Wheat : Cytology in the Genomics Era and its Relevance in Breeding

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Abstract:

Wheat is the second important global cereal studied extensively by researchers. We studied the mitotic metaphase plate of *Triticum aestivum* (bread wheat, 2n=42) with the aim of developing a simplest convenient method for karyotype analysis. Besides karyomorphological studies, much advancement has been made regarding genome sequencing in the last decade. Beginning with the sequencing of single arm of chromosome to whole plant genomes, research has advanced rapidly with development of next generation sequencing technology, providing necessary throughput to conquer the mammoth sized complex genome. We discuss the progresses and hindrances that have been experienced towards the full annotation of hexaploid wheat genome sequencing. We have outlined the available tools and methodologies that facilitate breeding of elite varieties of wheat in global agriculture.

Keywords: Wheat, cytology, whole genome sequencing, transgenics, breeding

Introduction

Common wheat or bread wheat (*Triticum aestivum* L.) is one of the most important global food crop. It was first domesticated in Western Asia during the early Holocene, and spread from there to North Africa, Europe and East Asia in the prehistoric period. Naked wheats (including *T. aestivum/durum/turgidum*) were found in Roman burial sites ranging from 100BCE to 300CE. Wheat first reached North America with Spanish missions in the 16th century, but North America's role as a major exporter of grain dates from the colonization of the prairies in the 1870s. As grain exports from Russia ceased in the First World War, grain production in Kansas doubled. Worldwide, bread wheat has proved well adapted to modern industrial baking, and has displaced many of the other wheat, barley, and rye species that were once commonly used for bread making, particularly in Europe. Bread wheat is not only an important cereal crop but also a model for study of an allopolyploid plant with a large, highly repetitive genome. Transgenic technologies have been applied in development of disease resistant, drought tolerant and biofortified varieties of wheat. Advances in next-generation sequencing (NGS) technology provide needed throughput to conquer the enormous size of the wheat genome. We highlight the available tools and methodologies for wheat functional genomics research developed with the assistance of NGS technology. Our work also involves the classical karyomorphological study of a local wheat variety. Study of the polyploidisation and subsequent evolution of wheat is now based the traditional methods (chromosome counting, analysis of meiotic chromosome pairing, and marker-based mapping) as well as the more sensitive genome sequencing. This provides a detailed roadmap containing all information needed to generate desirable agronomic traits for future food security.

Evolutionary aspects

Bread wheat is an allohexaploid (an allopolyploid with 3 sub genomes A, B, D each having 7 chromosomes). Hexaploid wheat originated from 2 individual hybridisation events. Hybridisation followed by doubling of chromosomes facilitates the formation of normal bivalent as well as enables the fertility in plants. In the first event, a primitive tetraploid species *T. turgidum* ssp. *diccocum* (genomes AABB 2n=4x=28) (Dvorak and Gorham 1992), originated from initial hybridisation between two diploid wild wheat species *T. urartu L.* (2n=14, A genome donor) and unconfirmed species (B genome donor). The above mentioned 2 wild diploid wheat species formed amphidiploids (AABB) which give rise to tetraploid wheat *T. turgidum* commonly known as emmer wheat. This hybrid occurred in the cytoplasm of B

genome. Although specific identity of B genome donor remain uncertain but till today it was found that B genome is closely interconnected to S genome of Sitopsis section (*A. speltoides*, *A. searsii* etc. of genus *Aegilops* l.). After studying several DNA hybridisation it was mostly accepted by the scientists that *A. speltoides* (or *T. searsii*, 2n= 2x=14) is the B genome donor and it also seems that the original donor of B genome is no longer present in the wild. Around 10,000 years ago a second events occurred, emmer wheat out crossed with another wild diploid wheat *A. tauschii* (formerly *A. squarossa*, 2n=14), the D genome donor. This resulted the formation of hexaploid wheat (2n = 6x =42) (Feldman et al 1995). Attempts to determine the timing of wheat speciation, using DNA data, suggest that the diploid progenitors of allopolyploid wheat have diverged from a common progenitor some 2.5–4.5 MYA (Huang et al 2002). Because of this fairly recent divergence, most of the diploid wheat species have relatively limited morphological and molecular variation, occupy only a few well-defined ecological habitats, and are distributed throughout relatively small geographical areas. In historical terms, allotetraploid wheat developed about 300,000 to 500,000 years ago (Huang et al 2002), while allohexaploid wheat was formed only about 10,000 years ago (Feldman et al 1995, Feldman et al 2012).

