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Review Article

THE AGE OF GENOMICS AND TRANSGENICS IN WHEAT - A REVIEW

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Wheat is a staple food in South Asia and many other parts of the world since it is a major cereal crop and a large source of calories, second only to rice. Before the "Green Revolution," widespread famine plagued several South Asian nations. The Green Revolution wheat genotypes rescued these countries from economic collapse and have sustained their agricultural output over the past half-century. Cutting-edge methods for identifying and using genes have become available as a result of advances in molecular biology and biotechnology, opening up a new window of opportunity for preserving wheat yields. In this chapter, we've tried to collect all the information that's been produced for wheat enhancement during the previous three decades. Some examples of these developments are molecular markers, gene mapping, marker gene sequencing, and marker-assisted selection. The remaining half described various efforts to genetically modify wheat for the purposes of study or improvement.

Wheat (*Triticum aestivum*) is the most complicated allohexaploid plant due to its large genome size of over 17 Gb. The *Triticeae* family includes this plant, along with the Poaceae genus. Wheat is the second most extensively cultivated crop after rice, despite being more nutrient-denser and eaten by more than 2.5 billion people worldwide. Over 215 million hectares worldwide, it is farmed more than any other crop each year. In Asia and North Africa, wheat is the most significant and dominant staple crop. It does well in a variety of settings and has distinct seasonal responses in spring and winter depending on whether it is in a temperate, tropical, or subtropical climate. When a critical food crop's output drops because of abiotic challenges like rising temperature that is not acceptable. The green revolution, whose main objective was to raise production of the most significant cereal crops in the world, had a substantial impact on wheat yields during the previous century (Awika, 2011). Global exports of wheat are worth about $50 billion USD.

DNA is a biomolecule with all of an organism's genetic information recorded in precise codes and sequences along its double helix structure. The contemporary era of genomics and transgenics began with the discovery of restriction endonucleases and the structure of DNA in 1958. People think that transgenic events are most likely to happen in wheat because its genome is more complicated than those of other monocots and has more copies of genes (Bourke *et al.,* 2018). By comparing the genomic data that is now available and finding new points of view that were not known before, knowledge gained from sequencing DNA and genomes helps to improve the genetic makeup of organisms. Functional genomics has shown the transgenesis road map by giving the necessary annotated information about genes that are naturally found in different species. By building on previous genomics studies that used molecular and morphological markers (Brenchley *et al.,* 2012), researchers have made progress toward understanding the complex wheat genome and making accurate physical and genetic maps of the hexaploid wheat genome. Wheat's functional and structural genomes are stored in GenBank, TIGR, and other sources (Abdurakhmonov, 2016). Exploration is the first step in manipulating the genome. Different changes have been made using both old and new biotechnological means for genetic engineering and editing the genome (Ceasar, 2016).

Studies being conducted around the world right now are mostly focused on increasing wheat production and nutritional quality. According to predictions, the demand for wheat would rise by 60% by 2050. This demand cannot be quickly met through conventional cross breeding; however, genomics-assisted breeding and genetic engineering of wheat genotypes with genes from related and unrelated sources may speed up breeding and produce the genetic improvements needed to feed the world's rapidly expanding population. Although genetic changes are of critical importance, concerns about the biosafety of goods containing them have delayed their commercialization. This assessment focuses on the state of the wheat crop since the start of the green evolution as well as the successive advances in science and technologies that have been produced over time. How have these innovations been used thus far and will they be employed in the near future to increase wheat yields and quality in order to supply growing populations with a nutritious diet?

Wheat data from around the world

More than 2.4 billion tonnes of wheat, or roughly 17% of worldwide production from 2000 to 2020, have been produced in China, the world's largest wheat producer. The vast majority of China's wheat supply is eaten within the country to help meet the rising demand for food. When it comes to wheat, China is the world's largest consumer. In 2020–2021, China ate almost 19% of the world's wheat supply. In terms of global wheat production, India ranks second. Over the previous two decades, India has accounted for 12.5% of global wheat production. Similar to

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China, India stores much of its wheat at home to meet the increasing demand for food across the country. Russia, the world's third-biggest wheat producer, is the largest exporter of wheat. In 2021, the country exported wheat worth about $7.3 billion, or around 13.1% of the global total.

Figure 1. Worldwide wheat production

Obstacles to producing wheat before and during the genomics era

Research into wheat varieties has always aimed to increase production and quality of the grain they produce through genetic modification. Multiple ineffective false breeding techniques were used at first, prolonging the process beyond what was reasonable (Guzman *et al.,* 2016). In the early 1980s, the genomic era began with the creation of recombinant DNA technology, which was a big step forward in biotechnology. Bioinformatics databases and tools have made it easier to look at the whole genomes of many species as these technologies have improved over time. Similar efforts were made to improve wheat, which has always been very sensitive to stressors like insects, rusts, and climate change. Over time, the focus has moved toward molecular breeding (Araya *et al.,* 2017), but the biggest problem is meeting rising demand. Before biosafety and morality changed, marker-assisted breeding was used a lot to do the manipulative work that was needed. But most study is now done on technology that doesn't use markers. Before the time of genetics, the Green Revolution used traditional methods of plant breeding to improve wheat. However, these methods don't seem to work as well now (Breseghello and Coelho, 2013, Vagndorf *et al.,* 2018). During the age of genomics, advanced techniques for molecular breeding and changing genes were used. Researchers have used a variety of genetic engineering and genome editing techniques to make wheat more resistant to these stressors, and they have also been able to add other features or qualities to wheat. Only *MON-71800*, or "*Roundup ready wheat*," which Monsanto developed in 2004 by altering the *CP4 Epsps* gene to make plants resistant to the herbicide glyphosate, has been commercially released.

