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Wheat in the Era of Genomics and Transgenics

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Abstract

Wheat, as one of the most important cereal crops in the world and second major caloric source in the world after rice, is the major staple food in South Asia and many other countries of the world. Prior to onset of “Green Revolution,” South Asian countries were facing the threat of severe famine. Green Revolution wheat genotypes brought out these countries from the crisis they were facing and has helped them to sustain their productions for more than half a century. With the emergence of molecular biology and biotechnology, another window of opportunity is opened to sustain wheat yields by using modern techniques of genes identification and utilization. Through this chapter, we have tried to gather information that was generated for wheat improvement in last 3 decades. These afforest included the development of molecular markers, mapping of genes, sequencing of markers genes, and their utilization through marker-assisted selection. The other part recorded various efforts to genetically transform wheat for traits improvements and/or to study their molecular control.

Keywords: wheat, marker-assisted selection (MAS), transformation, gene mapping, rust resistance

1. Introduction

Wheat (*Triticum aestivum*) belonging to *Triticeae* tribe of *Poaceae* family exhibits the most complex allohexaploid genome of approximately 17 Gb. Moreover, wheat is the second largest crop of the world after rice, but it has higher nutrition value than rice and is consumed by more than 2.5 billion people across the world. It is cultivated over a land of 215 million hectares worldwide every year, which is more than that of any other crop of commercial significance. Growing on a diverse range of environments and responding variably to temperate, tropical, and subtropical climates during spring and winter seasons, wheat is the most crucial as well as dominant staple food of Asia and North Africa. It is highly susceptible to abiotic stresses such as higher temperatures and depression in its yield could be resulted which is not permissible for a major food crop. Green revolution based upon a single objective to improve the yields of major cereal crops resulted in major yield shift for wheat during the previous century [1, 2]. Global export value of wheat is around 50 billion US\$ [3–5].

DNA, a biomolecule, is coiled in form of double-stranded helix which carries all the essential genetic information in specific codes/sequences for the proper functioning of an organism. Modern era of genomics and transgenics emerged after the identification of DNA structure in 1958 and discovery of restriction endonucleases later. By thorough analysis of complexity in wheat genome, it is believed that probability of transgenic events to occur is most likely within wheat as it is far greater than that of other monocots as it exhibits higher gene copies [6]. Information generated through DNA and genome sequencing lead to genetic improvement of the organisms by comparing the available genomics data as well as determining the undiscovered perspectives. Functional genomics have unlocked the roadmap of transgenesis by providing necessitated annotated information of genes naturally present in different organisms. By following up previous genomics studies done through molecular and morphological markers, researchers have taken a step toward exploring complex wheat genome and developing detailed physical and genetic maps of hexaploid genome of wheat [7, 8]. The functional and structural genomics of wheat is being stored in databases such as GenBank, TIGR, etc. [9]. The manipulation in genome comes next to exploration, and various alterations have been made by the implementation of conventional as well as advanced biotechnological approaches for the genome editing and genetic engineering [10].

Increasing wheat yield and nutritive quality are the major focus of studies going on currently in the world. It has been estimated that by 2050, the demand of wheat is going to increase up to 60%. Conventional cross breeding cannot fulfill this demand rapidly, only the genomics aided breeding and genetic engineering of wheat genotypes with genes from related and unrelated sources can speed up breeding and bring required genetic gains to feed rapid growing world population. Though genetic manipulations are of utmost importance yet biosafety is a great concern before commercializing products carrying genetic manipulations. This review focuses on the status of wheat crop since the very beginning of green evolution and the cascades of advancements that have been made upon progress in science and technology with the passage of time. How have these advancements been utilized by now and are going to be used in near future for increasing wheat yields and quality in order to provide growing populations a healthy food?

Global statistics of wheat:

Wheat crop have experienced rises and declines in its yield eventually over the years. **Figures 1** and **2** shows the graphical representation of global wheat production among last 6 decades (1961–2018). Before the onset of green revolution in 1960s, many wheat consuming countries were on the brink of famine due to insufficient yield. The issue had even worsened in South Asia, where wheat has been the major staple food and the area is thickly populated [13]. Norman Borlaug presented the idea of developing short-statured wheat genotypes by selective cross breeding techniques, which proved beneficial, and as a result dwarf varieties of wheat became rust free and the yield enhanced multiple times. Green revolution indeed sorted out the issue of crop yield, but many challenges remained consistent [2, 14]. With the emergence of green revolution, older wheat genotypes were almost eliminated and the diverse gene pools got weaker, as the sources of many essential genes were lost; in other words, the evolutionary process was shaken to a great extent. The older genotypes were taller, and relatively more vulnerable to rusts than the semi-dwarf green revolution varieties. Later on, the work over acquiring resistance by implementing DNA manipulative technologies was evidenced [15], which involve different native genetic markers and transgenes from other organisms.

