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Review

WHEAT GENOMICS: CHALLENGES AND ALTERNATIVE STRATEGIES

Syed Sarfraz Hussain[1,2] and Raheel Qamar[1]

[1,2]Department of Biosciences, COMSATS Institute of Information Technology (CIIT), H-8/1, Islamabad, Pakistan, and [1]Institute of Molecular Physiology & Biotechnology (IMBIO), University of Bonn, Kirschallee 1, D-53115, Bonn, Germany

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Abstract: The precise origin of the wheat plant as we know it today is not known. Wheat evolved from wild grasses, probably somewhere in the Near East. A very likely place of origin is the area known in early historical times as the Fertile Crescent - a region with rich soils in the upper reaches of the Tigris-Euphrates drainage basin. Wheat is one of the universal cereals of old world agriculture. Wheat has a complex polyploid large genome in the cereal crops. Efforts on wheat genome mapping using DNA markers started after formation of the International Triticeae Mapping Initiative (ITMI) in 1990. However, the progress in developing saturated map was slow because of large genome size of wheat, polyploid nature, high proportion of repetitive DNA and lack of sufficient polymorphism. For large-genome species, including crops such as wheat full-genome sequences may not be available in the near future. Therefore, it is necessary to devise alternative strategies to access genomes of wheat and its relatives on a large scale and thus ensure continued advances in the biology of wheat. In this paper, the availability of abundant, high-throughput sequence based markers which is the key for detailed genome-wide trait analysis has been reviewed.

Introduction

Wheat (*Triticum aestivum* L. em Thell.) is the first important and strategic cereal crop for the majority of world's populations. It is the most important staple food of about two billion people (36% of the world population). Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally [1]. It exceeds in acreage and production every other grain crop (including rice, maize, etc.) and is therefore, the most important cereal grain crop of the world, which is cultivated over a wide range of climatic conditions. Most wheat is grown for human food and about 10 percent is retained for seed and industry (for production of starch, paste, malt, dextrose, gluten). Wheat grain contains all essential nutrients; kernel contains about 12 percent water, including carbohydrates (60-80% mainly as starch), proteins (8-15%) containing adequate amounts of all essential amino acids (except lysine, tryptophan and methionine), fats (1.5-2%), minerals (1.5-2%), vitamins (such as B complex, vitamin E) and 2.2% crude fibers.

There is little doubt that worldwide wheat production will have to be increased in the near future to help feed the world's growing population. Because new arable cropland will not be available on a large scale, increases in production must come from genetic improvement, which must be expedited by advances in wheat genomics [2]. Using molecular markers is of great value for genetic and plant breeding purposes.

Hexaploid bread wheat is an allopolyploid with genome constitution AABBDD, formed through hybridization of *T. urartu* (AA) with a B genome diploid of unknown origin, and subsequent hybridization, only about 8000 years ago, with a D genome diploid *T. tauschii* [3]. In wheat, evidence for homoeology between the three

genomes had already been provided by the pioneer work of E. R. Sears, who assembled the first set of aneuploid genetic stocks in wheat [4,5]. The development of aneuploid stocks in wheat led to the discovery that an extra dose of a particular chromosome could compensate for the absence of another which means because of triplication of genetic material, wheat can tolerate the loss of whole chromosomes, arms, and segments [4-7]. This compensating ability of chromosomes of different ancestral origin defined their relationship, and resulted in the classification of the 21 wheat chromosomes into 7 homoeologous groups [8]. The ability of chromosomes to substitute for one another suggested that they carried similar genes. Chromosomes belonging to the same homoeologous group have similar gene content and gene order and can compensate for each other in nullisomic-tetrasomic combinations [2].

One of the main objectives of plant breeders is to improve existing cultivars which are deficient in one or more traits by crossing such cultivars with lines which possess the desired trait or by induced mutagenesis. Wheat is endowed with striking genetic, cytological and molecular versatility. Yet it is also a problem plant in the hands of breeders due to three features which add greatly to the complexity of breeding and selection, (i) wide range of end uses, each with differing but specific quality requirements, (ii) the complexity of the polyploid wheat genome, and (iii) low level of polymorphism in bread wheat [9]. This has imposed many constraints on wheat breeding programs and has, in many cases, restricted the diversity of germplasm that can be used in a specific breeding program. As a result, the level of diversity detected between commercial wheat varieties is generally lower than for many other species. Similarly, low level of polymorphism in wheat necessitates that a larger number of markers needs to be screened than is the case with other cereals [10-11]. The situation becomes worse by the fact that the level of polymorphism is not consistent across genomes or crosses.

The Genomes of Bread Wheat

The DNA content of a haploid common wheat nucleus is 18.5 picogram (Pg), which is equivalent to approximately 16 billion base pairs (16×16^6 Kb) [12] and is 6, 35 and 110 times larger than maize, rice and *Arabidopsis* respectively [2,13]. More than 70% of the genome consists of repeated DNA sequences with various degrees of repetition while less than 20% consists of low copy number or single copy sequences [14] and less than 1% consists of actual coding genes [12]. The average length of a wheat chromosome is 10 μM with a DNA content which equals one half of the haploid rice genome while three wheat chromosomes are equal to the haploid maize genome [15]. Nishikawa and Furata [16] showed that DNA content of the three genomes in haploid wheat is present in a ratio of 1.14=1.2=1.0. More than 85% of wheat genes are present in uninterrupted gene-rich clusters, interspersed by gene-poor regions, consisting of retrotransposon like repetitive sequences and pseudogenes. Each chromosome arm consists of approximately 6-8 gene-rich regions spanning less than 10% of the chromosome [17,18]. Each gene-rich region may be subdivided into 'mini' gene-rich and gene-poor regions.