Peoples from developing countries, like those in Africa and Asia, who live in hot, dry conditions often talk about these problems. Also, because climate change is happening almost everywhere, some of these problems are also happening in rich countries like the United States, Canada, and Australia (Chatrath *et al.,* 2007, Pretorius *et al.,* 2007).

DNA technology made it possible to find and make genetic mutations, but it also has flaws and unintended effects that could affect genes other than the one that was meant to be changed. Also, the rise of bioethics and biosafety worries led to the failure of established DNA technology that tried to change the genes of living things. People thought that these activities were meant to pollute and mess with nature, which of course had some bad results. Since then, transgenesis has been allowed for use around the world to change the genes of crops, as long as the biosecurity of the product is guaranteed (Khan *et al.,*2019). Because of the Bioethics and Biosafety Act, this is the case. New developments in genome editing and targeted or site-directed mutagenesis, both of which are very good and are sure to lead to the best results, will help a lot with the growth of sustainable agriculture.

The genome's complexity in wheat

Out of the 23 species investigated so far, wheat has one of the largest and most complex genomes, with a substantial variation in ploidy levels. Bread wheat (*Triticum aestivum*), Durum wheat (*Triticum durum*), Emmer wheat (*Triticum dicoccon*), Einkorn wheat (*Triticum monococcum*), Khorasan wheat (*Triticum turgidum* or *Triticum turanicum*), and Speltoid wheat (*Triticum speltoideum*) are the six most extensively grown types of wheat. Diploid Einkorn is distinguished from tetraploid Durum, Emmer, and Khorasan, as well as hexaploid Durum, bread wheat, and Khorasan. Except for *T. aestivum* and *T. durum*, which are only grown in a few locations (Mirosavljeviae *et al.,* 2020), all the species are descendants of old ancestors.

