutation research as it provides a method to knock out genes and allows the generation of variation without the need for transformation, greatly simplifying the regulatory process. The method and background has been recently reviewed by Slade and Knauf (2005) and by Comai and Henikoff (2006).

Concerns have been expressed regarding the utility of this technique in wheat since it was felt that polyploidy would hide mutations and complicate both the screening and the phenotypic assessment of mutant lines. However, the technique has proved highly successful in wheat (Slade et al. 2005; Weil 2005). Polyploidy appears to allow wheat to tolerate a far higher mutation load than diploid crops and this reduces the number of mutant families that must be screened. Therefore, Slade et al. (2005) were able to recover 246 alleles in the waxy genes from a screen of only 1,920 mutagenised lines. Given that wheat has only two functional waxy genes (granule-bound starch synthase I) this represents a surprisingly high success rate. Several groups around the world are now developing mutant or TILLING populations for bread and durum wheat, and this is likely to become a widely used technique in functional analysis of candidate genes.

22.4.3 Transformation as a Tool in Genomics

The success of genetic transformation depends on the proper introduction and insertion of the target gene into the nuclear genome and ensuring its expression in a heritable manner (Shewry and Jones 2005; Jones 2005). Usually, soft explant tissue derived from immature embryos is used as the source material for wheat transformation. Micro-projectile bombardment (Sparks and Jones 2004) has been extensively used in the past as the means of delivery of gene constructs. However, Agrobacterium-mediated transformation systems are preferred as they enable the delivery of single copy insertions (Wan and Layton 2006; Wu et al. 2006) and are subject to a lower frequency of transgene silencing (Hu et al. 2003). Alternative transformation methods are being investigated in an attempt to circumvent the tight intellectual property controls associated with biolistic and Agrobac-

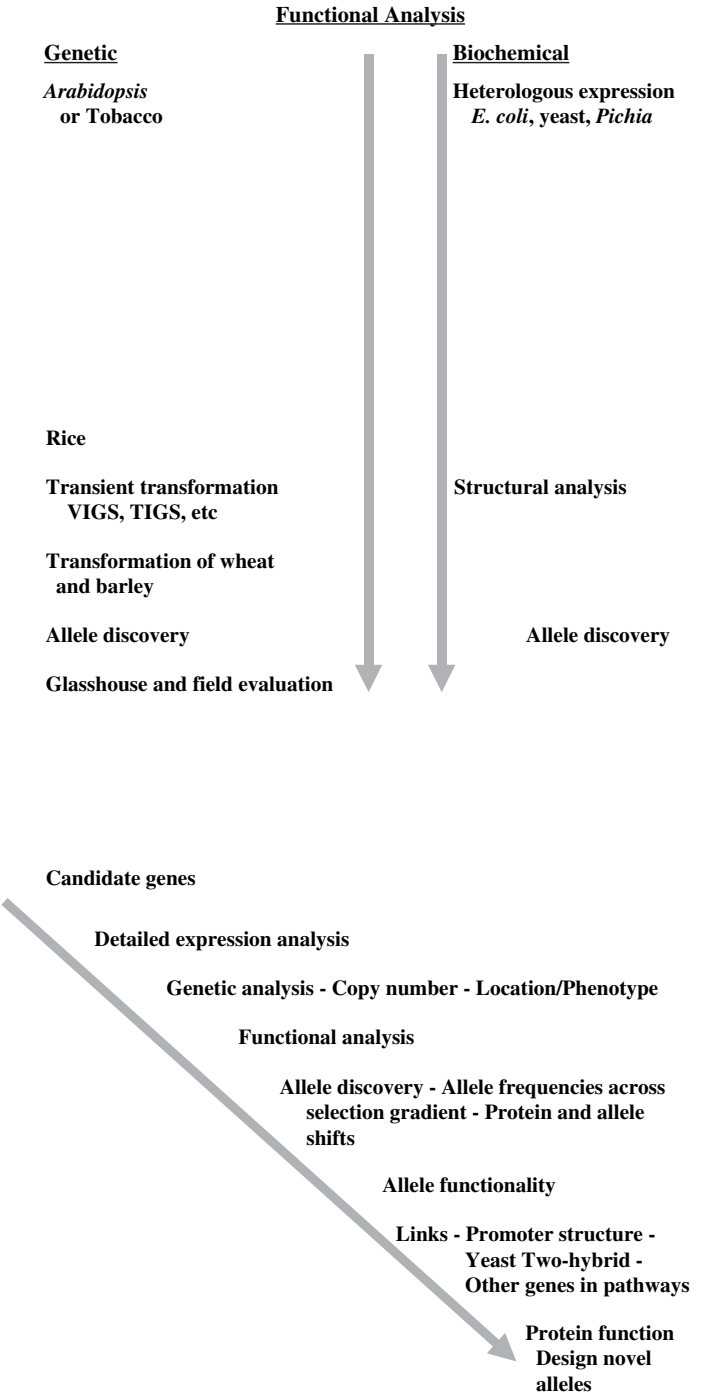

Fig. 22.2 An outline of various processes involved in functional analysis of genes and alleles

terium transformation protocols (for example, Badr et al. 2005). A summary of the selectable markers that are suitable for use in wheat transformation can be found in Goodwin et al. (2005).