It has also been found that the gene density and organization in gene-rich regions are not significantly influenced by the size of the plant genome. The gene-rich regions show some similarity in the physical location, structural organization and gene densities among the three genomes of bread wheat [19]. However, the gene-rich regions vary in the number of genes, gene density and the frequency of recombination [20]. The distribution of recombination is highly uneven over the Triticeae chromosome and it appears to be limited to distal chromosome regions [21]. When comparing physical maps to genetic

linkage maps, Gill *et al.* [22] found that recombination occurs only in gene-rich regions of the wheat genome. This was confirmed by Sandhu *et al.* [19] who found 82% recombination in the 1S0.8 gene-rich region, located on the group 1 short arm. However, the level of recombination varies within the same gene-rich region. A low level of recombination was found in the proximal 20-30% of wheat chromosomes, despite the presence of gene-rich regions due to the presence of the centromere [20,22]. As a result of non random distribution of recombination along the chromosome length, the bp/cM may vary from 118 Kb in gene-rich regions to 22 Mb in gene- poor regions [22].

Comparative Genetic Mapping

On the advent of plant molecular genetics, molecular markers were widely used to study the organization of plant genomes, and genetic linkage maps based on molecular markers have been assembled for many different plant species. The first preliminary studies concerning comparative mapping in plants were performed on the Solanaceae with the successful demonstration that cDNA markers were collinear along tomato and potato chromosomes [23]. The high conservation of gene sequences during evolution allowed the use of RFLP markers derived from one species to be used in genetic mapping experiments in closely related species. Later, the first comparative mapping studies were performed on grasses [24]. This development advanced the idea of synteny and colinearity in plants. During these attempts, a remarkable degree of genome conservation has been established in comparative genetic mapping experiments for the Poaceae family, despite the fact that genome sizes vary as much as 40-fold between some of the species [24]. Comparative genetic mapping in bread wheat revealed that most gene sequences are triplicated on A, B and D genomes and the three sets of the seven homoeologous chromosomes show overall colinearity. Evidence of a few translocations were, however, also found [25] which was later on confirmed by the ITMI project (a series of papers in Genetics, 2004).

Comparative genetic mapping experiments with rice, wheat and maize have indicated that genome colinearity is observed even in species belonging to different subfamilies of Poaceae [3,26-33]. Comparison of rice, hexaploid wheat and barley have led to the identification of colinear regions and established the genetic correspondence of the seven homoeologous groups of the triticeae genomes with the 12 rice chromosomes [3,27-29,31,33-34]. This gave birth to the concept of describing colinearity on the basis of rice and *Arabidopsis* sequences which has been very successful and has made possible the multiple alignments of chromosome maps for other crops like oats, sorghum, sugarcane, barley and pearl millet [24]. Most importantly, the recognition of putative orthology of monogenic or quantitative traits across different species is facilitated by comparative mapping experiments. The aligned maps can be exploited to identify many different markers from a variety of species for a given genomic region. This is especially useful for fine scale mapping or map-based cloning experiments. Such experiments also detect minor rearrangements which deviate from the overall colinearity.

Present Status of Wheat Maps

So far, maps of bread wheat were mainly developed from interspecific or wide crosses [10,35-40] (Table 1). The low level of polymorphism revealed by RFLP markers in wheat [10,11] has often hampered the establishment of intervarietal genetic linkage maps. To increase the level of polymorphism, several of these maps have been made by crossing relatively distantly related parental lines [36-38]. However, the development of such maps is a prerequisite for the dissection of complex agronomic traits through QTL analysis and the use of these QTLs in plant

Table 1.
Molecular maps of wheat (*Triticum* spp).

No.	Genome	Markers	# of mapped Loci	Mapping Population	References
1.	Bread Wheat	RFLP, SSR	262, 2	Chinese SpringXCourtot DH	[47]
2.	Bread Wheat	STMP	279	Opata85XW7984	[37]
3.	Bread Wheat	SSR	53	Chinese SpringXSynthetic	[175]
4.	Bread Wheat	AFLP, SSR	620, 42	Gamet X Saunders DH	[176]
5.	Bread Wheat	RFLP	197	Chinese Spring X *T. spelta* Var Dutamellanum	[35]
6.	Durum Wheat	RFLP, PCR	259	*T. turgidum* Blanco et al. 1998 (Messapix X MG4343)	
7.	Durum Wheat	RFLP, AFLP	88	*T. turgidum* (Messapix X MG4343)	[177]
8.	Einkom Wheat	RFLP, RAPD, ISSR	81	*T. monococcum* X *T.boeoticum* Spp. *boeoticum*	[178]
9.	Wild emmer Wheat	AFLP, RAPD STMS	543	*T.coccoides* var. Hermon X *T. durum* var Langdon	[179]
10.	D-genome	RFLP	546	*T. tauschii* (TA1691XTA1704) Proginator Aegilops *tauschii*	[49]
11.	D-genome	RFLP	127	*T. tauschii* (TA1691XTA1704)	[42]
12.	D-genome	STMS	55	Opata85XW7984	[180]
13.	Durum Wheat	RFLP, AFLP SSR	306	JennahXKhetifa	[50]
14.	Bread Wheat	RFLP, SSR	230	*T. aestivum* var Fomo X *T.spelta* var Oberkulmer	[38]
15.	Bread Wheat	SSR	1235	joined four genetic maps	[106]
16.	Bread Wheat	EST-SSR	3530	ESTs (ITEC)	[181]
17.	Bread Wheat	SSR	540	W7984Xopata85	[182]
18.	Bread Wheat	SSR, TRAP	700	Hard red spring wheat intervarietal Recombination inbred population	[183]
19.	Bread Wheat	EST-SSR	101	W7984Xopata 85, LumaiXHanxuan WenmaiXShanhongmai	[134]