Graphs, given above, showed that during the last 6 decades, production of wheat worldwide has exceeded 3.3 folds, i.e., from 222.1 metric tons to 755.9 metric tons. By 2050, it needs to be enhanced further over 60% for fulfilling the

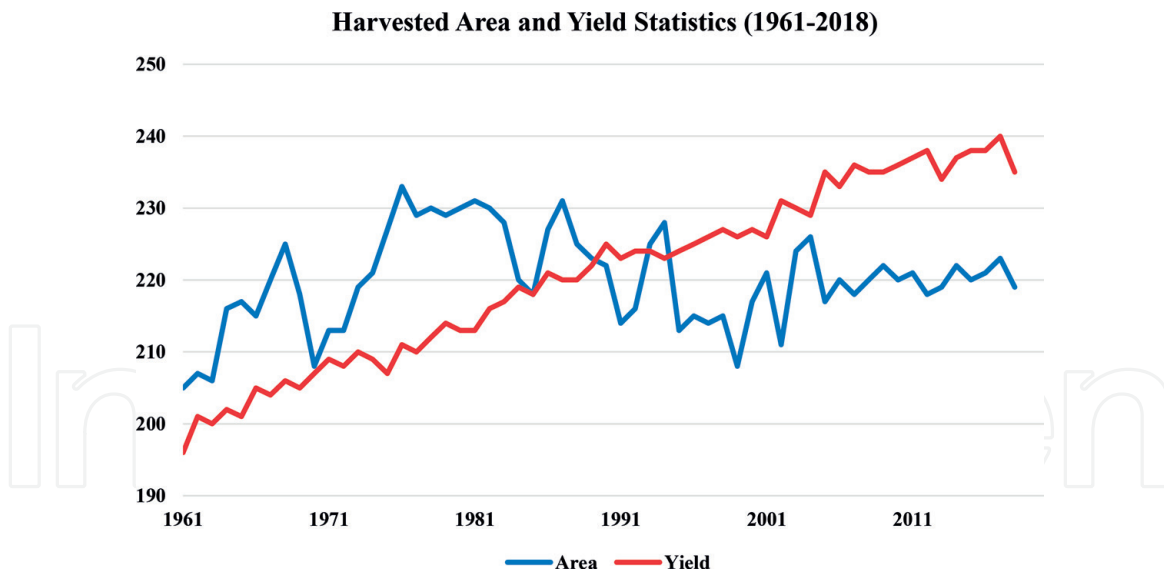


Figure 1.
Trend of wheat production worldwide (1961–2018) (Source: [11, 12]).

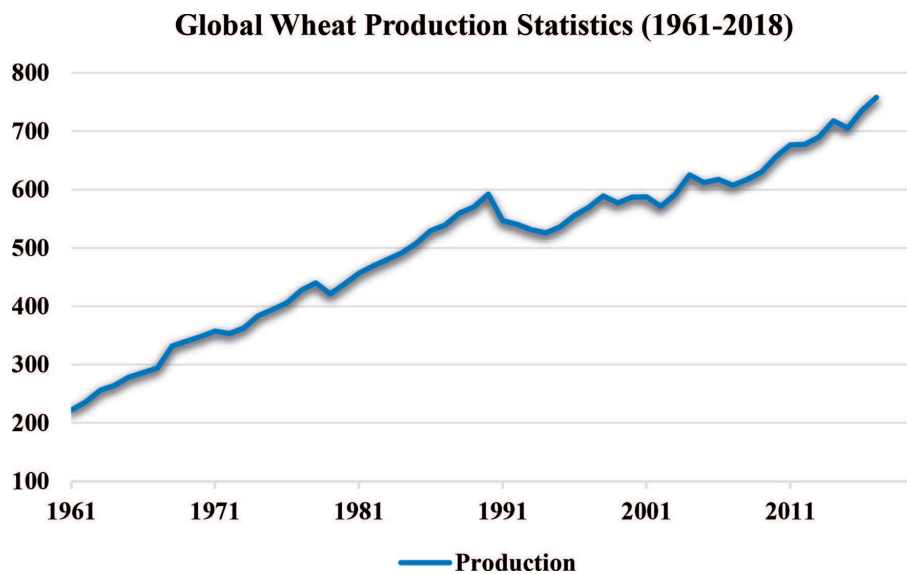


Figure 2.
Trend of area harvested and yield obtained from wheat worldwide (1961–2018).

consumers demand [16]. The global production of wheat has increased by 3.38% on average every year in each decade after 1970. A phase of declined production has been recorded after consecutive rise of production. This could be a result of several conditions combined, which involve exposure to insect/rust attack, harsh environmental conditions, and poor land management practices. After green revolution, the exponential growth in global wheat production can be clearly seen till the next decade of 1970s. First declined phase was experienced in late 1970s, prior to the beginning of genomic era. Following the genomic era from early 1980s, the wheat production started to grow exponentially once again for another decade. The major drop in production can be seen at the start of 1990s, which has been recovering ever since. The decade with the most variable production is 2000–2010. While, the decades of highest wheat productions is 1980s and the most recent one.