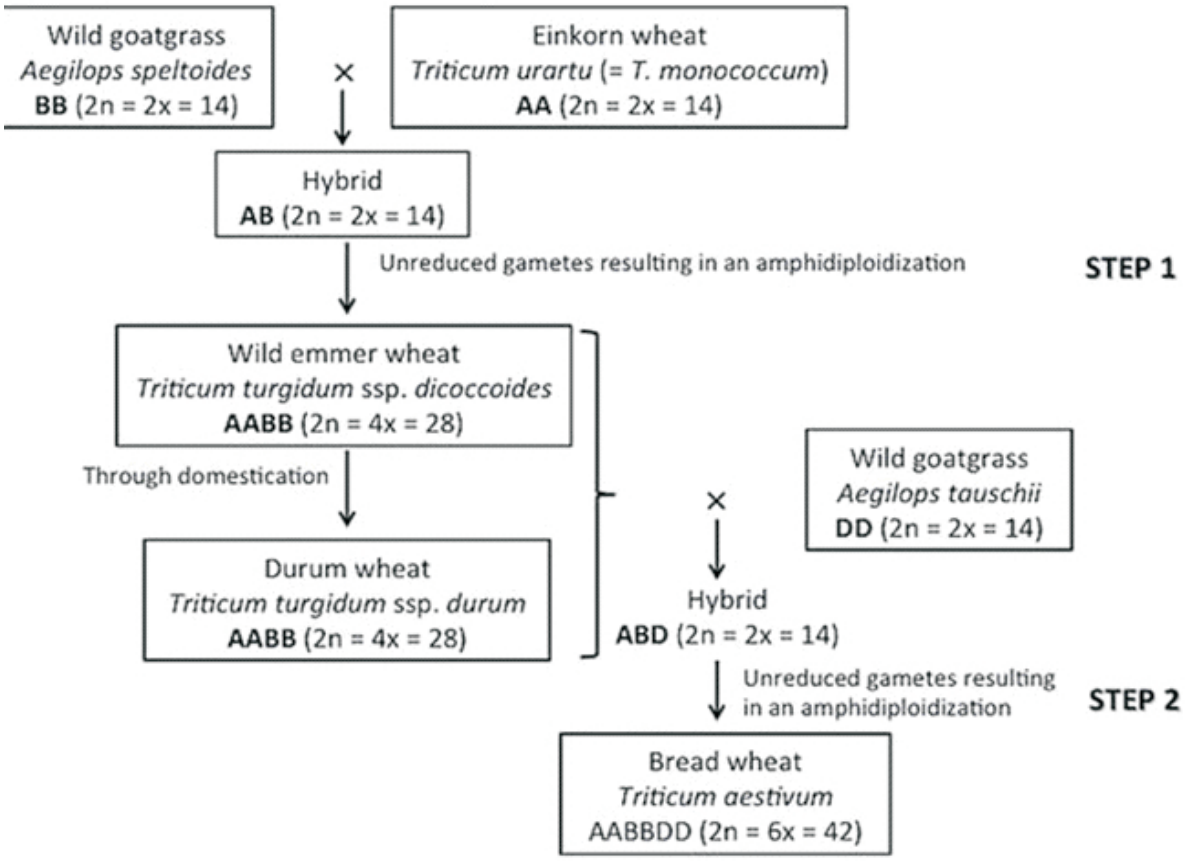

Figure 2. Complex wheat evolution

Wheat with three genomes (AABBDD) has a genome size of about 17 Gb and 164,000-334,000 genes; of those, 85% are located on about 10%. Wheat has six copies of every gene, as measured by its ploidy level. It is difficult to induce changes in the genome since the bulk of its features, including yield, are polygenic. Careful planning and individual targeting of each gene copy increases the likelihood of making these alterations, according to research (Berkman *et al.,*2012).

Recent developments in wheat enhancement from the genomics era

Genomic advancements have allowed for the wheat crop to be improved for long-term productivity. Molecular breeding, also known as marker-assisted breeding, was used to solve the problems that had to be solved in order to achieve this primary goal (Vagndorf *et al.,*2018). Screening wheat for resistance to aphids using RAPD and SCAR molecular markers revealed that multiple members of the aphid-resistant Dn gene family (Dn1, Dn2, Dn4, and Dn5) are responsible for this trait (Myburg *et al.,*1998). Screening and enhancing their expression using SNPs or modifying wheat via an appropriate technology for delivering into plant genome have both been reported as methods of using R and APR genes for rust resistance (Ellis *et al.,*2014, Nsabiyera *et al.,* 2016, Xu *et al.,*2016). Drought-tolerant inbred wheat lines were analyzed using microsatellite markers to pinpoint the underlying causal gene(s) already present in the wheat genome (Kumar *et al.,*2012).

Physical genome mapping of wheat

The complete physical map of all 21 chromosomes of bread wheat (*T. aestivum*) is accessible in the IWGSC database in addition to whole-genome profiling (WGS) and High Information Content Fingerprinting (HCIF) in the form of BAC libraries. Instead, all of the mapping data, such as BAC clone marker and position data and deletion bin data, is stored in the physical contigs themselves. Both linear topological contigs (LTCs) and fingerprinted contigs (FPCs) (Alaux *et al.,* 2018, Nelson *et al.,*2005) are two examples of the software tools included in the database that are essential for keeping the data on physical maps up to date and maintained. Various physical genome maps constructed by different countries are given in figure 3.

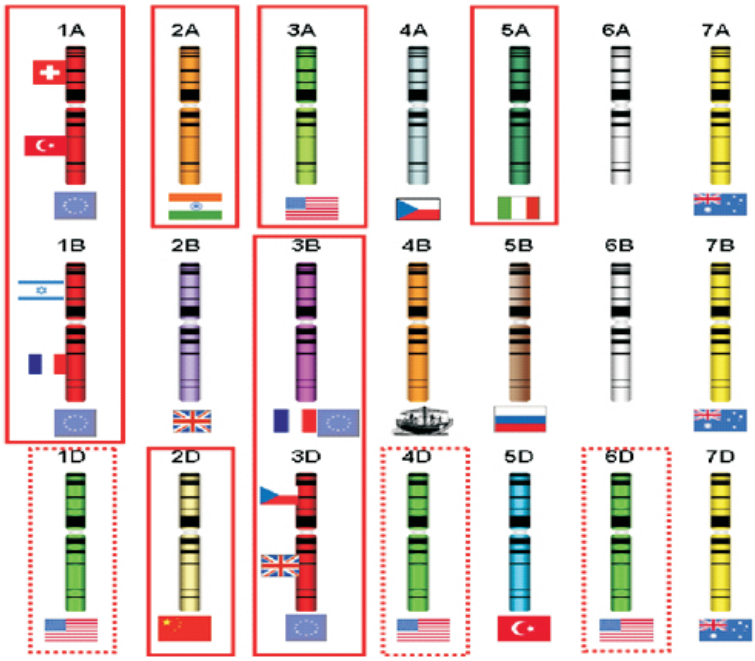

Figure 3. Countries contribution for Wheat physical genome mapping

Molecular markers based physical map

Physical maps of bread wheat have been made using molecular markers and compared to genetic maps made with the same markers. These maps allowed researchers to examine genetic and geographic distances, shedding light on variations in recombination frequencies and the possibility of cryptic structural modifications in certain parts of the genome. Many different methods have been used to make physical maps.

Deletion mapping

Wheat aneuploidy allowed for the precise mapping of genes to specific chromosomes (Randhawa, *et al.,*2004). In later years, physical mapping of molecular markers made extensive use of the wheat chromosome deletion lines developed by Endo and Gill (1996).