breeding (Table 2). The first intervarietal map of bread wheat, based on RFLP markers was published by Cadalen *et al*. [41]. Consequently, RFLP based genetic maps were developed for the 2x, 4x and 6x wheats and a diploid ancestor [10,25,42-51]. RFLP markers have provided the basic foundation of much of the mapping work in wheat and other species. Table 3 describes the research statistics in wheat. The number of whole genome maps developed from crosses between wheat cultivars or breeding lines is more limited. Extensive deletion for each of the 21 wheat chromosomes have been developed and anchored to the maps of the hexaploid wheat [22,52-57]. Recently, Kumar *et al*. [58] described six important QTLs which were pleotropic/coincident involving more than one trait in a study involving two mapping populations. These QTLs could be utilized efficiently for marker assisted selection (MAS).

In spite of all these genetic resources, map based cloning of genes has been arduous in wheat because of polyploidy and a large genome consisting of repeated DNA sequences; as a result only a few successes have been reported in literature [59-60]. Similarly, little DNA sequence information is available for wheat to take advan-

Table 2.
Tagging/mapping of gene/ QTL agronomic characters in wheat.

Trait	Gene/QTL	Markers	Population	Reference
Grain protein content	QTL	RFLP	65 RILs (F_7)	[184]
	Major locus	RFLP	3 RILs	[185]
	QTL	STMS	100 RILs (F_8)	[186]
Preharvest sprouting	QTL	RFLP	2 RILs (F_5)	[187]
tolerance	Major locus	STMS, STS	100RILs (F_5)	[188]
	QTL	RFLP, STMS	204RILs (F_5)	[189]
Vernalization response	Vrn 1	RFLP	-	[190]
		RFLP	Molecular mapping	[43]
		RFLP, STMS	114 F_2 lines	[191]
	Vrn-A^m 1 Vrn-A^m 2	RFLP	Molecular mapping	[192]
Bread making quality	Glu-D1	PCR	-	[193]
	QTL	RFLP, STMS	204 RILs (F_5)	[194]
	QTL	RFLP, Biochemical	187 DH lines	[195]
Grain/Kernal hardiness	Ha	RFLP	-	[44]
		RFLP	114 RILs (F_7)	[196]
	QTL	RAPD, ISSR	100 RILs (F_7)	[197]
Red grain colour	R_1, R_3	RFLP	Molecular mapping	[45]
Flour Colour	QTL	RFLP, AFLP	150 F_4 lines	[198]
	Major locus	STS	150 F_4 lines	[199]
Milling yield	QTL	RFLP, AFLP	150 F_4 lines	[200]
Amylose content	Wx-B1	RFLP	98 single chromosome substitution lines	[201]
Flour viscosity	QTL	RFLP	78 RILs	[202]
Noodle quality	GBSS	PCR	-	[203]
Kernal morphology & texture	QTL	RFLP, STMS, PCR	78 RILs	[204]
Grain yield & 50 grain weight	QTL	RFLP	3RILs	[205]
1000 grain weight	QTL	STMS	100RILs	[123]

tage of gene discoveries. Moreover, the disadvantages of RFLPs have limited their application to pragmatic breeding programs and their use is decreasing [9].

Alternative Approaches for Wheat Genomics

The large genome size of wheat makes it unrealistic to anticipate complete sequencing in the near future. Therefore, large scale discovery, isolation and deciphering gene function in wheat and its relatives must rely on other, less direct methods. The current approach of map based cloning, transposon mutagenesis, differential display techniques and other strategies of deciphering gene function are either not suitable for large genomes or are tedious and expensive because these deal with a single gene at a time. Therefore, it is necessary to devise alternative strategies (molecular marker) to access genomes of wheat and its relatives on a large scale and thus ensure continued advances in the biology of this immensely important plant.

Molecular Marker System

A series of different molecular marker systems, which became available during the last two decades, can be broadly classified into three classes (i) the first generation molecular markers, including RFLPs, RAPDs and their modifications; (ii) the second generation molecular

markers, including SSRs, AFLPs and their modified forms, and (iii) the third generation molecular markers including ESTs and SNPs [61]. In this review, we will discuss the third generation molecular markers because enough information is available on first and second generation molecular markers in the literature.

Expressed Sequence Tags (ESTs)

Investigators are working diligently to sequence and assemble the genomes of various organisms, including both animal and plants. In spite of recent increases in DNA sequencing capacity, it is improbable that the genomes of more than a few flowering plants with the small genomes will be completely sequenced in the near future, while the large genome size makes it quite difficult to anticipate complete sequencing of any of the Triticeae genomes in the near future. However, by partially sequencing large numbers of expressed genes, it is possible to obtain transcript information to search databases for similar genes in the same or other organisms.

Table 3.
Research Statistics of Wheat (91-09-07; www.gramene.org).

