5

Application of Genomics to Molecular Breeding of Wheat and Barley

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ABSTRACT

In wheat and barley, several generations of selectable molecular markers have been included in the genetic maps; and a large number of qualitative and quantitative traits were located in the genomes, some of which are being routinely selected in marker-assisted breeding programs. In recent years, a large number of expressed sequence tags (ESTs) have been generated for wheat and barley that have been used for development of functional molecular markers, preparation of transcript maps, and construction of cDNA arrays. These functional genomic resources combined together with new approaches such as expression genetics, association mapping, allele mining, and informatics (bioinformatic tools) possess potential to identify genes responsible for a trait and their deployment in practical plant breeding. High costs currently limit the implementation of functional genomics in breeding programs. The potential applications together with some examples as well as challenges for applying genomics research in breeding activities are discussed. Genomics research will continue to enhance the efficiency and precision for crop improvement but will not replace conventional breeding and evaluation methods.

I. INTRODUCTION

Wheat (*Triticum* spp.) and barley (*Hordeum* spp.) belong to the *Poaceae*, the largest family within the monocotyledonous plants; it includes other major cereal crops of the world such as maize, rice, and rye, as well as important forage grasses such as Ryegrass, Fescue, and Kentucky bluegrass. Among the food crops, wheat and barley are important sources of energy and proteins for the world population and are cultivated over a wide range of climatic regions. Both wheat and barley are among the most extensively studied crop species, particularly in the area of cytogenetics. An extensive catalogue of genetic and cytogenetic stocks such as aneuploid lines, deletion stocks, translocation lines, and so on is available for these crop species (Varshney *et al.*, 2004a,b, 2006a). While barley (*H. vulgare*) is a self-pollinating diploid with $2n = 2x = 14$ chromosomes (H genome), wheat has diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$), and hexaploid ($2n = 6x = 42$) species. However, most modern wheat varieties are hexaploid (*T. aestivum*), described as "common" or "bread" wheat and valued for bread making. Bread wheat is an allopolyploid containing the three distinct but genetically related (homoeologous) genomes—A, B, and D. Although both wheat (hexaploid) and barley are characterized by large genome size with 18,000 and 5000 Mb, respectively, more than 80% of the genome consists of repetitive DNA sequences (Schulman *et al.*, 2004). Such large genomes with the repeated sequences make

both genome analysis and crop improvement a challenging task (Langridge *et al.*, 2001).

In recent years, however, due to advances in the area of genetics and genomics, significant progress has been made, and high density molecular genetic as well as physical maps (cytogenetic stocks-based) have become available for wheat and barley. Molecular markers are increasingly being used to tag genes or quantitative trait loci (QTLs) of agronomic importance, offering the possibility of their use in marker-assisted selection (MAS) for breeding (Jahoor *et al.*, 2004; Varshney *et al.*, 2004b, 2006a). In addition to their use in MAS, molecular marker maps have proven to be instrumental resources for the isolation of genes via map-based cloning (Stein and Graner, 2004) and comparative mapping studies in cereal species (see Devos, 2005; Devos and Gale, 2000). Moreover, comprehensive resources, including largest sets of expressed sequence tags (ESTs), bacterial artificial chromosome (BAC) libraries, and DNA arrays, have been developed to facilitate a systematic exploration of the corresponding genomes on the structural and functional levels (Close *et al.*, 2004; Zhang *et al.*, 2004a,b). In this chapter, we review recent progress related to the applications and potential of genomics research in molecular breeding of wheat and barley. Significant emphasis has been laid on the impact of functional genomics and other recent approaches such as association mapping and genetical genomics applied to wheat and barley breeding.

II. MOLECULAR MARKERS AND MARKER-ASSISTED BREEDING

The identification and utilization of genetic variation form the basis of plant breeding. During the process of breeding new varieties, the breeder needs to make decisions at several key points, such as the identification of the most appropriate parents for crosses and the selection of the most desirable individuals among the progeny of the cross (Langridge and Chalmers, 2004). To assess the efficiency of the breeding and selection process, a key issue in any plant-breeding program is the number of lines carried through the evaluation and selection phases. For large wheat- and barley-breeding programs, hundreds of thousands of lines are often required to produce a new variety. In order to save the costs related to running extensive field trials and carrying out the evaluation of some traits, for example, components of grain quality and yield stability, molecular markers provide the opportunities for replacing the expensive and often unreliable bioassays in a cost-effective manner (Koebner *et al.*, 2001). Molecular markers are now widely used to track loci and genome regions in many wheat- and barley-breeding programs, as molecular markers tightly linked with a large number of agronomic and disease resistance traits are available in these species (Gupta *et al.*, 1999; Jahoor *et al.*, 2004; Tuberosa and Salvi, 2004;

Varshney *et al.*, 2006a). In fact, a variety of molecular markers, including restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellite or simple sequence repeat (SSR), have been used for gene tagging and QTL analysis. However, the consensus is that SSRs are presently best suited for the use in marker-assisted breeding (Gupta and Varshney, 2000; Gupta *et al.*, 2002b). RFLP is not readily adapted to high sample throughput, and RAPD assays are not sufficiently reproducible or transferable between laboratories. While both SSRs and AFLPs are efficient in identifying polymorphisms, SSRs are more readily automated (Shariflou *et al.*, 2003). Although AFLPs can be principally converted into simple PCR assays (STS), this conversion can become complicated in large genome templates, as individual bands are often composed of multiple fragments (Carter *et al.*, 2003; Shan *et al.*, 1999). Although use of rare cutter restriction enzyme, such as *Pst*I, in AFLP can increase the frequency of single copy AFLPs; but on the other hand this technique has the risk of detecting only methylation polymorphisms, which may not be stable within or between genotypes (Pellio *et al.*, 2005). Other classes of molecular markers, that are, single nucleotide polymorphisms (SNPs) or single feature polymorphisms (SFPs) are being developed and integrated to genetic maps (Cui *et al.*, 2005; Kota *et al.*, 2003; Rostoks *et al.*, 2005). The inclusion of many molecular markers, especially microsatellite markers on genetic maps of wheat and barley (Table 5.1), will ease their use for marker-assisted breeding.