The first cultivated wheat was the diploid *T. monococcum,* einkorn wheat selected from wild *T. monococcum*, and both wild and cultivated *monococcums* are reproductively isolated from *T. urartu* with interspecific hybrids being sterile, although the two wild species have similar morphology (Nevo et al 2012). *T. urartu* has only limited distribution in the western Mediterranean, and mainly on basaltic soils and often in mixed stands with wild einkorn (Damania et al 1998). The emmer wheats are tetraploid (AABB) genome, with the "A" genome derived from *T. urartu*, and the "B" genome from *A. speltoides* (Nevo et al 2012). The wild emmer (*T. dicoccoides*) is a subspecies of *T. turgidum* L., which includes several cultivated types of which the durum-free threshing wheats are the most prominent in agriculture. The *T. timopheevi* wheat is a tetraploid, but with an AAGG genome hybrids with *T. turgidum* L. are infertile.

Cytology

Seeds of *T. aestivum* were soaked in water for 12 hrs and germinated on the moist filter paper at 25-30! for 36-40 hrs under dark condition. The germination and cytological experiments were performed at the Cytogenetics laboratory, Bethune College, Kolkata.

The protocol for obtaining well scattered metaphase plate was standardised in the present work. Secondary or adventitious root were excised and pre-treated with saturated PDB for 3 hrs at 12!. The root tips were fixed in 1:3 aceto- ethanol solution (Carnoy's fluid) for overnight and followed by 45% acetic acid for 5 minutes. Root tips were then transferred into staining solution containing 2% aceto-orcein and 1N HCl (9:1) for 1 hour. Further towards proper staining the root tips can be kept for 1 day before squashing the root tips in 45 % acetic acid. The preparation was temporarily sealed and observed under compound light microscope. The selected cells were photographed. 50-60 metaphase plates were screened for karyotype analysis.

Length of chromosome in mm = (length of chromosome / magnification value) ×1000

Mitotic centromeric index (i) = (length of short arm / length of long arm) ×100

Chromosomes were classified into group according to their 'i' values and nature of primary constriction. A simple protocol for obtaining well scattered metaphase plate suitable for karyotype analysis was standardised in this present work. To the best of our knowledge this is the first report of a simple and easily reproducible protocol. Our results suggest that use of secondary root is more convenient for karyomorphological study of wheat.

The results of karyotype analysis represented in **figure 1** indicates the chromosome number 2n=42 and the morphological feature observed in these chromosomes was that they were strictly metacentric to sub- metacentric in nature. Secondary constriction was also observed.

Karyotype of *T. aestivum* consists 2n=42 chromosomes. According to i-value, the chromosomes can be grouped into three groups:

GROUP A: This group consists of '2' pairs of chromosomes. Size of chromosomes ranges from 12.14-14.29 µm. They are secondary constricted chromosome; A1, A1' and A2, A2'. Constriction is at nearly median region.

GROUP B: This group consists of '9' pairs of chromosomes. Size of chromosomes ranges from 10.5-24 µm. They are B1, B1'; B2, B2'; B3, B3'; B4, B4'; B5, B5'; B6, B6'; B7, B7'; B8, B8'; B9, B9. Constrictions is at nearly median region.

GROUP C: This group consists of '10' pairs of chromosomes. Size of chromosomes range from 7.86-17.50 µm. They are C1, C1'; C2, C2'; C3, C3'; C4, C4'; C5, C5'; C6, C6'; C7, C7'; C8, C8'; C9, C9'; C10, C10'. Constriction is at nearly sub- median region.

Fig. 1 (a-e)