Transformation has become an important tool in functional analysis either through ectopic expression of the transgenes or through gene silencing or reduced expression. There are now many examples in the literature where transformation has been used for functional analysis. One recent example was the characterization of the "Q" gene from wheat (Simons et al. 2006). This gene is responsible for the free-threshing character that was crucial for wheat domestication. Ectopic expression allowed both silenced and over-expressed phenotypes to be observed and was important in confirming the identity of the cloned gene.

Transient transformation has also been useful in functional analysis; an illustration is given by Srichumpa et al. (2005). In this case transient single cell transformation was used to study several alleles of the powdery mildew resistance locus, *Pm3*. A related technique has been the use of virus-induced gene silencing (VIGS) to specifically knock down the expression of candidate genes. In wheat this method uses barley stripe mosaic virus (BSMV) (Holzberg et al. 2002), but it has not yet been widely used for functional analysis. Nevertheless, Scofield et al. (2005) were able to use VIGS for functional analysis of the wheat leaf rust resistance gene, *Lr21*. An outline of various processes involved in functional analysis of genes and alleles is given in Fig. 22.2.

22.5 Applications of Genomics in Wheat Breeding

22.5.1 Developing an Effective Integrated Marker-Assisted Selection System

Once a marker is identified through linkage or association mapping analysis, its utility as an indirect selection tool must be validated in appropriate breeding populations. Validation failures can be due to an absence of polymorphisms at the locus in the target germplasm, different recombination patterns in the target germplasm causing loss of linkage between the marker and the target locus, or confounding effects of new epistatic interactions between the marked locus and the genetic background of the target germplasm. The practical value of a marker depends on how successfully it can be integrated into a breeding program and how easily it can be applied on a large scale in modern breeding programs. Marker systems such as RFLPs or AFLPs do not meet these criteria due to the laborious nature of their application. Thus, molecular breeding programs should focus on PCR-based assay systems such as STS, SSR, and SNP markers.

Simply inherited disease resistance is a common target for marker-assisted selection (MAS), particularly where breeding programs do not have ready access to disease hot spots and where there is a need to pyramid resistance genes. In wheat, there are a number of inter-chromosomal translocations from related species that carry useful

genes for which markers are available; these markers allow the translocated segment containing the target gene to be easily introduced into elite lines (http://maswheat.ucdavis.edu/; McIntosh et al. 2003). However, molecular dissection of loci that contribute to complex traits such as yield and abiotic stess tolerance remains a considerable challenge, even with the newly available marker technologies. MAS applications for complex traits are limited because of the rarity of QTLs of large effect with good stability across cropping environments and diverse genetic backgrounds.

Public wheat breeding programs generally use pedigree breeding methods or modifications thereof. Individual plants are selected in early generations to increase the frequency of simply inherited alleles for the target traits such as resistance to diseases, plant height and stature, agronomic type, etc. At more advanced generations, when a sufficient level of homozygosity is present, selections are then made among families for quantitatively inherited traits such as yield, drought, heat and salinity. The exact profile of target traits will be specific to the target region. For example, many wheat breeding programs in Australia use markers for race specific leaf and stripe rust resistance genes, where these genes are still effective. In contrast, CIMMYT's wheat improvement strategy is based on durable or race non-specific genes. Race-specific genes are avoided because the rust pathogen overcomes these resistances with time. However, race-specific genes are more effective if deployed in combination and breeding programs in many regions have attempted to do this. An example is the effort to pyramid race-specific resistance genes to counter a recent outbreak of a new strain of stem rust in eastern Africa (http://globalrust.org).

A number of wheat breeding programs have begun to use marker-assisted selection on a modest scale. Breeding programs have to develop pragmatic approaches to integrating MAS. The breeding strategies used will be dependent on breeding objectives, resource availability, and information from genetic characterization of different traits. For example, MAS may not be justified for simply inherited traits that can be reliably screened under field conditions such as disease resistance, unless there are extenuating circumstances. Thus, it may not be possible to reliably pyramid disease resistance genes without the use of markers. Similarly, it may be important to carry out MAS to retain disease resistance loci during single seed descent programs. Markers are also valuable for characterizing potential parental genotypes in order to assist in designing crosses.