A. Functional molecular markers

Most of the molecular markers developed, as mentioned above, have been designed from genomic DNA sequences, and therefore they could belong to either transcribed or nontranscribed regions of the genome. Such markers sample genetic variation in the genome more or less randomly and are sometimes referred to as "neutral" or "random" markers (RMs). However, over the last few years, functionally characterized genes, ESTs, and genome-sequencing projects have facilitated the development of molecular markers from the transcribed regions of the genome. Among the more important and popular molecular markers that can be developed from ESTs are SNPs (Rafalski, 2002), SSRs (Varshney *et al.*, 2002, 2005a), or COS (conserved othologous set—the markers that can be used across species, as sequences for such markers are highly conserved; Fulton *et al.*, 2002; Rudd *et al.*, 2005). Putative functions can be deduced for the markers derived from ESTs/genes using homology searches (BLASTX) with protein databases (e.g., NR-PEP, SWISSPROT, and so on). Therefore molecular markers, generated by utilizing (gene) sequence data, are known as "functional markers" (FMs; Andersen and Lubberstedt, 2003). FMs have some advantages compared with RMs as they are completely linked to

Table 5.1. Available SSR Markers in Wheat and Barley

Populations	Number and type[a] of SSR loci mapped	Designation of SSR	References
Wheat			
ITMI RILs (W7984 × Opata85)	279 gSSR	gwm	Röder *et al.* (1998)
F2s (Chinese Spring × Synthetic)	53 gSSR	psp	Stephenson *et al.* (1998)
ITMI RILs (W7984 × Opata85)	65 gSSR	gdm	Pestsova *et al.* (2000)
ITMI RILs (W7984 × Opata85), deletion lines	168 gSSR	barc	Song *et al.* (2002)
ITMI RILs (W7984 × Opata85)	66 gSSR	wmc	Gupta *et al.* (2002a)
ITMI RILs (W7984 × Opata85)	84 gSSR	cfd	Guyomarc'h *et al.* (2002)
ITMI RILs (W7984 × Opata85)	22 eSSR	DupW	Eujayl *et al.* (2002)
Consensus map based on four mapping populations (W7984 × Opata85, RL4452 × AC Domain, Wuhan × Maringa, Superb × BW278)	1108 gSSR	wmc, gwm, gdm, barc, cfa, cfd	Somers *et al.* (2004)
RILs (W7984 × Opata85; Wenmai6 × Shanhongmai), DHs (Lumai14 × Hanxuan10)	101 eSSR	Cwm or *GeneName*-SSR	Gao *et al.* (2004)
ITMI RILs (W7984 × Opata85)	149 eSSR	cnl, ksu	Yu *et al.* (2004a)
RILs (W7984 × Opata85)	126 eSSR	gpw/cfe	Nicot *et al.* (2004)
RILs (W7984 × Opata85)	48 eSSR	cwem	Peng and Lapitan (2005)
ITMI RILs (W7984 × Opata85)	>600 gSSR	gwm	Röder and Ganal (personal communication)

(*Continues*)

Table 5.1. (*Continued*)

Populations	Number and type[a] of SSR loci mapped	Designation of SSR	References
Barley			
DHs (Igri × Franka, Steptoe × Morex, Harrington × Morex, Harrington × TR306)	45 gSSR	HVM	Liu *et al.* (1996)
DHs (*H. vulgare* var Lina × *H. spontaneum* Canada Park)	242 gSSR	Bmac, Bmag, Ebmac, Ebmag, Hv*GeneName*	Ramsay *et al.* (2000)
F_2s (Lerche × BGRC41936), DHs (Igri × Franka)	57 eSSR	Hv*GeneName*	Pillen *et al.* (2000)
Consensus map based on three mapping populations (Igri × Franka; Steptoe × Morex; OWB Dom × OWB Rec)	185 eSSR	GBM	Thiel *et al.* (2003), Varshney *et al.* (2006b)
DHs (Igri × Franka, Steptoe × Morex)	127 gSSR	GBMS	Li *et al.* (2003)
DHs (*H. vulgare* var Lina × *H. spontaneum* Canada Park), F_2 (SuspTrit × Cebada Cepa)	65 eSSR	GBM	Marcel and Niks, Wageningen (personal communication)

[a]gSSR, derived from genomic DNA after isolating from genomic library; eSSR, derived from ESTs after searching ESTs for SSRs.

the corresponding trait allele (Varshney *et al.*, 2005c). Such markers may be derived from the gene responsible for the trait of interest and target the functional polymorphism in the gene, thus allowing selection in different genetic backgrounds without revalidating the marker–QTL allele relationship. Thus, they have also been referred as "perfect markers" or "diagnostic marker" even though different alleles with the same polymorphism (resulting from intragenic recombination, insertion, deletion, or mutation) may produce different phenotypes. A perfect marker allows breeders to track specific alleles within pedigrees and populations and minimize linkage drag—segregation of undesirable segments with gene of interest—flanking that gene.

In recent years, due to emphasis on functional genomics, an excellent collection of ESTs has been developed in wheat (Zhang *et al.*, 2004a) and barley (Zhang *et al.*, 2004b). In terms of numbers, wheat with 600,039 and barley with 419,146 ESTs rank number second and fourth among EST collections for plant species (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html, dbEST release 102805). Both genetic and deletion stocks (with various sized terminal deletions in individual chromosome arms, useful for sub-arm localization of genes/markers)-based physical maps of wheat and barley have been generated with EST-based markers, for example EST-SSRs (Gao *et al.*, 2004; Nicot *et al.*, 2004; Peng and Lapitan, 2005; Thiel *et al.*, 2003; Yu *et al.*, 2004a; see Table 5.1), EST-SNPs (Kota *et al.*, 2001; Somers *et al.*, 2003), EST-RFLPs (Qi *et al.*, 2004; Varshney *et al.*, 2005c), and EST-CAPS (K. Sato, Japan, personal communication). As a result, high-density transcript maps for barley with over 3000 EST loci (personal communications with K. Sato, Japan; R. Waugh, UK), and wheat with over 16,000 EST loci (Qi *et al.*, 2004), have or will be shortly available. Microarray-based gene-expression data between two genetically different lines can also be utilized to identify single feature polymorphisms (SFPs) for SNP detection in a highly parallel manner (Borevitz *et al.*, 2003) which can be exploited to develop FMs. In fact, in a study using 17 and 19 Affymetrix GeneChip expression datasets for two genotypes, >10,000 SFPs have been identified between the two genotypes of barley, a species with a large and complex genome (Rostoks *et al.*, 2005). By using another alternative method, with a smaller number (three) of replicate datasets and a different statistical method (robustified projection pursuit), about 2000 SFPs have been identified in another study of barley (Cui *et al.*, 2005). However, identification of SFPs involves the problem of sensitivity versus selectivity, that is, a large number of putative SNPs could not be confirmed (Rostoks *et al.*, 2005). Furthermore, the development of SNP markers in polyploid crop species such as wheat is complicated by the need to distinguish intragenome from intergenome polymorphisms, referring to the "three genomes" carried by this species: A, B, and D (Powell and Langridge, 2004).