Class	Species	No
Maps	*Triticum aestivum*	14
	Triticum turgidum	2
Markers	*Triticum aestivum*	1532166
	Triticum monococcum	10712
	Triticum turgidum subsp *durum*	8993
	Triticum turgidum	1722
	Triticum sp	604
	Triticum turgidum subsp *dicoccoides*	177
	Triticum urartu	126
	Triticum aestivum/Thinopyrum intermedium Alien addition line	36
	Triticum baeoticum	19
	Triticum turgidum subsp *dicoccum*	3
	Triticum aestivum X *Triticum timopheevi*	2
	Triticum timopheevi	1
QTL	*Triticum aestivum*	23
	Triticum turgidum	8
Genes	*Triticum*	598
Proteins	*Triticum*	3609

Therefore, an expressed sequence tag is usually a portion of an entire gene that can be used to help identifying unknown genes and to map their positions within a genome.

ESTs represent the transcribed portion of the genome (transcriptome) which serve as the templates for the synthesis of proteins and ultimately determine the shape, size and characteristics of an organism. The basic strategy for EST production and use was formulated by Craig Ventner's group (TIGR) in 1991 and is a rapid, efficient method for sampling a genome for active gene sequences. Typically, anonymous cDNAs are used to determine short DNA sequences (200-700 bp) in a single sequencing reaction [62]. These sequences are then used to search existing databases [63] to determine if a specific gene has been found in the same or other organisms and whether its function has been determined.

Compatibility of ESTs with Other High Throughput Techniques

The availability of EST databases was also essential to move onto the use of a number of new techniques, including cDNA and oligonucleotide micro-arrays, and SAGE (Serial Analysis of Gene Expression). The developing technology of DNA-chips is poised to revolutionize many areas of plant biology. In this technology, DNA is fixed onto a solid surface (glass or a membrane) and hybridized with a fluorochrome-labeled nucleic acid probe. The degree of hybridization to each DNA gives a measure of the amount of probe complementary to the immobilized DNA. In one report, microarrays of 1046 of human anonymous cDNAs were produced and used to monitor differential expression in a two-color hybridization assay [64]. In this study, the differences in expression were compared between heat shock versus control tissue and found that 17 individual array elements displayed altered fluorescence of 2-fold or greater. These 17 were sequenced and the sequence of 14 matched

known genes, mainly related to heat-shock, whereas three did not match any known gene. Oligonucleotide arrays are the versions of DNA microarrays where short oligonucleotides complementary to known genes or cDNA sequences can be directly synthesized and gridded on the glass slides [65, 66]. One nice example of the use of oligonucleotide arrays is that of [67] who arrayed 135,000 oligonucleotides complementary to the entire 16.6 kb human mitochondrial genome and were able to detect single base polymorphisms throughout the entire mitochondrial genome in single hybridizations.

In plants, DNA microarrays and chips have been applied for studies of stress tolerance and growth. These are very powerful systems for the study of model organisms such as Arabidopsis and rice because their complete genome sequences are available [68-70] and a large number of full length cDNAs have been collected [71,72]. Microarray analysis has allowed the investigation of gene expression related to important physiological [73,74] and agronomic trait including stress responses to high salinity [75-78] and drought [76,77,79-80]. Kawaura *et al*. [81] described the successful construction of a microarray containing 22,000 wheat oligo-DNAs and its utilization for transcriptome analysis of salinity stress response in common wheat.

The SAGE technique allows for both qualitative and quantitative profiles of gene expression by relying on short DNA tags to identify individual transcripts [82]. It is a rapid method of analyzing and cataloging tens of thousands of transcripts through the concatamerization and sequencing of gene tags. It offers the advantage of quantifying gene expression of thousands of genes without hybridization probes and is an alternative to DNA microarrays although the exact advantages and limitations of the two techniques for complex genomes are still to be determined. Velculescu *et al*. [83] analyzed 60,633 tags representing 4,665 yeast genes with expression levels varying from an average of 0.3 to more than 200 copies per yeast cell. One impressive feature of their SAGE results is the finding of genes that had not been predicted from previous analyses of the complete yeast genome sequence. Although SAGE can quantitate the abundance of individual transcripts without prior information on the genome under study, its full utilization depends on knowledge of mRNA sequences and the association of each tag with a known gene. Similarly, DNA microarrays depend on knowledge of at least partial gene sequences such can be obtained through EST programs.

Wheat ESTs

There was wide recognition of the need for the development of the wheat EST resources. During a meeting of approximately 300 wheat and barley researchers at the 8th International Wheat Genetics Symposium (Saskatoon, Canada, August 1998), it was decided to organize an international effort to develop Triticeae genomics resources and collaborations. Devel-

Table 4.
No. of Triticeae ESTs available to public (17 -09-2007).

Plant Species	No. of ESTs
Hordeum vulgare + subsp. *vulgare (barley)*	437,713
Triticum aestivum (wheat)	1,049,881
Hordeum vulgare subsp. *spontaneum*	24,161
Secale cereale	9,293
Triticum monococcum	11,190
Aegilops speltoides	4,315
Triticum turgidum subsp. *durum*	8,924
Triticum turgidum	1,938
Aegilops sp.	4,446
Hordeum sp.	461,906
Secale sp.	9,293
Triticum sp.	1,071,934
Other grass genera:	
Avena sp.	7,632
Brachypodium sp.	20,449
Oryza sp.	1,217,814
Pennisetum sp.	2,848
Saccharum sp.	255,964
Sorghum sp.	227,154
Zea sp.	1,271,325

oping ESTs has become a top priority for crop genomics worldwide [84]. Development and deletion mapping of ESTs from hexaploid wheat were conceived by a group of U.S. researchers as a cost effective approach and a short cut to gene discovery, comparative genomics and evolutionary genomics (a series of papers in Genetics, 2004). It is in this context that expressed sequence tag (EST) analysis has opened exciting prospects for gene discovery, irrespective of the genome size [63,85-90].