Using intergenomic polymorphism across the A, B, and D sub-genomes, these deletion stocks were used to map genes for morphological features to physical portions of wheat chromosomes, either directly for unique and genome-specific markers or indirectly for duplicate or triplicate loci.

In silico physical mapping

In silico physical mapping using sequence similarity with mapped EST loci at Grain Gene database (http://wheat.pw.usda.gov/GG2/blast.shtml) can be used to map markers with known sequences to wheat chromosomes, as indicated above (Kharbikar *et al.,* 2022). This method uses the 16000 wheat EST loci assigned to deletion bins. Using this technique, Parida *et al.* (2006) were able to assign 157 SSRs containing unique wheat sequences to chromosomal bins. The focused mapping of genes for useful traits, comparative genomics, and the sequencing of gene-rich areas in the wheat genome all rely on these bin-mapped UGMS markers.

Mohan *et al.* (2007) used in silico and wet-lab techniques to assign bins to 672 loci out of 275 wheat and rye EST-SSRs. Some wheat FHB resistance QTL cDNA clones were found using in silico mapping (Hill Ambroz *et al.,* 2006).

Radiation-hybrid mapping

Cox *et al.* (1990) employed radiation hybrid (RH) mapping to physically map humans and animals after its introduction by Goss and Harris(1975). Using this method, NDSU manipulated tetraploid durum wheat by inserting and removing chromosomes from the D genome.

The RH mapping of chromosome 1 utilized the alien substitution line DWRH-1D of durum wheat that carries the nuclear cytoplasmic compatibility gene scsae. These RH lines detected a total of 88 radiation-induced fractures, with 39 of these being indicators unique to 1D. One breaks every 199 kb of DNA was reduced on this 1D RH map because to the addition of 378 markers (Kalavacharla *et al.,*2006).

BAC-based physical maps

The diploid species *Aegilops tauschii* is being utilized to generate a BAC-based physical map of the wheat D genome, with the ultimate goal of identifying and mapping genes, and then sequencing the GRRs. The first step in this process involved fingerprinting and assembling a massive number of BACs into contigs.

Genetic mapping of wheat genome

Sequence-based mapping technologies can be used in conjunction with molecular markers such as amplified fragment length polymorphism (AFLP), expressed sequence tags (EST), quantitative traits loci (QTL), restricted fragment length polymorphism (RFLP), RAPD, SCAR, SNP, SNP haplotypes, SSRs, and sequence tagged sites to map the human genome. These events can be used in marker-assisted

breeding to improve a small set of closely related genotypes (Jae-Han *et al.,* 2014, Kumar *et al.,*2007, Rimbert *et al.,* 2018). They also contribute to the study of phylogenetic relationships and the identification of genes involved in certain phenotypes.

Wheat and sequencing technologies

Understanding phenotypic features, their molecular bases, and changes requires knowledge of genome sequences. The lower amount of genome conservation in plants has hampered comparative genomics studies aimed at improving wheat. Due to its importance in crop improvement, wheat genome sequencing has risen to the top of the sequencing priority list. There are significant restrictions on sequencing operations because of the genome's immense complexity and size. Using the chromosomes from both wheat genomes, multiple drafts of the wheat genome have been sequenced thanks to advances in next-generation sequencing technologies (Muthamilarasan and Prasad, 2014, Shi and Ling, 2018).

Random shotgun next-generation sequencing aided by the IlluminaHiSeq 2000, Genome AnalyzerIIx, and Roche 454 pyrosequencing technology was used to sequence the whole genome of *T. aestivum* cv. Chinese Spring (CS42). Genome data from *Aegilops, Aegilops tauschii, Aegilops speltoides,* and *T. monoccum* was compared to what was already known. This has the potential to expose 124,000 genes throughout the A, B, and D genomes (Berkman *et al.,* 2012). The two diploid wheat species, *T. urartu* and *A. tauschii*, were also sequenced, and their total gene counts were reported to be 34,879 and 43,150 (Jia, *et al.,* 2013, Ling *et al.,* 2013). The results of the research have made it easy to pinpoint duplicate genes in hexaploid organisms, whose evolutionary past was previously shrouded in mystery (Muthamilarasan and Prasad, 2014). Wheat *cv.* Chinese Spring (Hexaploid) whole-genome sequencing commenced in 2014 (Choulet *et al.,* 2014), except for chromosome 3B, which Choulet and his team finished working on independently.

Mapping based on sequence

Several low-cost strategies for high-resolution genetic mapping of complex genomes have become available thanks to advances in sequencing technology in the genomic era. Wheat's polyloidy presents challenges for genome sequencing despite the many prospective uses for these technologies. In a study, whole-genome shotgun NGS was used to map the DH wheat variety. Results from the wheat 9000 SNP iSelect test were compared to those from the variation mapping study, which were found to be consistent. There were many parallels between these findings. The study found that a total of 416,856 genetic markers, 2740 gene-linked SNPs utilizing the wheat iSelect test, 118 simple sequence repeats, and 1351 diversity array technologies were used to generate a reference map of the wheat genome. These markers were discovered to be 40–100 kb away from their nearest genes in the comprehensive analysis, which bodes well for the possibility of mapping the genome in order to identify genes. The presented information is highly helpful for a comprehensive examination of the wheat genome (Cavanagh *et al.,* 2013, Saintenac *et al.,* 2013) as it connects the genetic and physical maps of wheat.