B. Status of marker-assisted breeding

Prior to the deployment of DNA markers in plant breeding, the markers need to be validated, a process in which functionality is tested in a range of genetic backgrounds (Gupta *et al.*, 1999; Langridge and Chalmers, 1998). For instance in wheat, marker validation studies were conduced for QTLs for grain protein content by using near isogenic lines (NILs), the lines that are genetically identical except at one or a few loci (Singh *et al.*, 2001), for *Lr10* by using 16 wheat cultivars (Blazkova *et al.*, 2002), for the QTL for Fusarium head blight (FHB) resistance by using the progeny of crosses between the FHB-resistant spring wheat line and five European wheat varieties (Angerer *et al.*, 2003), or NILs from existing breeding populations (Pumphrey and Anderson, 2003) and in germplam collections (Zhou *et al.*, 2003). Similarly markers associated with preharvest sprouting (Kato *et al.*, 2001; Mares and Mrva, 2001), plant height (Ellis *et al.*, 2002), and barley yellow dwarf virus (Ayala *et al.*, 2001) were validated and used for enriching favorable allele frequencies in segregating populations and tracking donor parent alleles during backcrossing (Cakir *et al.*, 2003) in wheat. Molecular markers have also facilitated the pyramiding of multiple disease resistance genes in wheat and barley. For example, Liu *et al.* (2000) integrated three powdery mildew resistance gene combinations (*Pm2* + *Pm4a*, *Pm2* + *Pm21*, and *Pm4a* + *Pm21*) into an elite wheat cultivar called "Yang158." Similarly, marker-assisted backcross introgression of *Yd2* gene conferring resistance to barley yellow dwarf virus was conduced in barley (Jefferies *et al.*, 2003).

A particular effort to use MAS in wheat- and barley-breeding programs has been initiated in Australia. More than two dozen loci each in wheat and barley are currently being used in the Australian cereal-breeding programs (Langridge, 2005). For breeding programs in which molecular markers are actively being used, it has been estimated that over half the varieties currently being released have used markers at some stage during the breeding process. For instance, in the South Australian Barley Improvement Program markers were deployed to eliminate defects in elite varieties and a "Sloop type" variety with cereal cyst nematode (CCN) resistance was advanced to commercial release in less than 8 years (Langridge, 2005). Several other new Australian varieties have been developed through the use of markers. While using marker-assisted backcrossing in combination with the production of doubled haploids (DHs), the time from the initial cross to release of the variety has been almost halved when compared with conventional breeding. In fact in conventional breeding programs, 12 years were required on average from the first cross to the release of a wheat variety and 14 years for a malting quality variety in the breeding programs of South Australia. The barley variety "Tango," released in 2000 in the United States, is claimed to be the first barley variety developed by molecular MAS. It contains

two QTLs for adult resistance to stripe rust, a character difficult to handle by conventional phenotypic selection (Toojinda *et al.*, 1998). These were transferred into the 1970s variety "Steptoe" via two cycles of RFLP-aided backcrossing (Hayes *et al.*, 2003a). Although "Tango" has a good level of rust resistance, its yield is less than that of its long outclassed recurrent parent, and hence it is seen primarily as a genetically characterized source of resistance rather than as a variety in its own right. Just as for wheat, most of the proposed targets for MAS in barley relate to genes for disease resistance, although for many of these disease-efficient phenotypic screens are available. On the other hand, malting quality represents an important QTL target for MAS breeding in barley, because this complex trait is difficult to score and malting barley attracts a substantial price premium compared to feed barley (Ayoub *et al.*, 2003; Han *et al.*, 1997; Hayes *et al.*, 2003b).

The above examples demonstrate the potential of marker-assisted breeding concepts. Nevertheless, the best possible integration of marker-assisted concepts in a given breeding program depends on a variety of issues, such as the traits under consideration and the availability of closely linked markers, the costs for phenotypic versus MAS as well as the breeding scheme (backcross vs pedigree based approaches). On the basis of a simulation study, the combination of MAS at the BC_1F_1 and haploid stage was identified as the optimal strategy (Kuchel *et al.*, 2005). This study showed that incorporation of marker selection at these two stages not only increased genetic gain over the phenotypic alternative but also actually reduced the overall costs by 40%. Furthermore, as the unit marker assay costs are expected to decrease with the development of automated platforms and high-throughput marker systems, it is anticipated that MAS assays will become increasingly competitive. In a similar way, the deployment of MAS for wheat and barley breeding will benefit from development of additional FMs.

C. Whole-genome breeding

As mentioned above, in several cases molecular makers have been successfully utilized in trait-based breeding. Since extensive genetic information is available about a wide range of traits covering disease resistance, abiotic stress tolerance, and aspects of quality, it is possible, in principle, to target large numbers of loci at once in breeding strategies to manage the entire genome. This process is termed as "whole-genome breeding" (Langridge, 2005). There are several ways in which this can be achieved but it does require major shifts in breeding methodologies and is likely to be specific for each task being addressed. The overall concept is illustrated in Fig. 5.1. On the basis of molecular information derived from a large number of crosses, Jefferies (2000) proposed the concept of developing a genetic ideotype. The ideotype shown in Fig. 5.1 was prepared on the basis of known allele composition at 10 target loci or regions for 5 key varieties namely "Alexis" (European malt quality), "Sahara" (North African

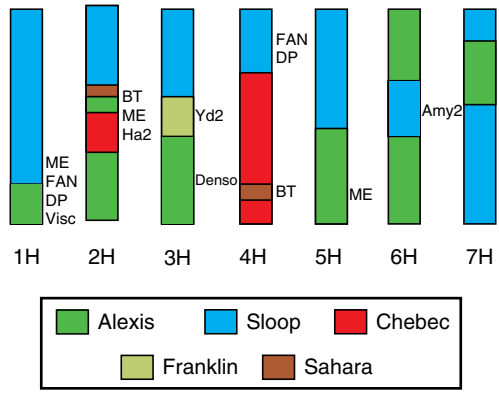

Figure 5.1. Genetic ideotype based around a set of five barley varieties. Each chromosome has been colored to reflect the desired region. The location of some key loci is indicated (ME, malt extract; FAN, free amino nitrogen; DP, diastatic power; Visc, wort viscosity; BT, boron tolerance; Ha2, cereal cyst nematode resistance; Yd2, barley yellow dwarf virus resistance; Denso, dwarf; Amy2, α-amylase) [adapted from Jefferies (2000)].

landrace), "Sloop" (Australian malt quality), "Chebec" (well-adapted Australian), and "Franklin" (Australian malt quality). It can be seen that in some cases there are blocks of loci all with the desirable alleles, the breeders seek to maintain or transfer these blocks into a new variety, for example the region on linkage group 1H. This linkage block can be sourced from "Alexis." The linkage group 2H is complex, and therefore several specific recombination events are required to bring together the desirable alleles from "Sahara," "Sloop," and "Chebec." For constructing such a variety, the populations should be designed so as to achieve the desired structure for each chromosome. Further, the population size can also be kept manageable by working with the chromosomes (linkage groups) one at a time and bringing them together only in the final variety.