As of September 2007, there are over one million Triticeae ESTs available to the public (http://wheat.pw.usda.gov/genome/). For individual entries see Table-4. ESTs have been developed for numerous organisms and sequences are deposited in GenBank (http://www.ncbi.nim.nih.gov/Genbank/index.html) and in species databases (Table-5).

EST Derived SSR Markers

The genomes of all eukaryotes contain iterations of 1-6-bp nucleotide motifs. This class of DNA sequences is known as microsatellites [91] or simple sequenced repeats (SSRs [92]). The ubiquity of SSRs in eukarutic genomes and their usefulness as genetic markers has been well established over the last decade. In mammalian systems, in particular, SSRs have been the marker of choice for several years, and well-developed SSR based linkage maps are available for a number of species [93-95]. A high level of SSR informativeness has also been demonstrated for a variety of plant species and this has prompted the initiation of SSR discovery programs for the majority of important crops [96-105]. A consensus map for microsatellite markers including WMC (Wheat Microsatellite Consortium), GWM (Gatersleben Wheat Microsatellite), GDM (Gatesleben D genome Microsatellite), CFA (Clermont-Ferrand A genome), CFD (Clermont-Ferrand D genome) and BARC (Beltsville Agriculture Research Center) SSR marker sets and totalling 1,235 markers has been developed [106]. There are several SSRs identified as linked to traits of interest. A few examples are the powdery mildew [*Erysiphe graminis* DM *f.sp. tritici* (Em. Marchal)] resistance gene *Pm5e* in common wheat on chromosome 7BL linked to two markers *Xgwm*783 and *Xgwm*1267 [59]. However, to date, a number of limitations have existed with SSR discovery in plants, including a lack of DNA sequence in databases, a perceived low abundance of SSRs and differences in the most common types of repeat found.

Previous analysis of plant DNA sequence database entries for all possible SSR motifs have revealed frequencies ranging from one every 29 kb to 50 kb, depending on species [107,108]. SSR

Table 5.
Plant specific EST databases in which significant value has been added to large collections of EST sequences.

Plant EST database	URL	Genomes Refs
TIGR Plant Gene Indices	http://www.tigr.org/tdb/tgi/plant.shtml	All large collections of plant ESTs
NCBI Unigenes	http://www.ncbi.nlm.nih.gov/UniGene/	11 plants with largest EST collections
MIPS Sputniks	http://mips.gsf.de/proj/sputnik/	All large collections of plant ESTs
PlantGDB	http://www.zmdb.iastate.edu/PlantGDB/	All large collections of plant ESTs
University Minnesota	http://www.ccgb.umn.edu/	Pt, Mt, Gm
B-EST barley database	http://pgrc.ipk-gatersleben.de/est/login.php	Hv
Kazusa EST databases	http://www.kazusa.or.jp/en/plant/database.html	Lj, At, Py, Cr
Solanaceae genomics	http://sgn.cornell.edu/	Different Lycopersicon and Solanum species
Chlamydomonas resource Centre	http://www.biology.duke.edu/chlamy_genome/	Cr

frequency in plants has also been assessed by oligonucleotide hybridization, and such studies have suggested figures in the range of one SSR every 5 kb to 80 kb [102,109]. These results contrast sharply with those for humans, with an estimate of one SSR every 1kb on average [110]. Despite this relative difference in abundance, the perceived advantages of SSRs as markers are such that plant geneticists have resorted to screening large numbers of clones [101,111] or developing selective SSR enrichment techniques [105] to generate sufficient numbers of SSRs for implementation in genetic research [37,112].

The numerous advantages of these types of markers, including their abundance and dispersion throughout the entire genome, high information content, co-dominant inheritance, reproducibility and genomic specificity, are well documented [108,113,114]. However, most genomic SSRs have neither a genic function nor close linkage to coding regions [115], similarly, development of SSR markers is expensive, labour intensive and time consuming, in particular, if they are being developed from genomic libraries. Still, due to the importance of SSRs, they have been developed in a large number of plants including major cereal species such as barley [116], maize [117], oats [118], rice [119,120], rye [121], sorghum [122] and wheat [37,123].

EST-SSRs have received a lot of attention recently because of the increasing number of ESTs being deposited in databases for various economically important plants, such as rice (*Oryza sativa*), maize (*Zea mays*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), barley (*Hordeum vulgare*) and rye (*Secale cereale*). EST-SSRs can be rapidly developed from the *in silico* analysis of EST databases at low cost, and due to their presence in expressed regions, they can lead to the development of gene-based maps which may increase the efficiency of marker-assisted selection (MAS) through the use of candidate genes. Assessments of the polymorphism, diversity and transferability of EST-SSRs has been carried out in rice [124], grape [125], sugarcane [126,127], tomato [128], loblolly pine [129], Alpine Lady-fern [130], pasture grass endophytes [131], barley [132] and rye [133]. With the rapid increase in bread wheat ESTs in the databases (1,049,881; http://www.ncbi.nlm.nih.gov/dbEST), EST-SSRs have become an attractive alternative to complement existing SSR collections, and 101 new EST SSRs loci from bread wheat have recently been added to the wheat genetic map [134]. Comparisons between genomic-SSRs and EST-SSRs have revealed that wheat EST-SSR markers have a lower level of polymorphism but produce higher quality patterns [135-137]. The genetic diversity has also been assessed in a collection of 52 elite exotic wheat genotypes [138], and the results suggest that EST-SSRs can be successfully used for a variety of purposes and may be superior to genomic SSRs for diversity estimation. Genomic SSRs are frequently not transferable to closely related species [139] and thus not suitable for comparative genomics studies. Varshney *et al*. [140] reported a relatively high level of transferability (78.2%) for barley EST-SSRs in wheat followed by 75.2% in rye and 42.4% in rice. The transferability of EST-SSR markers across related triticeae species makes them a valuable source for comparative genomics studies.