The major producers of wheat are China (134 mt), India (98 mt), Russia (85 mt), US (47 mt), France (36 mt), Australia (31 mt), Canada (29 mt), Pakistan (26.6 mt), Ukraine (26.2 mt), and Germany (24 mt). They produce approximately 70% of total wheat of the world [17].

2. Challenges in wheat production before and after genomics Era

Wheat grain quality and yield have always remained the foremost preference of research interest for getting the genetically improved crop with enhanced yield and better grain quality. Initially, the task was performed by implementing various artificial breeding techniques, which took longer than usual, and still the results obtained were not as efficient as anticipated [18]. The genomic era started in early 1980s with the discovery of recombinant DNA technology, a breakthrough in biotechnology [14]. With the passage of time, advancements in these technologies have eased up genome-wide analyses among different organisms by using Bioinformatics databases and tools. Similar struggle was done for the sake of wheat improvement, which have been highly susceptible to numerous stresses such as insects, rusts, and climate change, since the very beginning and gradually shifted toward molecular breeding [19]; while, the most serious challenge is to fulfill the demand with continuously increasing consumption. In past, marker-assisted breeding had been used extensively for getting the desired manipulative task done; but with the amendments in biosafety and bioethics, most of the research has been directed toward marker-free technology. Genomics era involved the advanced molecular breeding and genetic modification techniques for the wheat improvement, which was done by conventional plant breeding techniques under green revolution, prior to genomics era which no longer seems effective lately [20, 21]. A lot of work has been done over acquiring resistance against these stress factors, and researchers have also succeeded in developing such characters/traits within the wheat by utilizing a broad range of genetic engineering and genome editing technologies. Though genetically modified wheat presents high potential for trait improvement, only one GM event has been commercialized, which is MON-71800 or roundup ready wheat developed by Monsanto in 2004 for inducing glyphosate herbicide tolerance through *CP4 Epsps* gene transformation [22].

The challenges are commonly reported from developing countries; most probably, the ones with hot and dry climatic conditions, i.e., African and Asian regions. Besides, some of these challenges are also encountered in developed countries such as America, Canada, and Australia [23, 24], as the climate change is hitting almost everywhere on globe.

DNA technologies opened a gateway for the detection and induction of genetic mutations, but the inaccuracy of developed procedures as well as off-target outcomes have shown certain complications which might affect some other gene, apart from the targeted one. Besides, rise of bioethics and biosafety issues led to the failure of established DNA technology regarding the genetic manipulation of living organisms, as it was believed that these practices are meant to be contaminating and playing with nature which of course showed adverse effects in some of the cases. Later on, with the establishment of bioethics and biosafety act, the approach of transgenesis for genetic improvement of crops has been granted to be used globally as long as the biosecurity of product is ensured [25]. The recent advancements of genome editing and targeted or site-directed mutagenesis are very advantageous and destined to provide most promising results for the development of sustainable agriculture.

3. Complexity of wheat genome

Wheat exhibits one of the enormous and complex genomes with diverse ploidy levels among 23 different species. Out of which, 6 species cultivated across the globe are most common, i.e., Bread wheat (*T. aestivum*), Durum (*T. durum*), Emmer