The whole-genome breeding approach has already been used in Australian breeding programs. In fact, graphical genotypes developed for DH lines of a cross between "Alexis" and "Sloop" revealed one individual with the appropriate configuration for chromosomes 1H, 5H, 6H, and 7H (Fig. 5.1). Therefore, only remaining chromosomes of the barley genome (2H, 3H, and 4H) needed to be "redesigned," and this could be achieved by selecting lines with key recombination events and the reassembling all chromosomes in the final cross (Langridge, 2005). The strategy followed with some modifications was used to produce the variety "Flagship" that was released in 2004.

The genetic ideotype strategy is only one of many possible whole-genome breeding approaches that could be applied to wheat and barley improvement. Examples of further applications are underway in the Australian programs

that include work to combine desirable alleles at multiple malt quality loci from European, Japanese, and Canadian lines into a well-adapted and high-yielding Australian background (Vassos *et al.*, 2003) and to correct multiple deficiencies in a high-yielding wheat line (Kuchel *et al.*, 2003). Advances in marker screening and genotyping strategies will help the whole-genome breeding approach, making it possible to be used widely. Furthermore, the shift to association genetic studies (see later) in wheat and barley may provide a view of the key linkage blocks and haplotype structure of these species, which will be crucial in the next generation of whole-genome breeding strategies.

III. GENOMIC RESOURCES AND APPROACHES

Due to significant progress in the area of molecular genetics during the last two decades, enormous genomic resources have been developed for major crop plant species. For example, for wheat and barley, high-density genetic maps, cytogenetic stocks-, as well as contig-based physical maps, deep coverage large insert such as BAC libraries are available (Gupta and Varshney, 2004). These tools have facilitated isolation of genes or QTLs via map-based cloning approaches leading to sequencing and annotation of large genomic DNA fragments in these species (Salvi and Tuberosa, 2005; Stein and Graner, 2004).

A large collection of sequence data from genome- and EST-sequencing projects, combined with recent advances in the DNA sequence analysis (bioinformatics) and establishment of high throughput assays, have provided the framework for large-scale gene discovery and analysis of DNA sequence variation in plant species. The salient challenge of applied genetics and functional genomics is the correlation between genetic and phenotypic information and the subsequent identification of the genes underlying a trait of interest so that they can be exploited in crop improvement programs.

A. Transcriptome analysis

With the establishment of large-scale EST-programs in several laboratories around the world, a comprehensive resource has been created that provides direct access to genes of wheat and barley. In order to establish an inventory of expressed genes in *Triticeae* species, an international consortium (International Triticeae EST Cooperative (http://wheat.pw.usda.gov/genome/) under the umbrella of International Triticeae Mapping Initiative, http://wheat.pw.usda.gov/ITMI/) was established to trigger the development of a wheat and barley EST database. This effort provided the first serious collection of ESTs and led to other initiatives in the area of wheat and barley genomics.

For wheat, a project entitled "The Structure and Function of Expressed Portion of Wheat Genome" involving 13 laboratories was established in 1999 and funded by the US National Science Foundation (NSF) (http://wheat.pw.usda.gov/NSF/). The objective of this project was to decipher the chromosomal location and biological function of a large set of wheat genes. To this end, a total of 117,510 ESTs (101,912 are 5′ ESTs and 15,605 are 3′ ESTs, as of July 2003) from 20 cDNA libraries were generated (Zhang *et al.*, 2004a). Since ESTs reflect the transcriptional status of the tissue they were derived from, the sequences are inherently redundant (Varshney *et al.*, 2004c). EST clustering is applied to remove the redundancy and to sort the sequences into singletons and sequence clusters (Zhang *et al.*, 2004a). The sum of the numbers of singletons and clusters yields the number of tentative unigenes (TUCs). Computational analysis of the wheat EST dataset, as mentioned above, yielded 18,876 contigs and 23,034 singletons (http://wheat.pw.usda.gov/NSF/curator/assembly.html; Lazo *et al.*, 2004). In addition to these ESTs generated in NSF-sponsored projects, other public laboratories and private organizations, such as the DuPont Corporation, also generated wheat ESTs and submitted them to public databases. As a result 600,039 wheat ESTs are available in the public domain (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html, dbEST release 102805). A computational analysis of 580,155 wheat ESTs suggested the presence of 44,954 TCs (tentative consensus sequences or consensi) and 77,187 singleton ESTs, as per TIGR Wheat Gene Index Release 10.0, January 14, 2005 (http://www.tigr.org).

Similarly for barley, a large collection of ESTs was generated from over 80 cDNA libraries, covering virtually any tissue and growth stage as well as a series of physiological conditions (e.g., seed development or seed germination at different time intervals). This work has involved several laboratories worldwide including IPK-Gatersleben (Germany), Clemson University (USA), Washington Sate University (USA), SCRI (UK), Okayama University (Japan), and the University of Helsinki (Finland). As a result, 419,146 barley ESTs have become available in public domain as of late October, 2005 (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html, dbEST release 102805). Cluster analysis of 330,000 ESTs that were available in 2003 resulted in the definition of ca. 33,000 TUCs. A comparison of the available sequence data to 254 well-characterized barley genes from the SWISSPROT database and to 1.2 Mb of annotated BAC-sequence originating from several regions of the barley genome revealed an EST coverage of 87% for the SWISSPROT dataset and 45% for the genomic sequences. Thus, a preliminary estimate of the gene repertoire of barley will lie between 38,000 and 72,000 genes (Graner *et al.*, 2005; Zhang *et al.*, 2004b). However, the complexity of a genome is defined not only by the number of its genes but also by the number of its proteins. The latter may be influenced by alternative splicing, which is a common feature of the human transcriptome (Johnson *et al.*, 2003). In higher plants, alternative splicing may be much more

infrequent, since so far only a few cases have been described. Barley EST data revealed that about 4% of the barley genes show alternatively spliced isoforms; a similar figure as was reported for *Arabidopsis* (Brett *et al.*, 2002).

The outcome of cluster analysis (for defining the unigene set) depends on a series of parameters including the average sequence length of an EST, the quality of the sequences, and the contamination of EST data with sequences from other organisms, such as microbes or fungi. Moreover, the result of the analysis is influenced by the stringency of the cluster algorithm. The higher the stringency, the more singletons (which may be due to sequencing errors only) and thus the more unigenes will be defined. Nevertheless, the redundant EST dataset (extensive EST databases prepared from many different tissues) can be used to estimate gene expression levels by measuring the frequency of appearance of specific sequences, employing computational tools such as Digital Differential Display (http://www.ncbi.nlm.nih.gov/UniGene/info_ddd.shtml) or HarvEST (http://harvest.ucr.edu/). An example of the use of wheat ESTs from multiple cDNA libraries to study developmental processes was shown by Ogihara *et al.* (2003). After the analysis of 116,232 ESTs, generated from 10 wheat tissues, they identified correlated expression patterns of genes across the tissues. Furthermore, relationships of gene expression profiles among the 10 wheat tissues were inferred from global gene expression patterns. However, the use of EST databases to study expression profiles is limited by the availability of cDNA libraries used to develop ESTs and by the depth of EST sequencing. There are also problems in tracking genes that may be represented by several partial EST sequences.