In addition to the advantages of genomic SSR markers mentioned earlier, EST-SSRs show a high level of transferability to closely related species because they originate from conserved transcribed regions that are better conserved between the genomes; this consequently facilitates their use in comparative mapping [141], as do restriction fragment length polymorphism (RFLP) markers derived from cDNA [for a review, see 142]. The transferability of bread wheat EST-SSRs across 18 wild relatives and five cereal species (barley, rye, oat, rice and maize) was studied with 78 EST-SSR markers [138]. More than 80% of cross-species

transferability was observed with wild relatives; this rose to as high as 90% with at least one of the cereal species. Similarly, a relatively high level of transferability (55%) of EST-SSRs was found from barley to wheat [143]. In another study, 368 EST-SSRs derived from five different grass species (barley, maize, rice, sorghum and wheat) were developed and 149 loci integrated into a reference wheat genetic map; 80 of these were subsequently assigned to chromosomes using nullisomic-tetrasomic lines [144]. In wheat, *in silico* analysis showed that the frequency of EST-SSRs is 1 at every 6.2 kb of EST sequence [145]. Recently, Singh *et al.* [146] reported 21 anchored simple sequence repeat (SSR) primer pairs detecting SSR length polymorphism and 42 anchored SSR primers detecting microsatellite-anchored fragment length polymorphisms (MFLPs) 21 anchored simple sequence repeat (SSR) primer pairs detecting SSR length polymorphism and 42 anchored SSR primers detecting microsatellite-anchored fragment length polymorphisms (MFLPs) with average value of polymorphic information content (PIC) as 0.473 for SSRs and 0.061 for MFLP.

Single Nucleotide Polymorphism (SNP)

Whole-genome sequences of *Arabidopsis* and rice have provided a fundamental platform for the discovery of gene content and function in dicot and monocot plants. Research on the model species has provided a wealth of knowledge on universal biochemical and genetic processes, as well as the development of analytical tools that are applicable to other plant species [147-149].

Large-scale genome sequencing programs offer a potential solution to the scarcity of markers that can be used in elite populations. The Single nucleotide polymorphisms (SNPs) are a second class of genetic markers that can be mined from sequence data and are useful for characterizing allelic variation, genome-wide mapping, and as a tool for marker-assisted selection. In the field of human genetics, SNPs are a major focus of efforts to increase the efficiency of mapping [150-153] and are already being used for detection and mapping of a variety of diseases [154]. The frequency and nature of SNPs in plants is beginning to receive considerable attention.

A range of methods have been developed for SNP detection. The RFLP method can be considered to be the first, which was used for the detection of mutations in the restriction sites. Since the 1980s, a variety of more or less sophisticated methods have been developed to screen for SNPs. The methods of SNP analysis can rely on enzymatic cleavage, hybridization with allele-specific probes, oligonucleotide ligation or single nucleotide primer extension, and can exploit different resolution and detection methods. Allele-specific amplification, ligase chain reaction (LCR), heteroduplex analysis, dideoxy sequencing, pyrosequencing, single-nucleotide primer extension, real-time PCR analysis and microarray scanning remain the methods of choice, with different throughputs and costs of a single assay [155,156]. The abundance of SNPs in cereals makes them extremely important for creating high density genetic maps. The range of public databases of nucleotide sequences opens the possibility to design SNP assays for genes or sequences of interest. Projects of SNP detection and validation are run for maize (http://www.agron.missouri.edu/) and wheat (http://wheat.pw.usda.gov/ ITMI/WheatSNP/). In wheat, SNPs from ESTs are predominantly being identified in the frame of the International Wheat SNP Consortium.

Pyrosequencing was frequently used in cereals [157,158]. This method has been used for SNP discovery in regions flanking microsatellite markers and for validation of SNPs identified in the EST database leading to the detection of either intergenomic or intervariety polymorphism. A number of SNPs have been identified in g-glia-

din genes [159] and explored using an allele-specific amplification approach. The cleaved amplified polymorphic sequence (dCAPS) method [160] was used to detect SNPs in the common wheat gene *Wx-D1* [161] and direct sequencing was used to detect SNPs in the barley P450 cytochrome genes [162]. SNP in genes can result in a change of phenotype; thus, the detection of SNPs in plant coding sequences, with its potential for automation, can greatly accelerate molecular breeding [163]. An advantage in using SNPs in plant breeding applications is that genotyping can be automated using single nucleotide primer extension assays [164], thus offering a potential to increase both efficiency and throughput.

EST Derived SNPs

There are a number of methods for discovering and identifying SNPs within a genetic locus. What ever method is chosen to discover and detect the polymorphism, the initial step is almost always to determine the sequence of the locus for a reference genotype. Once determined, this sequence is used to design oligonucleotide primers for use in PCR, which forms the cornerstone of all subsequent SNP based technology [165]. However, sequence analysis is also the most time consuming and costly methodology. There are many problems related to direct sequencing, one of which is sequencing error. A second problem relates to heterozygosity or ploidy level higher than diploid (e.g. wheat).