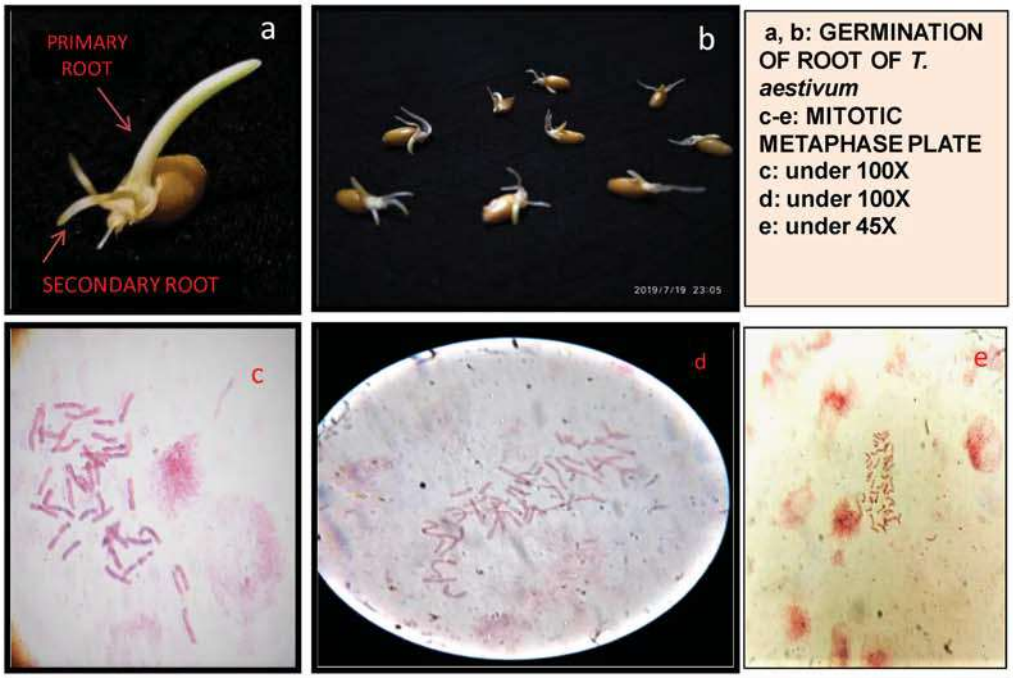
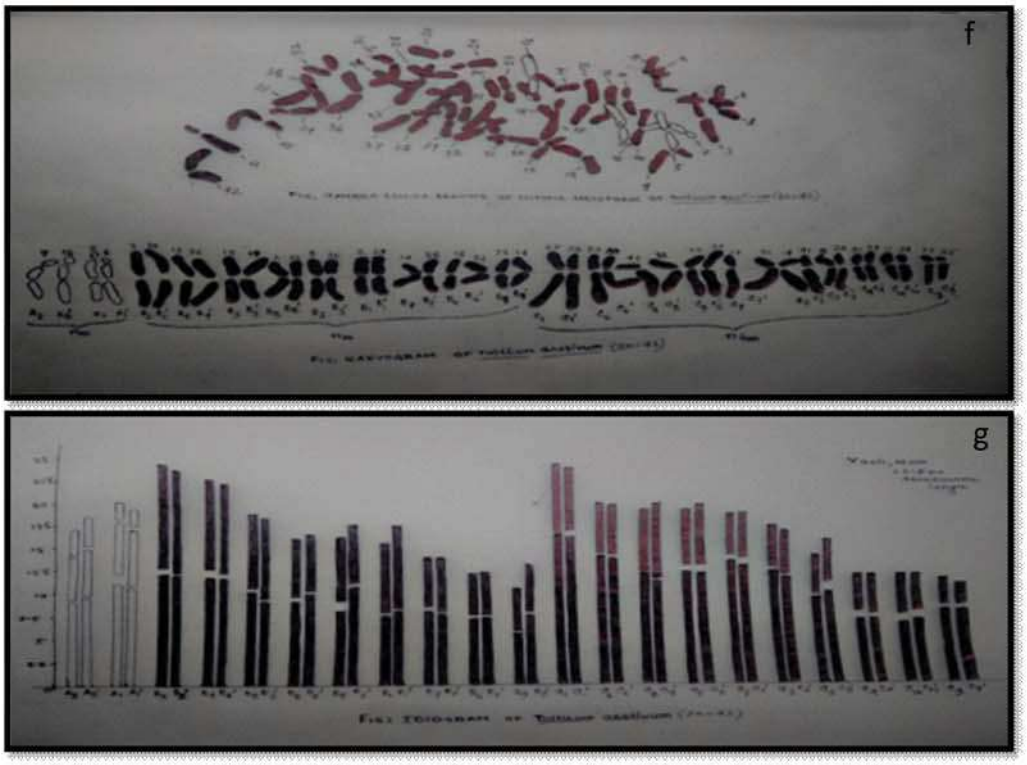


Fig. 1 (f-g)



f:THE KARYOGRAM DRAWING OF *T. aestivum* (from d); g:IDIOGRAM OF *T. aestivum* (from d)