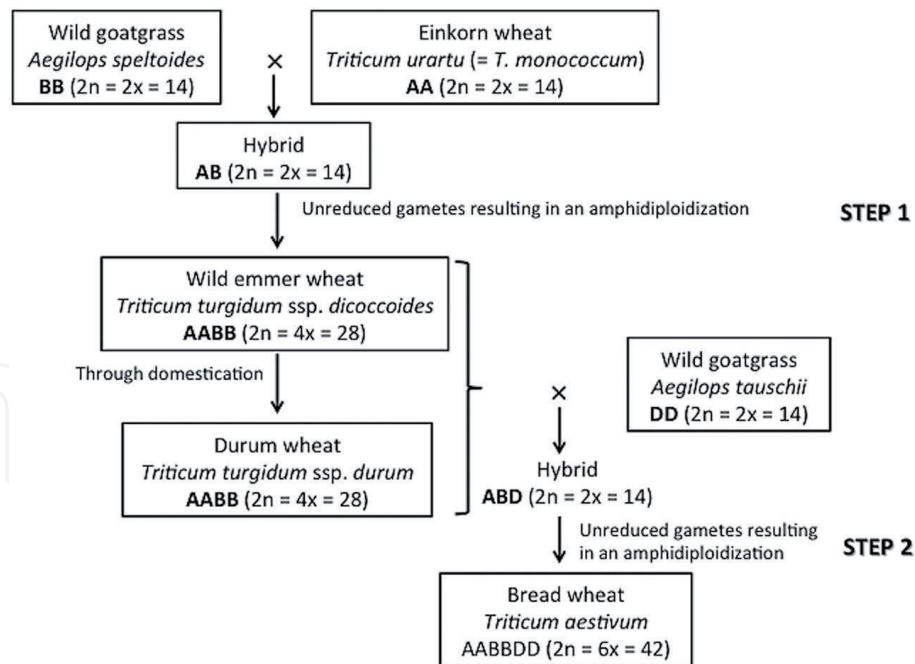


Figure 3. The complexity and evolution in wheat genome: the tetraploid wheat (*T. turgidum* spp. *Dicoccoides*) was developed by amphidiploidization between *T. urartu* and *A. speltoides* (diploids) (step 1), which later on hybridized into the allohexaploid wheat (*T. aestivum*) by amphidiploidization of *T. turgidum* spp. *Dicoccoides*, *T. turgidum* spp. *Durum* (tetraploids) and *A. tauschii* (diploid) together (step 2) [28].

(*T. dicoccon*), Einkorn (*T. monococcum*), Khorasan (*T. turgidum* or *T. turanicum*), and Speltoid (*T. speltoid*). As known, Einkorn is diploid; Durum, Emmer, and Khorasan are tetraploid; while Bread wheat and Durum are hexaploid. All the species other than *T. aestivum* and *T. durum* are ancient ancestors as they are cultivated at limited locations [26, 27] (Figure 3).

Wheat consisting of three genomes (AABBDD) exhibits genome size of approximately 17 Gb with 164,000–334,000 genes, 85% of which lies within <10% of all its chromosomes, i.e., 7. Wheat contains six copies of each gene as per its ploidy level and most of its traits are polygenic including the yield causing difficulty in inducing modifications in its genome. It has been reported that there are greater possibilities of developing these changes with extensive planning and targeting each copy of the gene separately [5, 29–31].

4. Recent advances of genomics era in wheat improvement

By the emergence of the genomic technologies, the steps toward improvement of wheat crop were taken to get sustainable production. For this core purpose, the posed challenges needed to be sorted out, which were done by using molecular breeding or marker-assisted breeding technologies [21]. Various members of aphid-resistant *Dn* gene family (*Dn1*, *Dn2*, *Dn4*, and *Dn5*) within wheat were found to be responsible for exhibiting some resistance against these insects by screening via RAPD and SCAR molecular markers [32]. *R* genes and *APR* genes were reported to be used for rust resistance either by screening and enhancing their expression through SNPs or by transforming wheat through suitable technique for gene delivery into plant genome [33–35]. Inbred lines in wheat having drought tolerance were evaluated by using microsatellite markers to get an idea of the responsible gene(s) present naturally within wheat genome [36].

5. Physical mapping of wheat genome

The International Wheat Genome Sequencing Consortium (IWGSC) database exhibits entire physical map of 21 chromosomes of bread wheat (*T. aestivum*) with High Information Content Fingerprinting (HCIF) and whole-genome profiling (WGS) in form of BAC libraries. Whereas, the physical contigs contain all the mapped information regarding markers, positions, and deletion bins of BAC clones. Different software programs, such as linear topological contig (LTC) and fingerprinted contig (FPC), are available within the database that are required for maintenance and update of data on physical map [37–39].

6. Genetic mapping of wheat genome

Molecular markers for genome mapping, such as amplified fragment length polymorphism (AFLP), expressed sequence tags (EST), quantitative traits Locus (QTLs), restricted fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), sequence of characterized amplified region (SCAR), single nucleotide polymorphism (SNP), simple sequence repeats (SSR), and sequence tagged sites (STS) along with the sequence-based mapping technologies, can be used as the efficacious tools for functional genomics of wheat. These phenomena help in getting comprehensive understanding of the genes responsible for certain traits and their phylogenetic analysis, which can thereby help in improvement of some closely related genotype through marker-assisted breeding [40–43]. **Table 1** shows some of the studies done in wheat to assess different populations and genotypes using molecular markers.