With the initiation of large scale plant expressed sequence tag (EST) sequencing program [166,167] also see http://www.wheat.pw.usda.gov/genome; http://www.wheat.pw.usda.gov/wEST/insf/title. html; http://www.zmdb.iastate.edu/zmdb/EST_project.html), a new and potentially rich source of SNPs has been uncovered. There are several reasons why ESTs might provide a number of SNPs for genotyping. The sequencing of ESTs provides sequence data for expressed genes. It is reasonable to expect that at least some of these ESTs will be responsible for the observed agronomic traits. Therefore, unlike anonymous markers such as microsatellites, the SNPs derived from ESTs could underlie the traits being examined.

In the mean while, a NSF funded project, executed by Prof. Jan Dvorak of University of California has the intentions to discover 1,800 SNPs and make their location on wheat chromosome and publicly available soon (http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0321757).

Future Perspective and Conclusion

There is no real substitute for a complete genome sequence: only with the elucidation of the sequence of the complete chromosomes can we dissect the gene complement and unravel the mechanistic pathways that make up the plant. Until new technologies become generally available that can produce longer sequence reads more cheaply, we will be limited to incomplete solutions. As long as ESTs continue to be actively sequenced to fill in knowledge gaps from the gene complement of the large plant genomes, our potential knowledge bases will continue to grow. EST sequencing certainly avoids the biggest problems associated with genome size and the accompanying retrotransposon repetitiveness. The EST sequence resources have been shown to have a wide range of applications and novel uses have been found for the resources. Although relatively, new in their concept, both SNPs and SSRs derived from ESTs are well on their way to becoming the dominant marker system in plant breeding. Given the large resources required to develop and utilize them, it is probable that their use in academic laboratories will require more time. However, due to the significant practical advantages and ability to examine polymorphism within EST's underlying different traits, there is no doubt that these will become the method of

choice in the near future.

International Wheat Genome Sequencing Consortium (IWGSC)

The International Wheat Genome Sequencing Consortium (IWGSC) is a collaboration focused on building the foundation for advancing agricultural research for wheat production and utilization by developing DNA-based tools and resources that result from the complete genome sequence of common (hexaploid) wheat. The IWGSC was established to facilitate and coordinate international efforts toward obtaining the complete sequence of the common wheat genome. To this end, the IWGSC will continue to refine the strategic roadmap, integrate existing international resources, and develop a sequencing strategy that will capture international participation and a broad funding base. IWGSC scientists from Australia, France, Italy, People's Republic of China, Turkey, United States, United Kingdom, and European Union are responsible for constructing physical maps of individual chromosomes or chromosome arms of the wheat genome as explained in the Fig.1.

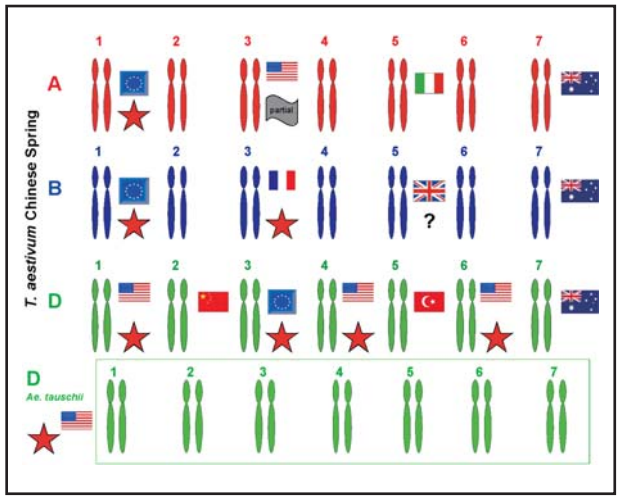

Fig. 1.
IWGSC scientists are responsible for constructing physical maps of individual chromosomes or chromosome arms of the wheat genome (Source: http://www.wheatgenome.org/).

International Genome Research on Wheat (IGROW)

IGROW was organized in 2002 to complement the IWGOC and ITMI and provide a focus for rapidly developing wheat genomics research. The immediate, urgent goal is to generate a draft sequence of the gene-rich regions of the wheat genome. Many people on behalf of IGROW have been very active in support of this mandate (http://www.k-state.edu/igrow/).

In the meantime, wheat genomics research is moving forward. The year 2003 was the last of a 4-year project funded by the NSF involving 10 universities on 'Structure and function of the expressed portion of the wheat genomes' (lead PI Cal Qualset, University of California, Davis) (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). As a result of this project and ongoing work elsewhere, wheat now ranks number one in plants with over 400,000 ESTs (http:/www.ncbi.nlm.nih.gov/dbEST) and also is the most densely mapped genome with over 20,000 EST loci mapped on the 21 chromosomes of wheat (see project website). Another NSF-funded project entitled 'Insular organization of the D genome of wheat' (lead PI Jan Dvorak, University of California, Davis) is constructing a global BAC-contig map of the D genome of wheat that is anchored to the EST physical map of D-genome chromosomes (project website: http://wheat.pw.usda.gov/PhysicalMapping). Jorge Dubcovsky is a lead PI (University of California, Davis) on a USDA-IFAFS project 'Bringing Genomics to the Wheat Fields,' which involves most of the public-breeding programs in the U.S. (project website: http://maswheat.ucdavis.edu/Production.htm). Jan Dvorak and Shahryar Kianian (North Dakota State University,Fargo) won awards for virtual wheat center proposals in 2003 from the highly competitive NSF Crop

Genome Research Program. Dvorak proposal will establish a virtual center at UC Davis in wheat SNPs, a new generation of markers. Shahryar's proposal will establish a virtual center in wheat mutagenesis and functional genomics at NDSU in Fargo. The above mentioned proposals are not only producing resources for the wheat genetics community, but have done much to bolster the position of wheat as a genetic.