Karyomorphological studies performed by Ehteamam et al. 2014 in different expression (T. aest- 47 T. aest- 74, T. aest-, t a s t 129, T. aest- 73 T. aest-97, T. aest- 96 T. aest 107, T. aest- 49 , T. aest- 82 and Chinese spring reveals the chromosome number of *T. aestivum* 2n= 42 and hexaploid efficient having 225 submetacentric chromosome in their karyotype and mean of the total chromosome length 240 1.002 the longest chromosome and the shortest chromosome length is found in *T. aestivum* in comparison with *T. urartu, T. boeticum , T. turgidum, T. monococcum.* The short arm length ranges between 17.38 to 24.77 % and the long arm of chromosome length vary from 12.93 to 19.0932 and the studies also reveal the highest asymmetry in the karyotype of *T. aestivum.* Karyotype analysis of monosomic, di-somic, tri-somic lines of Chinese spring and of F1 hybrid between monosomic and wheat identify the chromosomes and also reported that D genome are the shortest and the longest is the B genome. Two pairs satellite chromosome and 5 median ,10 sub median and 4 sub –terminal chromosome are found in the Chinese spring and the D genome is of 28 -22 %of the total chromosome length of hexaploid wheat. The story why synthetic and cultivated wheat by Rosyara et al. 2018 analyzed mitotic metaphase chromosome in altar 84 / *A. squarossa* (192) a hexaploid wheat collected by CIMMYT. The highest value recorded 99.1 µm in synthetic hexaploid wheat (Doy1/*A. squarossa* (458) with mean chromosome length of 5.67±0.54 µm. These results are in agreement with those of Kamel (2006) who reported the total chromosome length, mean chromosome length, and mean arm ratio varied among some genotypes of *T. aestivum.* The highest value for difference of range relative length was found in one of the synthetic hexaploid wheat (Altar 84/ *A. squarossa* (221)). Jahan and Vahidy (1984) analysed bread wheat Cv. Sarsabz and documented 2n=42 chromosomes and identify A, B, D genome based on total length, arm ratio and recorded 1B and 6B carrying NOR in the short arm and secondary constriction are proximal to the satellites. Ratio of chromosomes-arm in their experiment varies between 2.0 -1.1 as Gill reported same ratio in Chinese spring (1996). Karp and Maddock (1984) studied chromosomes of 19 regenerated plants derived from immature embryo callus culture of for hexaploid wheat cultivars. A total of 71 % of the regenerants carried the expected 2n=42 chromosomes and 29 % of the plants were aneuploid (2n= 38 to 45).

Marker assisted breeding

Bread wheat has no wild hexaploid progenitor in nature but has been domesticated by humans over the last 10,000 years from natural hybrids of diploid and tetraploid species (Salamini et al 2002). Diploid einkorn and tetraploid emmer wheat were cultivated by Neolithic humans and, together with wild species such as *T. urartu*, *A. tauschii*, and *A. speltoides*, formed part of the complex evolutionary path that led to the bread and durum wheats farmed today (Feldmann 2012). Over the last 50 years, developments in breeding methods, together with improvements in chemical inputs and farming practices, have permitted major advances in wheat breeding, exemplified by the many thousands of highly adapted wheat varieties now available with significant yield and quality benefits. Particularly significant was the exploitation of *Rht-B1* and *Rht-D1* dwarfing genes and the shuttle breeding methods used by the International Maize and Wheat Improvement Center (CIMMYT), Mexico, in the 1950s (Jones et al 2009). The use of wide hybridizations to generate substitution, addition, and translocation lines, together with information on how quantitative trait loci, specific genes or induced mutations influence end-use quality traits, is now well established in wheat breeding. Complementary progress has been made in research laboratories, where DNA transformation has had a significant impact on our understanding of the roles played by the specific genes controlling quality traits. Genetic modification using recombinant DNA, either originating from within the species gene pool (intra- or cisgenic) or from different species (transgenic), has the potential to drive major improvements in wheat quality (Jones et al 2009).

Marker Assisted Selection (MAS) is based on the concept which describes the loci located closely together on the same chromosome are more likely to be inherited in a recombination event during meiosis. Molecular markers are also used in MAS to detect the particular gene of interest underlying specific trait. It is the technique to identify different alleles (polymorphisms) between several individuals. Genetic linkage aids molecular markers to reveal if the linked allele is present or not in a line. There exist several molecular markers, based on the types of polymorphism. Nowadays MAS markers identifying single nucleotide differences are usually applied.

Increased global population, demand for superior quality grains, and rapidly evolving pathogens have necessitated the breeding of high-yielding, disease-resistant wheat cultivars. Significant improvements in breeding efficiency can be

made through advances in wheat genetics and genomics to develop tools that accelerate genetic gains in wheat. The identification of genes and quantitative trait loci for economically important traits and the development of associated molecular markers have the potential to improve selection efficiency. Marker-assisted selection enriches desirable allelic frequency, complements phenotypic data, and facilitates gene stacking. Molecular markers have been developed for various genes and quantitative trait loci conferring resistance to leaf rust, stripe rust, stem rust, *Fusarium* head blight, loose smut, common bunt, leaf spot, wheat blossom midge, and wheat stem sawfly ref. Markers are available for wheat grain and flour characteristics as well. Agronomic traits such as vernalization requirement, day-length sensitivity, and plant height can also be selected using molecular markers.

Single markers analyses (one trait assayed on one plant sample) have been developed and in modern technique it involves DNA amplifications. There are many useful technology platforms of assay SNP markers. Most straight forward is allele specific PCR (As-PCR). Some applications of MAS includes rust resistance, yellow rust resistance, *Septoria tritici* blotch, gluten content etc (Vagndorf et al 2018). So applying such MAS markers have proved as advantageous over conventional breeding as MAS marker can fix the desirable trait in early stages. Moreover, marker assisted breeding program and back crossing help to transfer important agronomic genes from wild relatives to cultivar wheat.