Mapped traits in wheat

Using forward genetics approaches in molecular markers like QTLs, numerous studies based on the wheat genome map have been conducted to learn how the plant reacts to biotic and abiotic stresses. It has been noted that environmental interactions are always crucial in QTL research (Kulwal *et al.,*2004, Kumar *et al.,*2007). Table 1 summarizes the research that has been conducted on key wheat characteristics, such as QTL or gene tagging.

Table 1. A summary of studies that have been done on QTL or gene tagging for major wheat properties

Trait	Gene/QTLs	Chromosome	Population	References
Aluminum tolerance	*ALMT1*	4D	DH	(Raman *et al.,* 2006)
Boron toxicity tolerance	*Bo1*	7BL	DH	(Jefferies *et al.,* 2000)
Drought tolerance	*DREB1*	3A	Barakatli-95	(Wei *et al.,* 2009)
Frost tolerance	*QTL*	5B	RSI	(Tóth *et al.,* 2003)
Photoperiod insensitive	*Ppd-B1*	2BS	RILs	(Mohler *et al.,* 2004)
Salinity tolerance	*QTL*	3A, 3B, 4,6 DL	RILs	(Ma *et al.,* 2007)
Russian wheat aphid resistance	*Dn1*, *Dn2*, *Dn5*	7DS	F2	(Liu *et al.,* 2001)
	Dn4, *Dn6*	1D, 7D	F2	(Liu *et al.,* 2002)
	Dn7	1B	F2	(Lapitan *et al.,* 2007)
	Dn8, *Dn9*, *Dnx*	7DS, 1DL	F2	(Liu *et al.,* 2001)
Stem rust resistance	*Sr2*	3BS	F3	(Spielmeyer *et al.,* 2003)
	Sr22	7A	F2	(Paull *et al.,* 1994)
	Sr38	2AS	NILs	(Seah *et al.,* 2001)
Leaf rust resistance	*Lr1*	5DL	F2	(Feuillet *et al.,* 1995)
	Lr3	6BL	F2	(Sacco *et al.,* 1998)
	Lr9	6BL	NILs	(Schachermayr *et al.,* 1994)
	Lr10	1AS	F2	(Schachermayr *et al.,* 1997)
	Lr19	7D	F2	(Cherukuri *et al.,* 2003)
Fusarium head blight resistance	*Fhb2*	6BS	RILs	(Cuthbert *et al.,* 2007)
	QTL	1B, 3B, 5A	RILs	(Buerstmayr *et al.,* 2002)
	QTL	2B	RILs	(Gilsinger *et al.,* 2005)
	QTL	4A, 5B, 6D	RILs	(Paillard *et al.,* 2004)

Arabidopsis genome comparison

Whole-genome sequencing has been performed on both wheat *Triticum aestivum* and *Arabidopsis thaliana* for the purpose of comparative genomics. To do this, we used *BLAST* and expressed sequence tags (*EST*s) to compare the genomic sequences of Arabidopsis and wheat endosperm clones (Accession Numbers: BQ605537-609969, Gen Bank) and determine the degree of identity and similarity between individual genes. Since the wheat genome is roughly 126 times larger than the Arabidopsis genome (Schachermayr *et al.,* 1997, Schachermayr e*t al.,* 1994). A comparison of nearly every 500 base pairs showed an error rate of less than 2% in terms of unresolved nucleotides. Clustering of ESTs was performed using the PHRAP software, and data for Arabidopsis was obtained from the TIGR nucleotide and protein databases. Wheat ESTs (4433 total) were also grouped into contigs using self-BLAST. Multiple sequence alignment produced an average alignment score that was greater because it represented a smaller proportion of simpler sequences that made up ESTs. There were 789 reported clustered ESTs (Contigs), and 1348 reported unclustered ESTs. As a result, a total of 2137 unique sequences were obtained and compared to the Arabidopsis genome, where it was found that the wheat ESTs clustered with 1130 unique genes, each of which was located on a different chromosome but shared a functional similarity with the wheat ESTs of about 75% (Benson *et al.,* 2000, Clarke *et al.*, 2003).

Genomic comparisons with different types of grass

Avena sativa, Hordeum vulgare, Oryza sativa, and *Zea mays* are just a few of the well-known grass species that belong to the *Poaceae* family. Wheat's genome is larger than those of oats (1.5 times), barley (three times), maize (six times), and rice (thirty-nine times). The *Triticeae* family consists of about 15 different genera and 300 different species. These include wheat and barley. It's possible that all grasses share the same number and quantity of genes (Barakat *et al.* 1998, Sandhu and Gill, 2002). The enormous genomes and high degree of genetic similarity among rice, maize, and wheat suggest they share a common ancestor from more than 50 million years ago (Kellogg, 1998). However, there is evidence of a conserved gene order, suggesting that evolution determines size in these creatures. While 62% of the markers are still present in maize and rice, 94% are present in wheat, barley, and oats. Research into the genetic similarities of plant species has revealed that the *Triticeae* family has more in common with itself than with the *Poaceae* family (Ahn *et al.,* 1993, Künzel *et al.,* 2000, Moore, *et al.,* 1995). It has been estimated that 7% of the genomes of wheat, 12% of those of barley, 17% of those of maize, and 24% of those of rice have these genes (Barakat *et al.,* 1997, Carels *et al.,* 1995), and it appears that this proportion is also present in the genomes of other *Poaceae* species.