Molecular markers	Population	Mapped loci	References
AFLP	RILs (Wangshuibai × Alondra's)	250	[44]
	<i>T. aestivum</i> (Chinese Spring × <i>T. spelta</i>)	24	[45]
EST	<i>T. aestivum</i>	22	[46]
QTL	RILs (Arina × Forno) F _{5.7}	8	[47]
	F ₂ (BC5 and BC9) <i>T. tauschii</i>	2	[48]
RFLP	<i>T. aestivum</i>	245	[49]
	<i>Triticum aestivum</i> L. emThell.	82	[50]
RAPD	Yangmai 5	180	[51]
	<i>T. aestivum</i>	71	[52]
SCAR	<i>T. aestivum</i> F ₂	43	[53]
	<i>T. aestivum</i>	23	[41]
SNP	<i>T. aestivum</i> (Chinese Spring × Renan)	3.3 × 10 ⁶	[43]
	RILs (Ning7840 × Clark and Heyne × Lakin)	3541	[42]
SSR	RILs (Ning7840 × Clark and Heyne × Lakin)	145	[42]
	DHs (Kitamoe × Munstertaler)	464	[54]
STS	DHs (AC Karma × 87E03-S2B1)	165	[55]
	RILs (Dream × Lynx)	283	[56]

RILs, recombinant inbred lines; DHs, doubled haploids; BC, back-crossed; F, filial generation.

Table 1.
Molecular markers for wheat genome mapping.

7. Sequencing technologies and wheat

The genome sequences play a crucial role in comprehensive understanding of phenotypic traits, their molecular basis, and alterations in them. The comparative genomics studies for enhancement of wheat have been constrained by its less genomic conservation. The wheat genome sequencing has become the utmost priority for the sake of crop improvement in order to know the genetic basis of traits controlled by a complex genome. The enormous complexity in genome and its size have caused several limitations of efforts in sequencing studies. Currently, several drafts of wheat genome have been sequenced on the basis of chromosomes of either of its genomes with the help of advancements in next-generation sequencing technologies [57, 58].

The entire genome including cDNA of *T. aestivum* cv. Chinese Spring (CS42) was sequenced by the application of random shotgun next-generation sequencing that involved Illumina HiSeq 2000, Genome Analyzer IIx, and Roche 454 pyrosequencing technology. The sequenced data were then compared with the previously identified genome sequences of *Aegilops*, *A. Tauschii*, *A. speltoides*, and *T. monococcum*. This could identify 124,000 genes distributed into A, B, and D genomes [7, 8, 59]. The diploid species of wheat, *T. urartu* and *A. tauschii* were also sequenced and reported to be having 34,879 and 43,150 genes, respectively [60, 61]. Indeed, the information obtained from all these efforts have caused a lot of ease in localizing the identical genes in hexaploid species, while the information regarding their evolution remained undiscovered for a while [57]. In 2014, IWGSC started to work over the whole-genome sequencing of wheat cv. Chinese Spring (Hexaploid) and started *de novo* assembly of each of the chromosomes except 3B, which was done independently by Choulet and his coworkers [8, 62].

8. Sequence-based mapping

The advancements in new sequencing technologies of genomic era have offered various cost-efficient approaches to carry out the genetic mapping of complex genomes with high resolution. The case of wheat genome sequencing, due to its polyploidy causes hurdles in spite of all the known promising applications of these technologies [63]. In a study, DH wheat variety was mapped by application of whole-genome shotgun NGS and the consistent outcomes were obtained from variant mapping and compared with the ones obtained from 9000 SNP iSelect assay of wheat. Significant resemblance was found among these results. As per findings, a reference map of entire wheat genome was developed, which consisted of 118 SSRs, 1351 diversity array technology, 2740 genes linked SNPs by wheat iSelect assay, and 416,856 genetic markers. By the detailed analysis, it was revealed that these markers reside within the range of 40–100 kb from their neighbor gene, hereby enhancing the possibility of genome mapping for gene identification. The given information is quite beneficial for the thorough study of wheat genome by linking both of its genetic and physical maps [63, 64].

9. Mapped traits in wheat

By the implementation of forward genetics approaches in molecular markers, such as QTLs, a large number of studies have been conducted based upon the genome mapping of wheat in order to identify response of plant against biotic and abiotic stresses. It is reported that while performing QTL study, environmental interactions always remain a significant factor [65, 66]. A short list of conducted studies for various traits in wheat regarding QTL or gene tagging is given in **Table 2**.