Whole genome sequencing

Genome is the haploid set of chromosomes in an organism, the genetic material containing DNA (or RNA). Whole genome sequencing is the process to figure out the order of DNA sequence, nucleotides or bases in a genome. Analysis of genome provides the valuable information on presence of genes, how the genes work together to direct the growth, development and regulate other necessary work in an organism.

Wheat occupies 17% of all crop area (in 2002, 210 million hectares *vs.* 147 million for rice and 139 million for maize). The trade value of wheat exceeds that of any other cereal species, including rice and maize: $31 billion of world trade in 2001 *vs.* $13 and $19 billion for rice and maize (FAOSTAT database). Besides being an important cereal crop it is an ideal model to study polyploidy nature. To develop the improved varieties of wheat and to meet the agronomic demand with the increase of population the sequencing of wheat genome becomes a fundamental requirement. But this basic necessity was lagged behind due to the complexity and mammoth size of wheat genome.

Common wheat is an allohexaploid consisting of seven groups of chromosomes, each group containing a set of three homoeologous chromosomes belonging to the A, B and D genomes, derived from a common ancestor. Despite their close homology, homoeologs are normally prevented from pairing by the *Ph1* gene on the long arm of chromosome 5B. Thus, common wheat functions much like a diploid organism, although it is able to tolerate aneuploidy due to the buffering effect of polyploidy. Sets of viable mono-, tri- and tetrasomic cytogenetic stocks were developed for all chromosomes, and nullisomics were developed for 11 chromosomes (Sears 1954). Since the loss of a pair of chromosomes can be compensated by two additional doses of a homeolog, 42 compensating nulli-tetrasomics were developed (Sears 1966). The monosomic chromosomes tend to misdivide and this property was exploited to produce a series of chromosome-arm aneuploids: monotelosomics, ditelosomics, tritelosomics, and iso-chromosome lines (Sears and Sears 1978). More recently, taking advantage of the gametocidal chromosome introduced from the *A. cylindrica* host, Gill et al (1996) developed 436 segmental deletion lines in Chinese Spring (CS). All of these genetic stocks, which are in the CS background, have been used to localize genes or markers to a specific chromosome, chromosome arm, or sub arm region and play a central role in wheat genetics and genomics.

Rice, maize, and wheat, which coevolved from a common ancestor, differ greatly in genome size. Among agricultural crops, common bread or hexaploid wheat (*T. aestivum* L., $2n = 6x = 42$, AABBDD) has the largest genome at 16,000 Mb, <8-fold larger than that of maize and 40-fold larger than that of rice (Arumuganathan and Earle1991). Amplification of transposable elements (TEs), coupled with duplication of chromosome segments, was a major driving force for cereal genome expansion, although polyploidization also contributed to the large genome size of wheat. About 90% of the wheat genome consists of repeated sequence.

UNDERSTANDING OF THE WHEAT GENOME AND ITS SEQUENCE

The 21 wheat chromosomes can be readily identified by heterochromatic banding (Gill e al 1999) or hybridization patterns using repetitive DNA probes (Pedersen and Langride 1997). A specific chromosome or chromosome arm

can be flow sorted at high purity using the genetic stocks (Vrana et al 2000). These sorted chromosomes have been used for construction of chromosome-specific BAC libraries (Safar et al 2004), together with other genetic and molecular resources for wheat genome sequencing.

Currently, ESTs (<500,000 to date) are the largest sequence resource for wheat. ESTs are cDNA clones, and as such they do not contain promoters, introns, and other functional elements. Until 2004, most completed genome sequences had been obtained through a clone-by-clone approach. Though the data provided was comprehensive, it was an expensive approach, especially if considered for a large genome such as that of wheat. In turn, other methods also had their limitations as whole genome shotgun sequencing was hindered by computational power, especially for genomes with large numbers of repeats and genomic filtration techniques such as methylation filtration and cot selection were not well tested enough to guarantee high percentages of gene identification. Ultimately, it was decided to sequence individual chromosomes of wheat, separated by flow cytometry and used to construct Bacterial Artificial chromosome.

ANALYSIS OF WHOLE GENOME SHOTGUN SEQUENCING

Grass genomes shows long-range conservation in gene order as they are highly dynamic due to the activities of repeat contents which contribute to vast variation in genome size, changes in local gene order and pseudo gene formation, particularly in larger genomes such as wheat. Analysing of BAC contigs on chromosome 3B, it is found that the 17-gigabase-pair (Gb) genome was composed of approximately 80% repeats, primarily retro elements, with a gene density of between 1 per 87 kb pairs and 1 per 184 kb. Information gained on the wheat genome from these studies and the importance of the wheat crop, a comprehensive genome-wide analysis of gene content has yet to be conducted due to its mammoth size, repetitive sequence and polyploid complexity.