Genomics application to wheat molecular breeding
Association mapping in wheat

Association of mapping using high-resolution linkage disequilibrium (*LD*) mapping, *QTL* promises genetic dissection of complex traits. (Flint Garcia *et al.*, 2006, Yu and Buckler, 2006). Some wheat chromosomes are superior for *LD*/association mapping for *QTL* discovery and fine mapping due to *LD* variation. *LD* decay over large distances helps identify phenotypic data with haplotypes in a chromosomal location, while *LD* decay over short distances helps fine map *QTL*. Recent wheat association mapping studies Kernel morphology and milling quality (Breseghello and Sorrells, 2006) and high molecular-weight glutenin quantity (Ravel *et al.,* 2006) were mapped. The genes/*QTL* influencing stem rust (*SR*), leaf rust (*LR*), yellow rust (*YR*), powdery mildew (*PM*), and grain yield (*GY*) were mapped in another investigation using 242 *DART* markers. Crossa *et al.,* (2007) used two linear mixed models to analyze marker-trait associations in five prev ously conducted *CIMMYT* elite spring wheat yield trials (*ESWYT*) in a variety of foreign settings. *DArT* markers 122, 213, 87, 63, and 61 are found in YR, GY, LR, SR, and PM, respectively. Association mapping provided 390-fold higher marker resolution than *QTL* analysis employing a *RIL* mapping population in the vicinity of a significant *QTL* for *Stagonosporanodorum* (glume blotch) resistance (Tommasini *et al.,* 2007). The identification of crop development objectives and the exploration of the genetic and biochemical bases of quantitative trait variation may be aided by improved statistical methods for high-resolution mapping of features *QTL* to individual genes.

MAS in wheat

Since a large number of marker-trait correlations have been discovered in the past few decades, a number of countries are utilizing molecular markers for marker-assisted selection (MAS) in bread wheat. Large-scale wheat MAS initiatives can be found in the United States, Australia, and *CIMMYT* in Mexico. In 2001, the United States brought together twenty separate wheat breeding programs to form a MAS partnership. This group worked together to use MAS in government-sponsored wheat breeding programs (Dubcovsky, 2004). MAS has improved the quality of bread and pasta by transferring 27 bug-resistant genes and 20 quality-enhancing alleles into 180 regionally-specific lines in the United States. Those apps disseminated 45 strands that were created using MAS (Sorrells, 2007). Several improved cultivars were produced after the Australian program strengthened 20 traits, including resilience to abiotic stress (Eagles *et al.,* 2001), Peter Langridge, personal communication). Agriculture Victoria now uses MAS instead of traditional bioassays when selecting for agronomically important traits like cereal cyst nematode resistance (Ogbonnaya *et al.,* 2001). QTL for transpiration efficiency and negative selection for traits like yellow flour color have both been introduced through backcross breeding using MAS (Landjeva and Börner, 2001). To achieve a desired result, Australian researchers simulated a marker-assisted wheatbreeding strategy. The research employed DH technology and a form of controlled backcrossing. Marker-assisted selection (MAS) at BC1F1 and MAS in haploids generated from BC_1F_1 pollen before chromosome doubling reduced marker-assisted breeding

costs by 40% (Kuchel *et al.,* 2005). This MAS approach was validated in a marker-assisted wheatbreeding study aiming to boost quality and rust resistance (Kuchel *et al.,* 2005). Twenty-five genes that regulate insect pest resistance, protein quality, homoeologous pairing, and other agronomic properties are marked in *CIMMYT*'s wheat breeding programs using markers (William and Crosby-Galvan, 2007). These methods rely on error-free markers constructed from DNA sequences. Isolating crucial genes for improved transgenic crops and "*perfect markers*" for MAS (Lange and Whittaker, 2001) will be aided by IWGSC's massive sequencing of GRRs (gene-rich regions).

Organization of organellar genomes

Over the past decade, scientists have also studied wheat's chloroplast and mitochondrial genomes extensively. The results of these studies will be discussed briefly here.

Chloroplast genome

Each mesophyll cell in bread wheat has between 130 and 155 chloroplasts. Each chloroplast has between 125 and 170 circular *DNA* molecules (135 kb), so there are between 16000 and 26000 copies of *cpDNA*. This is between 10% and 14% of the *DNA* in leaf cells and between 5% and 7% of the *DNA* in mesophyll cells. In diploid species, the amount of *cpDNA* in a mesophyll cell is between 4900 and 6600, and in tetraploid species, it is between 9600 and 12400.