B. Functional genomics

Functional genomics involves identification of functional allelic differences conferring an improved phenotype. In such an approach, the objective is to identify a sequence change conferring the improvement. The sequence change can then become the basis for a molecular marker that is specific for that allele. These types of (functional) molecular markers should/will always cosegregate with the trait of interest and should also be polymorphic in any cross, as discussed above. In general, such a marker will often be based on an SNP. Since SNPs can be detected by high-throughput systems, they bear the potential that large numbers of plants can be assayed for a particular allele (Rafalski, 2002). Thus, functional genomics can be linked or associated with plant breeding for crop improvement programs.

For carrying out the functional genomics studies, several techniques or platforms are available that allow the estimation of mRNA abundance for large number of genes simultaneously (Sreenivasulu *et al.*, 2002). The methods include serial analysis of gene expression (SAGE; Velculescu *et al.*, 1995), microarrays (Schena *et al.*, 1995), macroarrays (Desprez *et al.*, 1998), and massively parallel

signature sequencing (MPSS; Brenner *et al.*, 2000). SAGE, a logical extension of EST sequencing, can be used to study the expression patterns (Velculescu *et al.*, 1995). An improved variant to the conventional SAGE procedure called "Super-SAGE" was developed by using the type III restriction endonuclease *Eco*P15I for isolating fragments of 26 bp from defined positions of cDNAs (Matsumura *et al.*, 2003). Unfortunately, SAGE or SuperSAGE suffers from several problems. In particular, these experiments require large amounts of RNA and can be very expensive if many samples are to be analyzed, for example from a developmental series. As with MPSS (Brenner *et al.*, 2000; http://www.lynxgen.com/), the signatures generated can be difficult to assign to particular genes when the technique is applied to wheat and barley, where full genome sequences are not available.

Microarrays and macroarrays offer a technique for screening the expression profile of very large numbers of genes simultaneously. Furthermore, macroarrays have the advantage of ease of manufacture and low costs compared to microarrays, but macroarrays do not provide the same level of gene or probe density and specificity (Milligan *et al.*, 2004; Sreenivasulu *et al.*, 2002). On the basis of the available EST information, GeneChip arrays for both barley and wheat genomes have recently been constructed at Affymetrix (http://www.affymetrix.com/). The Barley 1 GeneChip is based on 350,000 high-quality ESTs from 84 cDNA libraries and contains 21,439 nonredundant genes (Close *et al.*, 2004). The Wheat GeneChip array contains 61,127 probe sets representing 55,052 transcripts for all 42 chromosomes in the wheat genome.

DNA arrays have been successfully utilized in many plant species, including cereals such as maize and rice (wheat and barley also), for understanding developmental processes, environmental stress responses, identification, and genotyping of mutations (Aharoni and Vorst, 2002; Milligan *et al.*, 2004; Potokina *et al.*, 2004). However, use of these technologies for applied aspects in plant breeding has been limited as it is not possible to simply estimate a difference in gene expression observed between two phenotypically contrasting lines (for agronomic trait of interest). With the possible exception of NILs (for which lines are genetically identical except a few loci), differential gene expression is not only due to the trait of interest but also due to variation in the genetic background. Therefore, background effects need to be eliminated in order to establish a functional association between the level of gene expression and a given trait. In this context, Potokina *et al.* (2004) established a strategy based on analysis of a representative number of well-described genotypes in terms of various phenotypic parameters for a given trait (malting quality). Subsequently, from a total set of genes which are differentially expressed between the lines by using macro/microarrays, only those genes are extracted whose expression profile accounts for phenotype-based relation between lines. Using this strategy with 10 barley genotypes characterized for 6 malting quality parameters, and a cDNA array with 1400 unigenes, Potokina *et al.* identified between 17 and 30 candidate

genes for each of the 6 malting parameters. This set of candidates contained genes that were previously supposed to be related to malting quality (e.g., cysteine proteinase 1), genes hitherto unknown to be related to this trait (such as a gene encoding 70-kDa heat shock protein) and genes of unknown function. Further, the observed linkage of five out of eight mapped candidate genes to known QTLs for malting quality traits underscores the potential usefulness of this approach for the identification of candidate genes for a trait under consideration (Potokina *et al.*, 2006). To reveal spatiotemporal expression patterns, cDNA microarrays containing ~9000 wheat cDNAs were used to monitor gene expression during the first 28 days of grain development following anthesis (Leader *et al.*, 2003). This study revealed 66 differentially regulated genes, which showed sequence similarity to transcription factors. The identified genes can be used for gene-specific marker development and synteny with rice to determine if any of the genes map within regions corresponding to QTL for grain yield or quality traits. Similarly, exploitation of cDNA microarrays is underway to identify genes controlling endosperm development (Shinbata *et al.*, 2003) for studying the Russian wheat aphid (RWA) defense response mechanisms (Botha *et al.*, 2003) and assessment after fungicide application (Pasquer *et al.*, 2003). Such studies (also see Milligan *et al.*, 2004; Sreenivasulu *et al.*, 2004) clearly demonstrate that the functional association strategy can provide an efficient link between functional genomics and plant breeding.

The results from DNA array experiments, however, need to be interpreted with caution. Different microarray platforms (e.g., Affymatrix, Agilent, and Amersham), with the same RNA sample or analysis of the same microarray gene expression data with different bioinformatic tools, may yield different sets of candidate genes (Larkin *et al.*, 2005; Miklos and Maleszka, 2004; Tan *et al.*, 2003). Therefore, to further confirm candidate genes obtained from DNA array analysis reverse-transcription (RT)-PCR may be employed, since it is at least 100-fold more sensitive than DNA arrays in detecting transcripts (Horak and Snyder, 2002). In this regard, Czechowski *et al.* (2004) developed a real-time RT-PCR-based approach for quantitative measurement of genes including TF genes. Thus, knowledge about where and when TF genes are transcribed and how such transcription is affected by internal and external cues will be valuable in elucidating the specific biological roles of the cognate proteins especially in response to environmental stresses.

C. Expression genetics and eQTLs

Jansen and Nap (2001) outlined the use of gene expression data in QTL analysis and the approach was termed "genetical genomics." However, we prefer to call this approach "expression genetics" as here the expression-profiling data is analyzed in the form of a genetic perspective (Varshney *et al.*, 2005b).