Using gene sequences from the diverse grasses low-coverage, long-read (454) shotgun sequence of the hexaploid wheat genome was analysed and from this, assemblies of wheat genes in an orthologous gene family framework was created by using diploid wheat relatives to classify homoeologous relationships, and clarify a genome-wide catalogue of single nucleotide polymorphisms (SNPs) in the A, B and D genomes. These analysis contribute a fundamental step genetic and genomic analysis of this crop.

Nine years after the initial conceptualization of a genome sequence for hexaploid wheat, Brenchley et al (2012) published the first draft sequence obtained through whole genome shotgun sequencing. The authors obtained lower coverage (5 fold) with longer read lengths on the Roche 454 sequencing platform. Using diploid wheat relatives and progenitors the authors could classify homoeologous relationships and build orthologous gene family frameworks. Ultimately, a catalogue of 132,000 SNPs was created in the various sub-genomes of wheat. Repeat elements were identified to make up 79% of the sequenced genome and the number of genes was estimated at 94,000 to 96,000. A representation of nearly all wheat genes could be identified as constructed orthologous groups matched 90% of metabolic genes in Arabidopsis and 92% of publicly available wheat full- length cDNA. Overall, a trend toward gene family size reduction was observed in hexaploid wheat despite its recent evolution as a hexaploid. Expanded gene families' overrepresented categories included proteins involved in energy metabolism, defence, growth and nutrition. The authors also found gene families to be classified as A, B or D-genome derived, which indicates that transcriptional regulatory networks are maintained in a genome-specific manner in wheat.

In order to decode the mystery of the wheat genome and to expedite molecular breeding in wheat, a group of scientists and breeders initiated the International Wheat Genome Sequencing Consortium (IWGSC) in 2005. To overcome the difficulties caused by genome size and complexities, the 21 chromosomes of common wheat landrace Chinese Spring were separated by flow cytometric sorting. Bacterial artificial chromosome (BAC) libraries and physical maps were then constructed for each chromosome or chromosome arm. Chromosome sorting, DNA isolation and BAC library construction for each chromosome arm were performed in the laboratory of Prof. Jaroslav Dolezel at the Institute of Experimental Botany in the Czech Republic. Subsequent physical map construction and BAC sequencing were assigned to different laboratories of the International Wheat Genome Sequencing Consortium (IWGSC). Numerous projects by different groups were undertaken to produce reference sequences of single chromosome or chromosome arms. Currently, all chromosomes/chromosome arms of Chinese Spring have been sorted and their physical maps have been constructed. Sequences of many chromosomes, or parts thereof, are publicly available, including 1AS,

1BS, 3DS, 5DS, 7DS, 1AL, 1BL, 4A, 5A, 6A, 6B, and 7B. In addition to the chromosome-based BAC-by-BAC sequencing strategy of IWGSC, Hall and colleagues in the UK applied a whole genome shotgun sequencing strategy with 454 pyro-sequencing technology to sequence Chinese Spring, and produced a five-fold coverage genome sequence of Chinese Spring in 2012.

In 2017, Bierman and Botha et al published an improved genome sequence of Chinese Spring. They used precisely sized mate-pair libraries and an optimized algorithm to generate a new assembly representing > 78% of the genome, much higher than the scaffold proportion (~ 49%) produced previously by IWGSC. IWGSC with 2400 members of 68 countries is an international collaborative consortium established in 2005 by academics, plant growers, scientist and private breeders public to generate a high quality Ref Seq of bread wheat. Its vision is to make a high quality genome sequence integrated with physical map which can serve as a basic for the advanced development of improved varieties to empower all aspects of basic and applied wheat science.

NEW DEVELOPMENTS AND NEXT GENERATION GENOME SEQUENCING

BioNano mapping or optical mapping (genome mapping in nanochannel arrays) becomes a useful tool to generate short sequence maps along DNA which stretches thousands of base pairs in length, thus proceed towards mapping and assembling gigabase sized genomes. Applying the BioNano mapping strategy the IWGSC wheat reference genome is produced by the sequencing of physical maps from individually isolated chromosomes.

Šimková et al. (2016) discussed the approach to develop a BioNano high resolution map for chromosome 7DS by generating 371 contigs with N50 of 1.3 Mbp. Chromosome 7DS sequence assemblies gained through clone-by-clone sequencing were anchored to the 7DS BioNano map.

Next generation sequencing

In model species a range of mapping-by-sequencing approaches have been developed to clone genes underlying both quantitative and qualitative traits. In polyploid wheat the cost of full-genome sequencing, and until recently the lack of a full genome reference, have delayed advances in this area. Most efforts have focused instead on reduced-representation methods which target specific sequences based on either RNA-seq, DNA capture platforms, or genotyping by sequencing. Initially, these methods were used for mapping of natural variation, but have recently expanded to identify causal polymorphisms based on forward screens of induced mutant populations. These methods, based on structured mutant populations, have been dubbed 'mutational genomics' and provide an innovative way to access variation in large and complex genomes such as wheat.