Like all other plant chloroplast genomes, the wheat chloroplast genome is broken into four equal pieces: two 21-kb regions containing inverted repeats and two single-copy segments (12.8 kb and 80.2 kb). Wheat chloroplasts have the same number of genes as rice and maize plastomes. However, unauthorized recombination between two short direct repeats and/or replication slippage caused structural differences in the gene coding regions, such as hotspots for length mutations. Based on loss patterns of open reading frames (*ORFs*) in inverted-repeat regions and the boundaries between them and small singlecopy sections, it is thought that wheat and rice are more closely related than maize.

In addition to identifying eleven distinct *cpDNA* types through *cpDNARFLP* analysis, researchers have found evidence of deletions, insertions, and inversions within the genus Triticum. Only with durum wheats, but not with any of the diploid species, does the bread wheat share its whole *cpDNA* type. *Ae. speltoides* is the most likely donor of the *B*subgenome of common wheat due to the high degree of similarity between its *cpDNA* and that of *Triticum aestivum, Triticum timopheevii*, and *Triticum zhukovskyi* (Newton, 1988).

Mitochondrial genome

There are at least 10 repeats in the 430 kb of wheat *mtDNA,* however it only encodes 30-50% of the polypeptides. About 50 genes transform the largely noncoding *mtDNA* into *RNA*(Newton 1988). From 25 gene-rich cosmid clones, the Chinese Spring mitochondrial genome was sequenced. There was a total of 55 genes identified, including 18 *ETS* genes, 20 ribosomal protein genes, 4 mitochondrial biogenesis genes, 11 ribosomal genes, 2 splicing and other function genes, 3 ribosomal *RNA* genes, and 24 transfer *RNA* genes. Multiple copies of a gene are tallied independently. In the mitochondrial gene maps of wheat, rice, and maize, only two to five genes showed significant synteny. Therefore, mitochondrial genes underwent a rearrangement throughout the development of cereals. The chloroplast genes of wheat, rice, and maize are completely syntenic with one another.

Transgenics for wheat improvement

Transgenics are creatures that have been genetically transformed or designed in order to carry an exogenous DNA fragment encoding for a specific protein. Transgenic methods allow for the in-depth study of a protein's role, expression, and interactions in the metabolism of a plant system other than its native (Viana and Sant'ana, 2017) at the molecular, *in vitro*, and *in vivo* levels. Herbicide tolerance was the only *GM* wheat event, according to the ISAAA. The *CP4 Epsps* gene, responsible for glyphosate resistance, was transferred from bacteria. The additional genes may have unintended consequences on other characteristics. In these cases, there are other factors to consider, all of which are crucial if one is to get to the bottom of the issue (Khan *et al.,* 2019).

Pellegrineschi *et al.* (2004) successfully transfer of the *DREB1A* gene from *Arabidopsis thaliana* into bread wheat, using the stress-inducible *rd29A* promoter, is a noteworthy achievement. The transgenic wheat plants expressing the *DREB1A* gene exhibited impressive resistance to water stress in a greenhouse setting. This was evident through their ability to withstand water deprivation, as they showed delayed wilting and leaf bleaching compared to the wild type wheat plants. In contrast, it observed that the non-transgenic plants exhibited a noticeable change in coloration after a period of 15 days of being submerged in water. (Zhou *et al.,* 2022), Successfully transferred *GmTDN1* (*DREB*-like transcription factor gene) into two current winter wheat varieties, cv Shi4185 and Jimai22, to improve drought tolerance and N-use efficacy. Overexpressing *GmTDN1* in wheat improved drought and low-N tolerance in greenhouse drought and N-deficiency environments. Both Shi4185 and Jimai22 *GmTDN1* transgenic lines were agronomically superior to wild-type plants and produced significantly greater yields under drought and N-deficient conditions in field trials conducted at three locations over two to three years. Zhang *et al.* (2022) reported that cloned and successfully transfers gene *TaCOL-B5* (Emmer Wheat) encodes a *CONSTANS*-like protein orthologous to plant COL5. Constitutive overexpression of the dominant *TaCol-B5* allele in a common wheat cultivar without the B-box region increases spikelet nodes per spike and produces more tillers and spikes, increasing grain output in transgenic plants under field conditions. Allelic variation in *TaCOL-B5* causes amino acid changes that affect *TaK4* protein phosphorylation. The *TaCol-B5* allele is found in emmer wheat but rare in current wheat cultivars worldwide.

Agronomic character enhancement with transgenics

Attempts to alter wheat date back to the 1980s, but it wasn't until 1991 that researchers led by Vasil and his colleagues announced the first effective transformation employing biolistic transformation. To induce gene expression, the chloramphenicol acetyltransferase (*CAT*) gene was electroporated into wheat protoplasts from a bacterium, (Ou-Lee *et al.,* 1986). To introduce a selectable marker into the wheat genome, *PEG*-mediated genetic transformation of *T. monococcum* protoplasts was performed (Lörzand Schell, 1985), this allowed the insertion of the *Tn5-aminoglycoside* phosphotransferase *type II (NPTII*) gene. Transforming early boot stage wheat with a few spikes in planta via the pollen tube pathway and Agrobacterium-mediated floral-dip transformation with high and *NPTII* transgene insertion as selection markers. The pattern of inheritance of this alteration was also evaluated over generations T1 and T2 (Zale *et al.,* 2009). It has been claimed that the *CRISPR-Cas9* technology was used to create the first transgene-free mutants of wheat by editing the genome at a specified region. (Shan *et al.,* 2013, Zhang *et al.,* 2016). This is despite the fact that the transgene-based transformation utilizing CRISPR/Cas has significant obstacles due to the complexity of the wheat genome.