The "expression genetics" or "genetical genomics" approach combines QTL mapping with expression/transcript profiling of individual genes in a segregating (or mapping) population. Genes controlling a particular trait that are differentially expressed between two genotypes are used to record the corresponding expression data on each individual of the mapping population. The level of expression of a gene is treated as a quantitative trait. Assuming that each gene showing transcriptional regulation is mapped within the genome or the species of interest, the expression data is subjected to QTL analysis. The QTLs identified using this approach are popularly called as "e(xpress)QTLs." The dedicated software tool "Expressionview" has also been developed to combine visualization of gene expression data with QTL mapping (Fischer *et al.*, 2003). This tool can be used to utilize gene expression data in the form of QTL analysis to identify the eQTLs.

Because eQTL analysis uses segregating populations, it is possible to determine whether expression of a target gene is regulated in *cis* (mapping of the differentially regulated candidate gene within the eQTL) or *trans* (the candidate gene is located outside the corresponding eQTL). The latter gene product (second order effect) is of interest because more than one QTL can be connected to such a *trans*-acting factor (genes acting on the transcription of other genes) (Schadt *et al.*, 2003). Thus, mapping of eQTLs allows multifactorial dissection of the expression profile of a given mRNA/cDNA, protein, or metabolite into its underlying genetic components, and also allows localization of these components on the genetic map (see Jansen, 2003; Jansen and Nap, 2001). Subsequently eQTL analysis, for each gene or gene product analyzed, can underline the regions of the genome influencing its expression. Furthermore, for plant species such as *Arabidopsis* and rice, for which whole-genome sequences are available, the annotation of those genomic regions, which correspond to an eQTL, will be helpful for the identification of the genes as well as their regulatory sequences (Sreenivasulu *et al.*, 2004).

The genetical genomics approach has already demonstrated its utility in dissection and uncovering the regulatory pathways of complex traits in humans, fruitflies, yeast, and some plants (for reference see Varshney *et al.*, 2005b). This approach holds great potential to pinpoint genes involved in expression of agronomic traits based on the hypothesis that the expression of a quantitative phenotypic trait is a function of the expression level of the underlying genes. The colocalization of candidate genes with QTLs controlling a particular phenotype supports the use of the candidate gene as a potential source for developing "perfect marker(s)" for selecting the phenotype in marker (genomics)-assisted breeding. The availability of large EST collections and GeneChip arrays for genome-wide expression profiling and analytical tools for molecular marker analysis in wheat and barley will accelerate the use of this

approach for understanding the genetic control of different agronomic traits for plant breeding.

IV. COMPARATIVE GENOMICS

Significant genomic collinearity in plants has been revealed by comparative genetic mapping, although plant genomes vary tremendously in size, chromosome number, and chromosome morphology. For example, genomic collinearity or conservation of synteny on chromosomes among related species is well known for the *Poaceae* (Ahn *et al.*, 1993; Devos, 2005; Devos and Gale, 1997, 2000), *Solanaceae* (Bonierbale *et al.*, 1988; Tanksley *et al.*, 1992), and between *Arabidopsis* and *Brassica* species (Lagercrantz, 1998). The availability of a large number of ESTs for cereal species including wheat and barley, and the complete genome sequence of rice has allowed sequence comparisons between and among cereal genomes and opened a new area of comparative genomics. The use of DNA sequence-based comparative genomics for evolutionary studies and for transferring information from model species to related large-genome species has revolutionized molecular genetics and breeding strategies for improving these crops (Paterson, 2004). Comparative sequence analysis methods provide cross-reference of genes between the maps of different species, enhance the resolution of comparative maps, help to explain patterns of gene evolution and can be used to identify conserved regions of the genomes, and facilitate interspecies gene cloning (Stein *et al.*, 2000).

Despite the benefits arising from the exploitation of syntenous relationships between genomes, it must be kept in mind that millions of years of genome evolution has left its traces. As a result, marker and gene collinearity are frequently interrupted, and chromosomes represent patchworks of collinear and non-collinear segments. In a comprehensive study, 5780 ESTs physically mapped in wheat chromosome bins (using deletion stocks) were compared by BLAST analysis to 3280 ordered BAC/PAC clones from rice, and numerous chromosomal rearrangements were observed between wheat and rice genomes (La Rota and Sorrells, 2004; Sorrells *et al.*, 2003). In addition, the physical locations of nonconserved regions were not consistent across rice chromosomes. Some wheat ESTs with multiple wheat genome locations were found associated with the nonconserved regions. An average of 35% of the putative single copy genes that were mapped to the most conserved bins matched rice chromosomes other than the one that was most similar overall (Singh *et al.*, 2004). As noted above, interruption of microcollinearity, due to rearrangements, was observed in other studies when extensive comparisons were made across smaller regions between collinear chromosome (/arm) of wheat and rice (Distelfeld *et al.*, 2004; Guyot *et al.*, 2004; Singh *et al.*, 2004).

Sequence comparison (BLASTN analysis) of 974 mapped ESTs from a transcript map of barley with the available 1,369,683 ESTs of six cereal species and 286,255 ESTs of three dicot species showed the presence of barley homologs in all species examined (Varshney *et al.*, 2005c). Among cereals, barley EST-derived markers showed *in silico* transferability of 95.4% to wheat, 70% to rice, 69.7% to maize, and 66.2% to sorghum. A lower transferability of only 38.7%, which was observed for rye, and 3.8% for oats may be attributed to the small datasets (9194 ESTs in rye and 501 ESTs in oats) that were available for analysis and which may be biased regarding the content of conserved sequences. Even significant homology of barley ESTs with an average of 15% ESTs of dicot species suggests that a COS of markers could be developed as demonstrated earlier by Fulton *et al.* (2002).

Despite the frequent observation of disturbed microsynteny between the genomes of *Triticeae* species and rice, at the sequence level, the cross-transfer information from rice (and other cereal genomes) to wheat and barley has greatly facilitated the isolation of genes via map-based cloning approaches in wheat and barley (see Stein and Graner, 2004). In addition, molecular marker resources that are available for wheat and barley have been used to improve the marker density of the genetic maps of other cereal species, such as rye, which lack the corresponding resources (Khlestkina *et al.*, 2004; Varshney *et al.*, 2004c). A summary of selected comparative mapping studies based on molecular marker and/or sequence comparison is given in Table 5.2. These studies will prove useful in comparative mapping among fairly divergent genomes and therefore may also prove useful for taxonomic studies, such as deducing phylogenetic relationships between different genera and species.