Trait	Gene/QTLs	Chromosome	Population	References
<i>Abiotic stress tolerance</i>				
Aluminum tolerance	<i>ALMT1</i>	4D	DH	[67]
Boron toxicity tolerance	<i>Bo1</i>	7BL	DH	[68]
Drought tolerance	<i>DREB1</i>	3A	Barakatli-95	[69]
Frost tolerance	QTL	5B	RSI	[70]
Photoperiod insensitive	<i>Ppd-B1</i>	2BS	RILs	[71]
Salinity tolerance	QTL	3A, 3B, 4,6 DL	RILs	[72]
<i>Russian wheat aphid resistance</i>	<i>Dn1, Dn2, Dn5</i>	7DS	F2	[73]
	<i>Dn4, Dn6</i>	1D, 7D	F2	[74]
	<i>Dn7</i>	1B	F2	[75]
	<i>Dn8, Dn9, Dnx</i>	7DS, 1DL	F2	[73]
<i>Stem rust resistance</i>	<i>Sr2</i>	3BS	F3	[76]
	<i>Sr22</i>	7A	F2	[77]
	<i>Sr38</i>	2AS	NILs	[78]
<i>Leaf rust resistance</i>	<i>Lr1</i>	5DL	F2	[79]
	<i>Lr3</i>	6BL	F2	[80]
	<i>Lr9</i>	6BL	NILs	[81]
	<i>Lr10</i>	1AS	F2	[82]
	<i>Lr19</i>	7D	F2	[83]
	<i>Lr20</i>	7AL	F2	[84]
	<i>Lr21</i>	1DS	F2	[85]
	<i>Lr22a</i>	2DS	F2	[86]
	<i>Lr24</i>	3DL	F2	[87]
	<i>Lr34</i>	7D	RILs	[88]
	<i>Lr35</i>	2B	F2	[89]
	<i>Lr37</i>	2AS	NILs	[78]
	<i>Lr40</i>	1DS	F2	[85]
	<i>Lr47</i>	7A	BC1F2	[90]
	<i>Lr52</i>	5B	F2	[91]
<i>LrTr</i>	4BS	F2	[92]	
<i>Fusarium head blight resistance</i>	<i>Fhb2</i>	6BS	RILs	[93]
	QTL	1B, 3B, 5A	RILs	[94]
	QTL	2B	RILs	[95]
	QTL	4A, 5B, 6D	RILs	[96]

Table 2.

Studies for various traits conducted in wheat regarding using QTL and gene tagging.

10. Comparative genomics with *Arabidopsis*

The genome-wide analyses in wheat (*Triticum aestivum*) and *Arabidopsis thaliana* have been performed for their comparative genomics studies. For this purpose, the ESTs as well as BLAST have been compared to evaluate the identity and similarity

index within certain genes of their genome sequences taken from *Arabidopsis* and endosperm clones of wheat (Accession Numbers: BQ605537-609969, GenBank). As the wheat genome is approximately 126 folds greater than that of the *Arabidopsis* [97, 98]. The error rate regarding unresolved nucleotides was recorded to be less than 2% during comparison of almost every 500 base pairs. The data of *Arabidopsis* were recruited from the TIGR databases of nucleotide and protein, and ESTs were clustered by PHRAP program. ESTs of wheat (4433) were clustered by self-BLAST as well into the contigs. The result of multiple sequence alignment represented relatively lower percentage of the sequences with low complexity of ESTs constituents; hence, higher score of alignments was produced on average. The number of clustered ESTs (Contigs) reported were 789, while that of unclustered ESTs was 1348. Therefore, the number of unique sequences obtained was 2137, which were proceeded for further genomic analyses and comparison with genome of *Arabidopsis* that revealed the grouping of these wheat ESTs with 1130 unique genes of *Arabidopsis* dispersed randomly within its genome upon different chromosomes which resembles in approximately 75% in their functions to wheat ESTs [99, 100].

11. Comparative genomics with other grasses

The most common members belonging to *Poaceae*, the grass family, include *Avena sativa*, *Hordeum vulgare*, *Oryza sativa*, and *Zea mays*. The wheat genome is 1.5 folds larger than Oat, 3 folds than Barley, 6.8 folds than maize, and 39 folds than rice. *Triticeae* is a tribe of this particular family consisting of over 15 genus as well as 300 species including barley and wheat. Number and size of the genes present within members of grass family are most likely to be same [101, 102]. Apart from genome size, the genes present within all these species are closely related to each other, and hence it clarifies the fact that rice, maize, and wheat have diverged over 50 million years ago and belong to a common ancestor [103]. The conservation of gene order is evident among these organisms, while the evolution is responsible for extent. The percentage of conserved markers among wheat, barley, and oat is 94%, while that of maize and rice is 62%. The greater resemblances have been observed among the members of *Triticeae* than that of the *Poaceae* family, while performing their comparative genomics studies [104–107]. The gene containing proportion among all the species of *Poaceae* seems to be similar, while the estimations upon presence of this particular's fraction within their genomes have been made, i.e., wheat (7%), barley (12%), maize (17%), and rice (24%) [5, 108, 109].