Marker-assisted breeding strategies can also be designed to rapidly and efficiently generate fixed lines for a target gene or combination of genes. Considering the relatively high cost of DNA extraction and subsequent marker assays, it is important to identify the optimum points for MAS interventions in the breeding process to increase the efficiency and effectiveness of the breeding program. Coordinated programs for MAS have been established in various countries:, the National Wheat Molecular Marker Program (NWMMP) in Australia, established in 1996 (Eagles et al., 2001); the national wheat MAS consortium in the USA, established in 2001 (Dubcovsky 2004); similar initiatives in Canada (R. DePauw and C. Pozniak, pers. comm.); and cooperatives established among breeding companies in Europe (Koebner and Summers 2003). Target traits for MAS include a range of disease and pest resistances and quality traits.

Although genetically modified (GM) forms of wheat are not currently in commercial use, ongoing gene discovery projects will likely find candidate genes with potential for future transformation programs. Some cultivars are clearly more receptive to transformation than others. When the cultivar with the best agronomic type is not the most receptive to transformation, it is possible to transform a more receptive cultivar (Pellegrineschi et al. 2002) and then introgress the gene into the target background using diagnostic markers for the transgene. This type of MAS aided line conversion can be accomplished for any crop species including wheat. Marker-assisted introgression of transgenes into a range of desired backgrounds is commonly practiced in the private sector for crops such as maize.

22.5.2 Marker-Assisted Selection in the CIMMYT Wheat Breeding Program

An important feature of CIMMYTs marker implementation program is the systemic integration of molecular genotyping with field-based screening. Currently, reliable markers are used for a limited number of traits. These traits are relevant to the breeding program's goals and therefore justify the investment in MAS. To keep the number of assays manageable within the breeding program, markers are used once in the early generations to favorably skew allele frequency and again on the advanced progeny to confirm the presence of the target alleles in the genetically fixed material. When two or more genes are targeted using markers, the segregating progeny are usually screened using MAS at the F_1 top-cross or F_2 generations. Tissue sampling is delayed as long as possible in the field to allow the breeder to first select for disease reaction and agronomic type; materials are then screened for presence/absence of the target alleles using markers. This strategy reduces the time available to run large numbers of marker assays as tissue sampling occurs later in the growth cycle and the breeder requires the gene profiles before harvest; an alternative strategy is to sample plants in the seedling stage, this extends the time available to provide the marker data but results in the screening of many plants with unsuitable background genes and agronomic type. Only fixed lines positive for the target markers are advanced to expensive replicated multilocational yield and quality evaluation (William et al. 2007). The extent of MAS investment at CIMMYT is determined by the importance of the target trait to the breeding program, the reliability of alternative phenotypic screens, and the additional selective power provided by the assays.

22.6 Key Challenges for Molecular Breeding of Wheat

The improvements in wheat yields attributable to the Green Revolution were achieved by radically changing the crop architecture to maximize yield under high-input conditions. It is unlikely that a similar modification for any other single trait

will lead to such dramatic increases in yield again. Thus, it is expected that continued progress in productivity will come through incremental improvements. Yield potential and crop adaptation are constrained by a number of factors including: available genetic variability for yield enhancing traits; the complexity of inheritance of economically important traits such as yield potential and drought tolerance; climate change and erosion of productivity in many farming systems. The large-scale use of resynthesized wheat lines in the CIMMYT breeding programs has led to dramatic improvements in both yield potential and adaptation to multiple stresses (Trethowan et al. 2005a) and adaptation around the globe (Dreccer et al. 2007; Lage and Trethowan 2007). At CIMMYT, improvements in drought stress adaptation attributable to resynthesized wheat were achieved by improving the heritability of drought screening procedures (Trethowan and Reynolds 2006) and the understanding of the physiological basis of adaptation to drought (Reynolds et al. 2007).

It is likely that the negative effects of climate change on wheat production in countries at lower latitudes such as India and Pakistan will be much greater than in developed countries where production may even increase as lands at high latitude are brought into production (Rosenzweig and Hillel 1995). According to Trethowan et al. (2005b), the area in India currently regarded as close to optimal for wheat production will halve over the next 40 to 50 years as temperatures increase. Wheat breeding can help mitigate some of the effects of climate change, largely by improving adaptation to higher temperatures and increasing drought tolerance and/or water use efficiency.