V. EXPLOITATION OF NATURAL VARIATION AND ALLELIC DIVERSITY

Existing genetic variation in germplasm collections has been utilized for decades by plant breeders in creating new varieties improved for desired agronomic traits. However, during the process of domestication, the genetic base of crop species has been narrowed (Tanksley and McCouch, 1997). Thus modern breeding is now returning to the wild ancestors of crop plants and employs some of the diversity that was lost during domestication to further improve agricultural performance (Zamir, 2001). Utilization of wild germplasm tends to be complex, as the target loci in the wild material are often transferred in large linkage blocks that adversely affect performance of the adapted parents; the phenomenon is called "linkage drag" (Gur and Zamir, 2004; Tanksley and McCouch, 1997). Although there may be many tools or strategies in modern genetics or genomics that may manage and exploit unused natural-variation potential of wild,

Table 5.2. Comparative Mapping and Genomics Studies Revealing the Syntenic Relationship Between Wheat and Barley and Other Cereal Species

Species	References
Barley, wheat	Dubcovsky *et al.* (1996), Hernandez *et al.* (2001), Hohmann *et al.* (1995), Namuth *et al.* (1994), Salvo-Garrido *et al.* (2001), Varshney *et al.* (2005b), Weng and Lazar (2002)
Barley, rye	Varshney *et al.* (2005d), Wang *et al.* (1992)
Barley, rice	Han *et al.* (1998, 1999), Kilian *et al.* (1995, 1997), Perovic *et al.* (2004), Saghai-Maroof *et al.* (1996), Smilde *et al.* (2001), Varshney *et al.* (2005d)
Barley, wheat, rye	Börner *et al.* (1998), Devos and Gale (1993), Devos *et al.* (1993b), Gudu *et al.* (2002)
Barley, wheat, rice	Dunford *et al.* (1995), Gallego *et al.* (1998), Kato *et al.* (2001)
Barley, oat, maize	Yu *et al.* (1996)
Wheat, rye	Devos *et al.* (1992, 1993a), Khlestkina *et al.* (2004)
Wheat, maize	Devos *et al.* (1994)
Wheat, rice	Francki *et al.* (2003), Kato *et al.* (1999), Kurata *et al.* (1994), Lamoureux *et al.* (2002), La Rota and Sorrells (2004), Laubin *et al.* (2003), Li *et al.* (2004), Liu and Anderson (2003), Sarma *et al.* (1998, 2000), Singh *et al.* (2004), Sorrells *et al.* (2003), Yu *et al.* (2004b)
Wheat, maize, rice	Ahn *et al.* (1993), Moore *et al.* (1995b)
Wheat, maize, oat, rice	Van Deynze *et al.* (1995a,b)
Wheat, foxtail-millet, maize, rice	Moore *et al.* (1995a)

unadapted, as well as cultivated germplasm resources for crop improvement, two main approaches are listed below.

A. Advanced backcross QTL analysis

In order to exploit the potential of wild species in breeding programs, efforts were made in past to introduce alien or exotic genes from wild species into cultivated varieties. For example, 57 genes for resistance to diseases and pests were introduced into wheat from other genera of the *Triticeae* via alien translocations (transferring of chromosomal segments from wild or other species that carry disease resistance genes). In many cases, the size of the alien fragments and the translocation breakpoints have been precisely determined by genomic *in situ* hybridization (for review see Friebe *et al.*, 1996). For transferring the QTLs for important traits from a wild species to a crop variety, an approach named

"advanced-backcross QTL analysis (AB-QTL)" was proposed by Tanksley and Nelson (1996). In this approach, a wild species is backcrossed to a superior cultivar, and during backcrossing cycles, the transfer of desirable gene/QTL is monitored with molecular markers. The segregating BC_2F_2 or BC_2F_3 population generated during backcrossing (F_2 or F_3 stages) is then used not only for recording data on the trait of interest, but also for genotyping with polymorphic molecular markers. These data are then used for QTL analysis, leading to simultaneous discovery of QTLs. Once favorable QTL alleles are identified, only a few additional marker-assisted generations are required to develop NILs that can be field-tested and used for variety development. Therefore, a cycle of AB-QTL analysis (i.e., QTL discovery, NIL development, and testing) represents a direct test of the underlying assumption of QTL breeding: that beneficial alleles identified in segregating populations (such as BC_2 or BC_3 in the case of AB-QTL) will continue to exert their positive effects when transferred in the genetic background of elite lines (Grandillo and Tanksley, 2005).

AB-QTL analysis has been used in some studies of wheat and barley, showing that certain genomic regions (QTLs) derived from wild or unadapted germplasm have the potential to improve yield. For example, after genotyping 72 preselected BC_2F_2 plants derived from a cross between a German winter wheat variety (Prinz) and the synthetic hexaploid wheat line (W7984), Huang *et al.* (2003) identified a total of 40 putative QTLs involved in yield and yield component traits. For 24 (60%) of these QTLs, alleles from the synthetic wheat W7984 were associated with a favorable effect on the analyzed traits, despite the fact that synthetic wheat was overall inferior with respect to agronomic appearance and performance. For four of the seven QTLs to associate with yield, the wild-type (WT) allele had an effect that increased total yield, and the increases associated with the WT allele ranged from 5 to 15%. By using 111 BC_2F_1 lines from another cross of the wheat variety Flair with the synthetic wheat line XX86, a total of 57 QTLs were identified for seven agronomic traits analyzed (Huang *et al.*, 2004). For 24 (42.1%) QTLs derived from XX86 line, a positive effect was observed on traits such as 1000-grain weight and number of grains per year.

In barley, the first AB-QTL study was conducted on 136 BC_2F_2 families derived from the cross of the German spring barley variety Apex and Israeli wild barley accession ISR101–23 (Pillen *et al.*, 2003). A relatively high proportion (36%) of the 86 QTLs identified for 13 quantitative traits measured in a maximum of six environments had favorable effects derived from the exotic parent for 7 of 13 traits investigated. Interestingly, in one case, the exotic parent allele was associated with a yield increase of 7.7%, averaged across the six environments tested. To validate the QTL effects, a second AB-QTL study was undertaken by using the same wild accession (ISR101–23) but with the German spring variety Harry as the recurrent parent; 101 BC_2F_2 families were

evaluated for the same 13 quantitative traits (Pillen *et al.*, 2004). In this study, a total of 108 putative QTLs were detected, and altogether 52 (48%) favorable effects were identified from the exotic parent. The comparison of these two AB-QTL studies using the common exotic donor parent showed that in total 26% of the putative QTLs could be detected in both AB populations. Wild barley germplasm (accession HOR1508) has also proven to be a good source of QTL alleles with favorable effects on yield and other agronomically important traits under conditions of water deficit in Mediterranean countries (Forster *et al.*, 2000; Talamè *et al.*, 2004). Of the total 80 significant QTLs identified by Talamè *et al.* (2004), 42 (52%) had beneficial alleles derived from the donor wild parental line. After genotyping 181 BC_3DH lines (var Brenda × wild accession HS213), Li *et al.* (2005) identified a total of 25 QTLs for yield, yield components, and malting quality traits. In contrast to the previous studies, most positive QTLs originated from the recurrent parent "Brenda." One QTL each for yield and heading date (derived from Brenda) explained 18.3 and 20.7% of the phenotypic variation, respectively. This may provide a first hint that not any accession of wild germplasm will show a positive effect on a given agronomic trait.