International Wheat SNP Consortium (IWSC)

It is now feasible to envisage the development of SNP markers in wheat, due to the explosion in the availability of ESTs. The hexaploid nature of the wheat genome makes such analysis more complex than it would be in species with simple genomes. The starting point for the SNP development is a collection of relatively over-assembled contigs of ESTs that enable PCR primers to be designed that will amplify the sequences present at one locus only, and also enable the verification that the SNP is real, and not a sequencing error. An open international consortium of institutions (public and private) is attempting to mine the contigs in a coordinated way, pooling information on validated SNPs and avoiding duplication of effort. The current status of wheat SNP development in the consortium will be evaluated. Progress in this project can be found at http://wheat.pw.usda.gov/ITMI/2002/WheatSNP.html.

Diversity Array and Microarray

DArT markers can be used as any other genetic marker. With DArT, comprehensive genome profiles are becoming affordable for virtually any crop, regardless of the level of molecular information available for the crop. DArT genome profiles will enable breeders to map QTL in one week, thereby allowing them to focus on the most crucial factor in plant breeding: reliable and precise phenotyping. Once many genomic regions of interest are identified in many different lines, DArT profiles accelerate the introgression of a selected genomic region into an elite genetic background. Furthermore DArT profiles can be used to guide the assembly of many different regions into improved varieties. Therefore, Diversity Array Technology (DArT), is a novel method to discover and score genetic polymorphic markers with capability to discover hundreds of markers in a single experiment is now getting popularity among scientific community for use in wheat in the near future. On the other hand, DNA Microarrays have become indispensable for functional genomics because they can systematically investigate transcriptional profiles. A variety of DNA microarray and chips have been developed and applied to transcriptome analysis of important organisms. Wheat microarray chips will also be available shortly for global gene profiling of the response to abiotic stress tolerance. Such chips for global gene profiling studies should help to elucidate the mechanisms of abiotic stress tolerance in wheat.

Role of MicroRNA under stress conditions

MicroRNAs (miRNAs) are a class of recently discovered non-coding endogenous small RNAs [168]. They have emerged as key elements of genetic control in organisms as evolutionary distant as plants and mammals [168,169]. Different lines of evidence has shown that these non-coding RNA molecules are regulatory elements involved in a wide variety of processes related to gene regulation during cell growth and development. miRNAs are naturally occurring, endogenous 21–24 nucleotide RNAs derived from genomic loci and consequently are not cleavage products of RNA silencing inducers, such as transposons, transgenes or viruses [168,169]. They are transcribed from transcriptionally autonomous units through precursor molecules with the potential to form self-complementary fold-back structures [168,169]. Growing evidence suggests that miRNAs have important roles in controlling gene expression in eukaryotes [170].

As negative regulators, they act on their target mRNAs to trigger either mRNA cleavage or inhibition of protein synthesis. Plant miRNAs generally interact with internal regions of target mRNAs through perfect or near perfect base pairing to trigger mRNA cleavage within the region of complementarity in a manner that resembles siRNA guided degradation [171,172].

In addition to their roles in development, plant miRNA seem to have an important function in adaptive responses to abiotic stresses. miRNAs appear also to play a role in these mechanisms. Zhang *et al.* [173] reported that 25.8% of ESTs containing miRNAs were found in stress-induced plant tissues. Although no experiments have confirmed that these miRNAs were only obtained from stress-induced tissues, the large percentage of ESTs containing miRNAs is indicative of miRNAs playing some role in plant responses to environmental stresses [174]. Therefore, it is speculated that miRNAs appear to be involved in plant responses to a variety of abiotic environmental stresses. Environmental Stress induces certain miRNAs to be over- or under expressed, or plants may have evolved some mechanism to synthesize certain miRNAs to help cope with these stresses. The study of specific responses of miRNAs to environmental stress will help us improve plant resistance to environmental stresses, especially drought stress.

Significant progress in the field of wheat genomics has already been made during last few years. For example, the availability of a variety of molecular markers facilitated the preparation of high-density maps, which proved useful in the identification of molecular markers linked with genes and/or QTLs for a variety of economic traits, including those conferring tolerance to biotic and abiotic stresses. Development of functional molecular markers as a by-product of available sequence data will be useful for marker–trait association studies and examining the functional diversity in breeding germplasm collections or natural populations. Furthermore, genome and/or gene space or EST sequencing provides the sequence data to identify candidate genes for agronomic traits, either through *in silico* approaches, with the help of bioinformatics tools, or 'wet' laboratory experiments such as transcript profiling using micro- or macro-arrays. More interestingly, exploitation of association mapping approaches and expression genetics might provide the best molecular markers (e.g. functional markers) for a trait of interest, which can be used across different genetic backgrounds in MAS. These types of (functional) molecular markers should and/or will ideally co-segregate with the trait of interest. In general, such a marker will often be based on a SNP.

Integration of the above-mentioned genetic and genomic approaches, together with transcriptomics, proteomics, metabolomics and tools of bioinformatics is essential for the effective use of genomics in breeding. However, wheat genomics still faces many challenges. Some of these challenges include precise phenotyping, low heritability of traits, epistatsis, epigenetics, regulatory variation, technical difficulties and cost-investment issues. Indeed, in the post-genomic era--owing to the availability of high-throughput approaches combined with automation, the rapid increase in sequence data in the public domain and good expertise and tools in the area of bioinformatics--genomics holds great potential to provide solutions to problems of wheat community.

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