Genome-wide association studies in wheat

Genome-wide association study (GWAS) is an alternative approach to detect genetic variation in natural populations. It indicates multiple genetic recombination events which have occurred during divergence under both wild and domesticated conditions. In common wheat, sequence-based GWAS are still rare. Jia et al (2018) estimated from recent studies that 723 landraces from 10 Chinese agro-ecological zones were evaluated for 23 agronomic traits.

Functional annotation of wheat genes by Targeted Induced Local Lesions in Genomes (TILLING)

For functional analysis, each of the three homoeologs has to be individually evaluated and their combined effect on phenotypes should be considered. In this regard, TILLING has become a major approach for wheat gene functional annotation of genes in wheat. The first TILLING platform was developed in the diploid wheat *T. monococcum*, a cultivated A-genome diploid wheat species. *T. monococcum* is a model to study traits, genes, and alleles in bread wheat. In theory, knowledge of gene function in diploid wheat should be transferrable to hexaploid wheat, but polyploidization may complicate the mode of function due to the multiple homoeologous copies. Thus, it is necessary to make TILLING populations in polyploid wheat. The complete editing of all three homoeologs of target genes has

been reported. Further optimization should establish an efficient genome editing system for wheat, with more versatile applications such as allele replacement or targeted gene insertion (Jia et al 2018).

Dynamic wheat transcriptomes

The wheat genome sequence is the first mean to understand its physical structure and composition and to provide genomic infrastructure for mapping actively transcribed regions, especially during plant development and adaptation to biotic and abiotic stresses. Transcriptomes profiling is to identify expression patterns of all genes and their products in the wheat genome. Such information provide insights of regulation and their interacting networks in complex biological processes. For a long time, cDNA microarrays have been played the fundamental tool to study the wheat transcriptomes.

DATABASE AND TOOLS

To ensure that wheat breeding and research programs can make the most of this extensive genomic resource, the IWGSC endorsed the establishment of a data repository at URGI (Unité de Recherché Génomique Info/research unit in genomics and bioinformatics) from INRA (Institute National de la Recherche Agronomique/French national institute for agricultural research) to develop databases and browsers with relevant links to public data available worldwide. To manage data, URGI has built this data repository for the wheat community with the following specific aims: (1) to store resources for which no public archive exists (e.g. physical maps, phenotype information); (2) to enable pre-publication access to specific datasets (e.g. sequence assemblies and annotations, physical maps, markers); and (3) to enable rapid release of integrated resources upon publication. (Alaux et al 2018).

Through its concerted efforts to achieve a high-quality, functionally annotated reference wheat genome sequence, the IWGSC has generated a variety of resources for the bread wheat (*T. aestivum* L.) accession Chinese Spring. The IWGSC data repository fall into four broad categories: (1) physical maps, (2) sequence assemblies and annotations, (3) gene expression data, and (4) variation data.

In addition to sequence data, the Wheat@URGI portal hosts, within GnpIS-coreDB, several sets of genetic and phenomic wheat data that have been produced from French, European, and international projects since 2000]. A significant amount of these data is available without restriction. However, access to restricted data can be obtained through a material transfer or intellectual property agreement. Presents the types and number of genetic and phenomic data hosted in the GnpIS-coreDB database.

Genetic information corresponds to genetically mapped markers, quantitative trait loci (QTLs), genetic resources (germplasms), and genetic studies (genome-wide association studies (GWASs)). Genomic information consists of variations from SNP discovery experiments, genotyping, comparative genomics (synteny), and expression data (microarray, RNA-Seq). Phenomic data are available as whole trials including phenotypic and environmental observations recorded using variables from ontologies with Minimum Information About a Plant Phenotyping Experiment (MIAPPE) compliant metadata.

Germplasm data were mainly provided by the French small grain cereals genebank maintained by INRA at Clermont-Ferrand but also by partners of several European Union (EU) projects. They were linked together with related genotyping or Phenotyping characterisation data. Generally, genetic and phenomic data have been produced by INRA and its partners in large collaborative projects (Michael Alaux et al 2018). GBrowse displays the physical maps generated by the IWGSC members. A clickable image on the top of the browser gives access to all versions of the physical map for each chromosome. The browser displays physical contigs, BACs, deletion bins, and markers. From the BACs track, it is possible to order BAC clones directly at the INRA French plant genomic resource centre. From the BACs and markers tracks, one can go directly to the corresponding region in the IWGSC RefSeq v1.0 browser.