Many farmers have introduced conservation agriculture (reduced or zero-tillage and crop residue retention) to reduce erosion, improve crop water use, and reduce costs, thereby improving overall profitability and sustainability of farming. These changes have significant implications for wheat breeders. For example, the spectrum of wheat diseases changes with stubble retention, such as diseases like tan spot (*Pyrenophora tritici-repentis*) and crown rot (*Fusarium pseudograminearum*), become more prevalent (Duveiller and Dubin 2002; Mezzalama et al. 2001). In addition, evidence also exists of a cultivar x tillage practice interaction for both yield and quality (Gutierrez 2006). Although characters such as coleoptile length do explain some of the variation in crop emergence and establishment in these systems (Trethowan et al. 2005a), most of the variation remains unexplained. Clearly, the key traits required for good performance in resource conservation systems must be identified if cultivars are to be bred that are better adapted to such farming systems.

22.6.1 Future Prospects for Wheat Molecular Breeding

If the rates of advance in wheat yields are to be maintained or even increased, our understanding and ability to manipulate the underlying genetic control of complex characters such as yield and abiotic stresses must be improved. The search for QTLs influencing yield and stress tolerance has been confounded by poor quality phenotypic data, the inappropriate nature and size of mapping populations, or

the inadequate density of molecular markers. Genotype x year interactions are frequently the single largest source of variation in the analysis of multi-environment trials. Therefore, it is not surprising that many QTLs are not consistent across seasons, locations, or populations.

Traditional QTL mapping using genetic populations generated by crossing two genotypes contrasting for a trait of interest has been useful in establishing the putative genomic location of the genetic factors contributing to the trait and for partitioning the variation in to single Mendelian genetic factors. However, this mapping approach is slow and expensive and can elucidate only the relative effects of the two alleles contributed by the two parental genotypes. Moreover, the resultant markers are often population dependent, thus suffering a substantial level of redundancy when validated in breeding populations. Association analysis has the potential to overcome these problems and improve the cost efficiency and speed of marker identification for certain important agronomic traits. Although linkage mapping in biparental populations is likely to remain important for some traits and where fine mapping is required.

The existence of phenotypically well characterized breeding populations combined with new cost effective genome-wide scan technologies (such as DArT) and association analysis approaches offers powerful new opportunities. For example, advanced CIMMYT wheat breeding lines have been distributed annually to around 100 global locations for the past half century. Yield and agronomic data have been collected from these trials and returned to CIMMYT for analysis and collation in public access databases. Seed of all these materials was kept in the CIMMYT gene bank and is now being used for genotyping and pilot testing of association analysis using breeding material (Crossa et al. 2007). It is hoped that this approach will identify genomic regions with a putative influence on yield potential and other complex agronomic traits.

The large-scale use of markers in wheat breeding is still limited due to a lack of markers for high value traits and the absence of low cost high throughput analytical platforms appropriate to the needs of wheat molecular breeding. Marker detection through capillary electrophoresis offers significant incremental advances in throughput and unit costs, but dramatic progress will have to await appropriate SNP-based systems. Large-scale EST sequencing projects will undoubtedly lead to the generation a large number of SNP gene-based markers. SNP markers developed in this way will then provide an important source of candidate gene-based markers for molecular breeding and allele mining. There are a number of potential high throughput platforms for large-scale low cost simultaneous genotyping of less than one hundred SNP markers, which may be appropriate for the next generation of wheat molecular breeding applications scenarios: (i) Luminex (http://www.appliedcytometry.com/starsupport/docs/STarBase.pdf) which currently offers simultaneous detection of up to around 50 SNP polymorphisms per DNA sample based on bead hybridization and detection coupled with flow cytometry; (ii) SNPWave (http://www.keygene.com/techs-apps/technologies_snpwave.htm) which is based on highly multiplexed allele discrimination using capillary electrophoresis and may allow selective simultaneous detection of nearly 100 SNPs; (iii) TaqMan

(http://www. appliedbiosystems.com) which is based on allele discrimination using RT-PCR technology based on 5' nuclease activity that has been adapted for high throughput applications (Ranade et al. 2001). Recent advances of the technology have enabled deployment of 384-well based platforms; (iv) MassARRAY (www.sequenom.com) technology combines primer extension reaction chemistry with mass spectrometry based on MALDI-TOF for rapid and cost effective characterization of SNP polymorphisms. In the human diagnostics arena, researchers have been able to reduce the average cost of SNP genotyping from US$1 to 10 cents per data point (Roses 2002). Although this is based on intensive investment in optimization of a range of candidate SNP markers, similar advances will ultimately be possible for wheat molecular breeders. The added advantage of SNP-based marker systems is the avoidance of gel-based allele separation for visualization and their potential for automation in high throughput assay platforms. This ongoing research will inevitably lead to the development of more robust, simple and cost effective high throughput assays (Jenkins and Gibson 2002). The challenge is establishing an intimate and iterative collaboration between molecular biologists and wheat breeders such that the results of whole genome scanning and association genetics can be rationalized and deployed in wheat breeding programs. These techniques have the potential to substantially improve parent selection for crossing, the rate of genetic gain, and the time taken to develop new cultivars.

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