Overall, these results have demonstrated that the AB-QTL strategy represents a very effective way to unlock valuable wild alleles and transfer them into elite cultivars to improve their performance. It is, however, important to note that in early backcross generations plants still contain a number of wild species chromosome segments which can mask the magnitude of some of favorable effects of introgressed alleles (Septiningshi *et al.*, 2003). On the other hand, by utilizing AB-QTL approach it has been demonstrated in tomato that the pyramiding of independent yield-promoting segments introduces a number of alleles, with favorable effects, into a given genetic background after generating segmental introgression lines (ILs). These studies led to production of novel varieties that reproducibly increase productivity relative to leading commercial genotypes both under normal cultivation conditions and in the stressed environment (Gur and Zamir, 2004).

B. Association mapping based on linkage disequilibrium

Another approach toward exploiting the potential of unadapted germplasm (natural population) is utilization of the germplasm in association mapping, based on linkage disequilibrium (LD). Unlike conventional segregating (or mapping) populations such as DH, F_2, or RILs that have been used in past for identification of genes or QTLs for trait of interest for plant breeding, the natural populations are the products of many cycles of recombinations and have the potential to show enhanced resolution of QTLs. Association analysis based on LD may offer more power than linkage analysis for identifying the genes

responsible for the variation in a quantitative trait (Buckler and Thornsberry, 2002; for review see Flint-Garcia *et al.*, 2003; Gupta *et al.*, 2005). LD is the nonrandom association of markers in a population and can provide high resolution maps of markers and genes. The extent of LD around a locus determines the resolution of association analyses and the number of markers that would be required to scan the entire genome (Rafalski and Morgante, 2004). Because genetic recombination is not evenly distributed over the genomes of most species, the linkage distance between markers and candidate genes varies widely (Philips and Vasil, 2001). Simulations estimating the power of detecting the association of variation in a candidate gene with the phenotype indicate that population size is important (Long and Langley, 1999). For a population size of 500, there is a high probability of detecting the association, even when the gene accounts for as little as 8% of the variation. For a population size of 100, only gene effects accounting for at least 15% of the variation can be detected.

LD in a germaplasm collection is affected by several factors such as recombination rate, mating system, genetic isolation, population size, population admixture, and natural and artificial selection (Rafalski and Morgante, 2004). Because of these matters, in wild species LD may extend only to a few kilobases (see Wall and Pritchard, 2003); but LD in cultivated and inbred species, such as wheat or barley, frequently extends across large linkage blocks (often almost entire chromosome arms) that have been maintained over by selection. This is particularly important in wheat where large chromosome segments from wild relatives have often been used in modern varieties, and these can show very low levels of recombination (e.g., Paull *et al.*, 1994). However, this may also help in the localization of genes from wild relatives. For instance, Paull *et al.* (1998) used coefficients of parentage in an association mapping study to identify the positions of several disease resistance genes from wild relatives in wheat.

In contrast to the extensive use of LD-based association mapping in human genetics, the potential of LD-based association mapping has not been realized adequately in plant species. One of the reasons for this involves occurrence of the structured populations. In this context, Pritchard *et al.* (2000) proposed a population-based method that allows for large-scale assessment of allele/trait relationships in structured populations. By using this approach, association mapping based on LD has been demonstrated in maize for the *Dwarf8* gene, which is involved in flowering time (Thornsberry *et al.*, 2001) and yellow endosperm color (Palaisa *et al.*, 2003).

In some studies, population structure has been analyzed in details in wheat as well as in barley for conducting the association mapping. For instance, after examining the levels of LD within and between 18 nuclear genes in 25 accessions from across the geographic range of wild barley, Morrell *et al.* (2005) demonstrated the following: (1) For the majority of wild barley loci, intralocus

LD decays rapidly, that is, at a rate similar to that observed in the out-crossing species, *Zea mays* (maize). (2) Excess interlocus LD was observed at 15% of the two-locus combinations; almost all interlocus LD involved loci that showed significant geographic structuring. However, in a collection of 134 durum wheat lines, after genotyping with 70 SSR markers, high levels of LD were found at both linked and unlinked locus pairs (Maccaferri *et al.*, 2005). Further, the information obtained from LD analysis was successfully utilized in some cases for association mapping studies. Using 236 AFLP markers in a set of 146 modern spring barley cultivars, 18–20 markers that accounted for 40–58% of the variation for yield and yield stability traits were identified (Kraakman *et al.*, 2004). Likewise in wheat, after analyzing the population structure and LD, association of 62 loci on chromosomes 2D, 5A, and 5B with kernel morphology and milling quality has been analyzed (Breseghello and Sorrells, 2005). Significant marker associations for kernel size were detected on the three chromosomes tested, and alleles potentially useful for selection were identified. This result was in agreement with previous QTL analysis.

Such high-resolution mapping of traits/QTLs to the level of individual genes will provide a new possibility for studying the molecular and biochemical basis of quantitative traits variation and will help to identify specific targets for crop improvement. It seems that association mapping approaches are viable alternative to classical QTL approaches based on crosses between inbred lines, especially for complex traits with costly measurements. However, in our opinion, though LD-based approaches hold great promise for speeding up the fine mapping, conventional linkage mapping will continue to be useful particularly when one tries to assess QTLs and the effect of a QTL in isolation (Rafalski and Morgante, 2004). In some studies, the utility of an approach involving the use of conventional linkage mapping along with LD has been recommended for the construction of molecular maps and for QTL analysis (Nordborg *et al.*, 2002; Zhu *et al.*, 2002).

VI. CONCLUDING REMARKS

Similar to other major crop species, the genetic maps of wheat and barley have benefited from the availability of large numbers of mapped markers. These have become a core resource for QTL analysis, trait-based molecular breeding, and whole-genome breeding. However, further development of existing breeding concepts will critically depend on the completion of our knowledge on genes, which underlie agronomic traits of interest. There are several ways that can be applied for the identification of candidate genes, each of which has pros and cons. Association mapping might provide a viable alternative to map-based cloning, in case there is sufficient decrease of LD around the locus of interest. At the level of functional genomics, transcript profiling may provide candidate

genes for agronomic traits. However, despite the tremendous progress in structural and functional genome analysis, the rate-limiting step regarding the isolation of candidate genes will be population development and accurate phenotyping. Additional efforts are required to develop an infrastructure for phenotypic analysis of large numbers of individuals under highly standardized and reproducible conditions. Similarly, more knowledge about the physiology, cell biology, and biochemistry of the individual traits is required to break down complex traits into components that show a high heritability and can be measured accurately. Once the target genes have been identified, their genetic diversity can be studied to identify superior alleles. Notwithstanding these limitations, the availability of a comprehensive portfolio of resources for genome analysis in wheat and barley has laid the groundwork to efficiently complement existing breeding concepts and to develop knowledge-driven strategies to further adapt these cereals to our needs.

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