In order to boost wheat grain production and quality, numerous transgenic technologies have been developed to date Salt tolerance, disease resistance, herbicide tolerance, and drought tolerance are just a few examples of the biotic and abiotic stress tolerance genes that contribute to these traits. High-molecular-weight glutenin subunits (*HMW-GS*) and low-molecular-weight glutenin subunits (*LMW-GS*) could be incorporated into the wheat genome by genetic engineering to boost wheat grain quality.Biolistics-mediated transformation of zygotic embryos was used to introduce the *1Ax1 HMW-GS* component into the wheat *cv*. *Bobwhite*. Because of this, the amount of gluten in *GM* grains rose by 71% (Altpeter *et al.*, 1996).

Marker-free transgenic wheat development technologies

Transformative processes have been greatly enhanced by the inclusion of selectable markers. Developing marker-free transgenics is essential because of the numerous health and environmental dangers connected with these genes. Several methods, such as co-transformation, site-specific recombination, and transposon-mediated elimination (Permingeat *et al.,* 2003, Puchta, 2003,Srivastava and Ow, 2004), can be used to remove selectable markers from plant systems. Furthermore, *pCLEAN* vectors have been developed specifically for the transformation to transport many transgenes without inserting any unwanted *DNA* sequences into the plant genome (Hellens *et al.,* 2003). It has also been discovered that *pCLEAN* vectors for gene delivery improve transformation efficiency (Thole *et al.*, 2017). The use of plant-derived genes for selection purposes has recently been included into the process of genetic transformation in wheat. Arabidopsis thaliana's *AtMYB12* is a gene involved in visible selection, while *Oryzasativa*'s *AlSAP* and *Aeluropuslittoralis*'s*ALS* are both herbicide-tolerant genes.

The acceptability of transgenic wheat in the market

To a similar extent as other commercial transgenic crops such as tomato, maize, rice, cotton, *etc.*, *GM* wheat has been adopted. European countries are opposed to *GMO* farming, in contrast to the United States and other less developed countries. The threat of famine is ever present in countries like Pakistan where wheat has traditionally been the primary food source. The governments of these countries are eager to adopt innovations that would ensure the long-term viability of staple crop production. Stakeholders cannot forsake transgenic crops despite widespread opposition from farmers, governments, the market, and trade organizations (Fox, 2009, Babar *et al.,* 2019).

Increased agricultural productivity, improved grain quality, drought tolerance, and insect and rust resistance are only some of the goals of transgenic wheat research and development. Claims have been made that introducing a gene responsible for a particular feature can improve the analysed problems by as much as 40 per cent. This is proof of the significance and efficacy of this technique. The world's population is expected to double by 2050, and by that time, transgenic crops could use up to 70 per cent of all farmland (Babar *et al.,* 2019). Most of the pushback against transgenic wheat stems from fears of contamination of wild-type and organic wheat with GMOs, but this doesn't seem likely to be a problem anytime soon, especially since herbicide-tolerant wheat has been on the market since 2004 and no other solutions have been reported that can handle demand and production issues on their own (Birzer and Badgery, 2006).

Wheat is the most resistant to tissue culture and genetic modification compared to other main cereal crops worldwide and in the United States (Bhalla, 2006, Jones, 2005). It is also genetically dependent on foreign *DNA* given by *Agrobacterium*. *Bayer Crop Science*, an agribusiness company, recently announced a partnership with the Commonwealth Scientific and Industrial Organization (*CSIRO*) to improve wheat's quality and stress tolerance (Xia *et al.,* 2012) Monsanto has also expressed interest in genetically modified wheat and plans to commercialize it sooner.

Future potential

Wheat, being a primary dietary source, holds immense global importance as a crop. To meet the growing demand for wheat, it is crucial to explore avenues for enhancing its production. One potential approach is the utilization of transgenic wheat and leveraging genomics to introduce favourable gene combinations into commercially viable varieties. This strategy holds particular promise, especially in developing nations. Agribusiness enterprises have exerted significant efforts towards the commercialization of transgenic wheat, despite encountering numerous challenges. These challenges have been addressed through the development of marker-free

transgenic approaches (Goutam *et al.,* 2013). The progress made in the field of genomics and transgenics during the present era has significantly contributed to the preservation of agriculture, health, and the environment on a global scale, despite the potential risks associated with these advancements. There is a current need for a transgenic revolution, akin to the one witnessed in the 1960s, to facilitate the development of high yielding varieties that can sustainably enhance production. This necessitates the utilization of the latest and most efficient technologies for genetic manipulation.

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