Impact of genome sequencing on wheat crop development

T. aestivum serves as the staple food for the 30% global population, rich in carbohydrate proteins and minerals and essential nutrients but due to the global warming posing a serious threat on the production of the wheat ± 2^{o}C temperature variation has resulted in 50% decrease in which production (Asseng et al 2015). Rise in greenhouse gases inflicts a steady increase in the global temperature which has been projected to rise up 24.5° C by 2080 (IPCC

2012). This is expected to the impose an enormous negative impacts on productivity of wheat and substantial rise to global food production and the security and this urged scientific research community to work towards genetic improvement of the wheat so as to impart durable stress resistance and economic traits in the major cereal (Muthamilarasan and Prasad 2013).

The ability of the high quality reference genome and the annotation of the wheat genome data would facilitate the crop improvement programme.

Transgenic wheat

Nature and human have combined to effect the progression of genotypes and differences in trait arrangement. This turned the landraces of wheat to be more complex, unstable and genetically varied in response to biotic and abiotic stresses and it identifies the landraces giving poor yield than breeder-developed high yielding genotypes. Mostly this high yielding genotypes cultivated by breeders leads to loss of attainable gene pool and that becomes a crucial restraint in breeding programme.

So it is very important to identify those genes which are tolerant to biotic and abiotic stresses through the species, genera and kingdom. Then using recombinant DNA technology to manipulate or incorporate these gene/s to develop transgenic wheat with desired traits is a significant approach. Transgenic development is not only based on recombinant DNA technology also on plant genetic transformation which helps in improving particular characteristics.

Creation of the first transgenic wheat coincides with third green revolution and among the three most important cereals wheat is the last one to be developed as transgenic. This was achieved by biolistic methods in 1992 and in 1997 by *Agrobacterium* mediated gene transfer

First herbicide tolerant wheat commercially known as Clearfield wheat is released in Canada in the year 2007. It is a product of mutation breeding and is developed in such a way that it can withstand imidazolinone herbicide. This herbicide blocks the activities of enzyme acetohydroxy acid synthase (AHAS), which is the first enzyme on the biosynthetic pathway of essential branched amino acids for plant growth (according to data provided by iaass.org). In field trial of USA this wheat shows similarities with parental lines in case of disease resistance, seed maturity, tendency to weed lines etc. Through genetic engineering Monsanto first developed herbicide tolerant wheat named Mon71800 event, commercially known as Roundup ready wheat. A glyphosate tolerant wheat line was produced by incorporating a gene from soil bacterium *Agrobacterium tumifaciens* to wheat. This gene actually codes for the production of novel form of the enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This enzyme functions in the shikimate pathway and a pathway responsible for synthesis of aromatic amino acids and aromatic compounds essential for growth. This glyphosate tolerant wheat is proven as safe and nutritious. Monsanto decided to commercialize the roundup ready wheat.

Conclusion

The tribe Triticeae is economically the most important group of the family Gramineae. It has given rise to cultivated wheats, barleys, ryes, oats, and a number of important range grasses. Hybridization among genera within the tribe has allowed the exchange of genetic material and given rise to polyploidy in the form of amphiploidy. The wheats (genus *Triticum*) comprise a series of diploid, tetraploid, and hexaploid forms, the polyploids having arisen by amphiploidy between *T.* species and diploid species of the genus *Aegilops.* Modern wheat cultivars belong primarily to two species: (1) hexaploid bread wheat, *T. aestivum* (2n = 42 chromosomes), and (2) tetraploid, hard or durum-type wheat, *T. turgidum* (2n = 28). Here we aimed to establish the karyotype of a local wheat variety by a simple and reproducible method. In 2017 the International Wheat Genome Sequencing Consortium, consisting of 68 countries worldwide, published the reference genome sequence of bread wheat and demonstrates the importance of BAC libraries, physical maps, and chromosome-based resources. The work reviews the techniques to accelerate wheat improvement thereby increasing profitability throughout the industry.

Future prospects

Future effort may be directed in generating multiple reference genomes, strategies to attain genome-wide genetic variation, genome-wide association studies, mutant population generation, and gene cloning and functional characterization. These resources and platforms will lay a solid foundation for wheat research, leading to a new era of wheat functional genomics that will bridge the gap between genotype and phenotype. Dissection of wheat genomes and gene functions should assist in genomics-assisted selection and facilitate breeding of elite varieties for sustainable agriculture. The use of the genome sequencing technology and informatics combined with information on genetic variation will provide the thrust for wheat to catch up with other crops in studies on functional genomics. The time for genomics-assisted wheat breeding is finally arriving. In the year 2001 Swaminathan stated that "we are now in a state of transition from Mendelian to molecular breeding; we are standing on the brink of the green revolution with explosive progress being witnessed in the area of the functional genomics and molecular manipulation".

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