12. Transgenics for wheat improvement

Transgenics refer to genetically engineered (GE) or genetically modified (GM) organisms carrying some exogenous segment of DNA that is responsible for encoding some protein. The function, expression, and interaction of particular protein in metabolism of plant system other than its native one under variable conditions can be evaluated at molecular level, *in vitro*, as well as *in vivo* by using transgenic approaches [132]. According to ISAAA [22], only one GM wheat event got wheat approved for commercialization, and that for herbicide tolerance. *CP4 Epsps* gene was taken from a bacterial source which created resistance against glyphosate herbicide. The inserted genes might perform differently and affect some other trait negatively. In such cases, multiple factors are involved and all of them are of crucial significance, which have to be taken care of simultaneously in order to discover the exact cause of encountering issue [133].

13. Transgenics for improvement of agronomic characters

Attempts to transform wheat were started in mid-1980s, and first successful wheat transformation was reported in 1991 by Vasil and colleagues using biolistic transformation. Wheat protoplasts were transformed with chloramphenicol acetyltransferase (*CAT*) gene from a bacterium in order to get the gene expression by application of electroporation transformation technique [134]. PEG-mediated genetic transformation of *T. monococcum* protoplasts was performed for introducing Tn5-aminoglycoside phosphotransferase type II (*NPTII*) gene into wheat genome as a selectable marker [135]. Wheat, at its early boot stage with a few spikes, was transformed *in planta* by *Agrobacterium*-mediated floral-dip transformation which involved the pollen tube pathway for the insertion of *hgh* and *NPTII* transgenes as selectable markers. The inheritance pattern of this transformation was also evaluated into further two generations, i.e., T1 and T2 [136]. First transgene-free mutants of

Transformation technique	Vector	Gene	Trait	Transformation efficiency	References
<i>Agrobacterium</i> -mediated (Callus)	pROK2	<i>CptI</i>	Insect resistance	N/A	[110]
<i>Agrobacterium</i> -mediated (Seed)	pWUbi	<i>TaMATE1B</i>	Heavy metals tolerance	N/A	[111]
<i>Agrobacterium</i> -mediated (in <i>planta</i>)	pVecNeo pWBvec8	<i>Lr67</i>	Disease resistance	N/A	[112]
	pBI121	<i>CspA</i>	Drought tolerance	N/A	[113]
<i>Agrobacterium</i> -mediated (embryo)	pCMV35S	<i>AtNTX1</i>	Salt tolerance	5.7–7.5%	[114]
	pGH215 pVS1	<i>Gfp</i> <i>Hpt</i>	Visual selection Antibiotic resistance	2–10%	[115]
	pZP201 pPTN290	<i>Gus</i> <i>Bar</i>	Visual selection Herbicide tolerance	2.7–37.7%	[116]
	pB1101	<i>Act1</i> <i>Bar</i>	Quality improvement Herbicide tolerance	1.28%	[117]
	pGA482	<i>Gus</i>	Selectable marker	27%	[118]
	pIG121Hm	<i>Gus</i>	Selectable marker	52–56%	[119]
	pCAMBIA3301 p3SSGUSINT	<i>Bar</i>	Herbicide selection	0.84–1.16%	[120]
	pWVec8	<i>Bar</i>	Herbicide tolerance	1.5–51%	[121]
	pCAMBIA pGreen	<i>Bar</i> <i>NptII</i>	Selectable markers	1.4–1.8%	[122]
	pLC41Hm pLC41bar	<i>Bar</i> <i>Hpt</i>	Herbicide tolerance Antibiotic resistance	40%	[30, 31]

Transformation technique	Vector	Gene	Trait	Transformation efficiency	References
Biolistics-mediated	pVst-HarChit pVst-Harcho	<i>HarChitHarCho</i>	Disease resistance	0.17–0.26%	[123]
	pLNU-SG	<i>TaGSL3</i> <i>TaGSL8</i> <i>TaGSL10</i>	Disease resistance	Less than 1%	[124]
	pAHC20 pAHC17	<i>SSI-IV</i> <i>GBSS</i>	Heat tolerance Yield improvement	N/A	[125]
	pAHC25	<i>HMV-GS 1Dx5</i>	Quality improvement	0.4%	[126]
	pUba	<i>sGfp</i>	Visual selection	0.8%	[127]
	pHAC20	<i>Gfp</i>	Visual selection	5–10%	[125]
CRISPR/Cas-mediated	P6U	<i>Lr34</i>	Disease resistance	N/A	[128]
CRISPR/Cas-mediated	pA9mRFP pU6sg	<i>GW2</i>	Improved grain quality	20%	[129]
CRISPR/Cas-mediated	pB1121	<i>Inox</i> <i>Pds</i>	Quality improvement	8–12%	[130]
PEG-mediated	pAHC25	<i>Gus</i> <i>Hpt</i>	Visual selection Antibiotic resistance	1–5%	[131]

Table 3.
 Transformation events targeted for improving agronomic traits.

wheat were reported to be generated by application of CRISPR/Cas9 system by editing the genome at directed site, while the transgene-based transformation through CRISPR/Cas faces certain hurdles due to complexity in wheat genome [137, 138].

Several transgenic technologies have been developed so far for the sake of improvement of major agronomic characters (**Table 3**) leading to increased grain yield and quality in wheat. These traits include genes for biotic and abiotic stress tolerance, including herbicide tolerance, drought tolerance, salt tolerance, disease resistance, etc. By the genetic transformation of wheat genome, numerous low-molecular-weight glutenin subunits (LMW-GS) and high-molecular weight glutenin subunits (HMW-GS) could be added to wheat genome, which plays a vital role in improvement of wheat grain quality [139–141]. Wheat cv. Bobwhite was transformed with 1Ax1 HMW-GS subunit through biolistics-mediated transformation of immature zygotic embryos. As a result of which, 71% improvement in gluten contents was observed in transformed grains [142].

14. Technologies for developing marker-free transgenic wheat

Selectable markers, as a crucial component of transformation procedures, have played significant role in enhancing the transformation efficiency. Various hazards in these particular genes upon environment and health have been feared, which need to be addressed by developing marker-free transgenics. Different strategies, such as co-transformation, site-specific recombination, and transposon-mediated

elimination, tend to be proved advantageous in the removal of selectable markers from plant systems [143–145]. Besides, customized marker-free vectors, known as pCLEAN vectors, for the transformation have been designed for delivering multiple transgenes specifically without adding any superfluous DNA sequences within plant genome [146]. The enhanced transformation efficiency has also been reported by the use of pCLEAN vectors for gene delivery [147]. Researchers have started to use the plant-derived genes for selection purpose as well while performing genetic transformation in wheat, i.e., *ALSAP*, drought and salinity-tolerant gene from *Aeluropus littoralis*; *ALS*, herbicide-tolerant gene from *Oryza sativa*; and *AtMYB12*, visible selection gene from *Arabidopsis thaliana* [148–150].

15. Transgenic wheat and its commercial acceptability

The level of acceptance of GM wheat is similar to that of other commercial transgenic crops, i.e., tomato, maize, rice, cotton, etc. European countries are reluctant to grow GMOs, while American and less developed countries are in favor. In countries like Pakistan, where wheat has been the traditional staple food, there is always a fear of famine on wheat crop failures. The governments in such countries are ready to adopt technologies ensuring yield sustainability of staple. Even due to the opposition of public, market, governments, farmers, and trade organizations to transgenic crops, the stakeholders cannot switch from them until any competitive alternative attracts the attention [151, 152]. The goals in wheat transgenics include biotic and abiotic stress tolerance, nutritive quality of grain, and herbicide tolerance, as described in **Table 3**. The core purpose of transgenic wheat development is to overcome global issues by providing best possible as well as permanent solutions, such as enhanced crop yield, improved grain quality, drought tolerance, and insect and rust resistance. It is reported that insertion of a gene responsible for particular trait causes variable improvements ranging from 20 to 40% and even higher in some cases, but still not complete eradication of issues under study. This illustrates the significance and efficacy of this technology. It is estimated that world population will be doubled by 2050, and transgenic crops will cover up to 70% of the cultivation land [153, 154]. Transgenic contamination in wild-type and organic wheat varieties is the most emerging issue that has been aroused for opposing transgenic wheat, but the acceptability of transgenic wheat does not seem to be a major issue in near future as the approved event of herbicide-tolerant wheat is being commercialized since 2004, and no other approaches have been reported so far that could deal with meeting up demand and production hurdles single handedly [22, 155].

Apart from global significance, wheat is last among all the major cereal crops that have been transformed depending upon various parameters, such as dependency of genotype upon exogenous DNA delivered by *Agrobacterium* and recalcitrance in tissue culturing [156, 157]. The companies of agribusiness, like Bayer Crop Science, have announced the alliance of Commonwealth Scientific and Industrial Organization (CSIRO); Monsanto have indicated their interest in GM wheat and planned to commercialize it sooner, while Syngenta have formed the alliance with CIMMYT for working over wheat improvement regarding its stress tolerance and quality [158].

16. Future prospects

Wheat, as a staple food, is the most significant and demanding crop all over the world and the remarkable enhancement in its production needs to be done, which is only possible by the commercial application of transgenic wheat and smart use

of genomics for bringing desirable gene combinations in commercial varieties, at least in developing countries. The companies of agribusiness have been working on it really hard to get transgenic wheat commercialized in spite of all the challenges being faced and working over sorting out the challenges by developing the marker-free transgenic approaches [159]. The advancements in current era of genomics and transgenics have played a crucial role in the maintenance of agriculture, health, and environment in the world, even though several hazards regarding them could be possible. A transgenic revolution similar to that of 1960s is now required in order to develop all the high yielding varieties for the sustainable production by implementing the most recent and efficient technologies for genetic manipulation.

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