

## Advances in Wheat Genomics and its Potential in Ensuring Food Security in the Scenario of Climate Change

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Prevalence of hunger and under-nutrition among the world population at alarming rates challenges the food security. Further, the instability in global wheat production in past two years and the projection of continuing decrease in wheat inventories predominantly due to climate change add-up to food insecurity. The global climate change imposing biotic and abiotic stress made the crop improvement more challenging. However, the timely release of wheat genome sequence holds the promise of evading these tribulations, provided the researchers and breeders pertinently utilize the genome information. In view of this, this short-review highlights the intact insights for the wheat research community on how to implement the genome sequence data in breeding and engineering transgenic wheat with enhanced biotic and abiotic stress tolerance.

**Key Words:** Climate Change; Genetic Engineering; Marker-Assisted-Breeding; Next-Generation Sequencing; Wheat; Whole Genome Sequence

### Introduction

Wheat (*Triticum* sp.) is the largest cultivated cereal globally, serving as food for a considerable population around the world. In 2009, wheat was ranked second [685.61 million tonnes (mt)] in terms of global production, next to maize (818.82 mt) (FAOSTAT 2009; <http://faostat.fao.org/site/567/default.aspx#anchor>). However, its global production is drastically decreasing which ultimately questions the food security (Semenov *et al.*, 2012). The production status in 2010 and 2011 showed a severe decrease resulting in the third position with 653.63 mt and 704.08 mt, respectively (FAOSTAT 2010, 2011). In 2012, the global wheat production was 674.88 mt (FAOSTAT 2012; <http://faostat.fao.org/site/567/default.aspx#anchor>). Moreover, the recent forecast of Global Information and Early Warning System (GIEWS) predicted that the world wheat inventories might reduce to 163 mt, 11% slide from their opening level and 2% (3 mt) less than it was reported in November

2012 (<http://www.fao.org/GIEWS/english/index.htm>).

Wheat is extremely sensitive to heat and its vulnerability is best illustrated with the 50% decrease in production recorded in Australia due to the  $\pm 2^{\circ}\text{C}$  temperature variation (Asseng *et al.*, 2011). According to Intergovernmental Panel on Climate Change (IPCC), the temperature may increase up to  $4.5^{\circ}\text{C}$  by 2080 depending on the progress of mankind, which would tremendously exacerbate the agricultural productivity (<http://www.ipcc.ch/>). Adding to this saddle, the FAO Hunger Report (2012) depicts that, about 12.5% of the global population (one in eight people) is starving, excluding 100 million children under the age of five. Apart from the adults, about 2.5 million children die every year due to starvation, which ultimately impedes human potential. Being a rich source of protein than other cereals, bread wheat (*Triticum aestivum*) constitutes the staple food of the world since its domestication

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occurred ~10,000 years ago and therefore its improvement has massive implications for food security. The global demand for wheat is expected to rise by 60% by 2050, whereas the climate change is anticipated to negatively affect the production by 29% in the same vicinities (Dixon *et al.*, 2009). Thus, the tough task of feeding the world on one hand and the drastic reduction in prime agricultural produce due to global climate change on the other emphasizes the timeliness of an intense agricultural research at both breeding and molecular level.

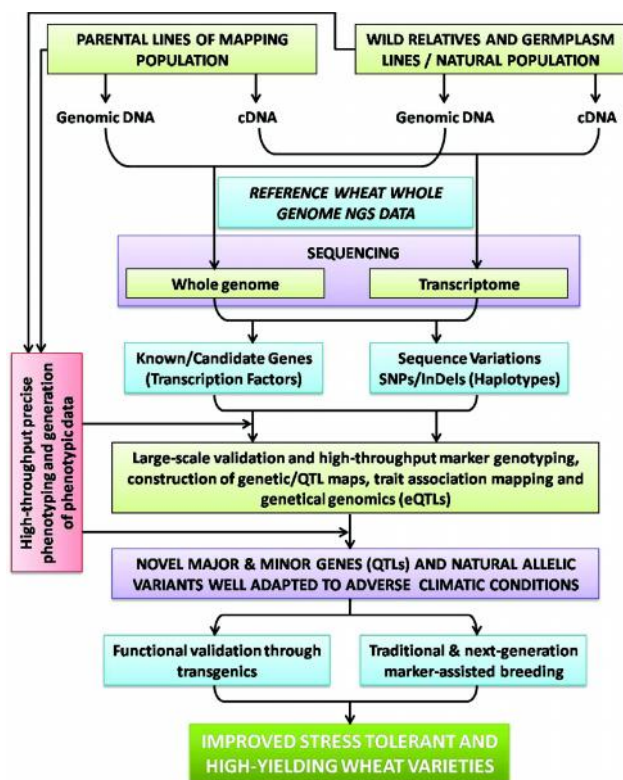
### Wheat Genome and its Potential

The size and complexity of the bread wheat genome ( $2n = 6x = 42$ , AABBDD allohexaploid; ~17 Gb: five times the size of human genome) hampered the progress in wheat genomics and its application towards crop improvement. However, the recent release of draft genome sequence (Brenchley *et al.*, 2012) provides a direct access to all 96,000 genes and 132,000 single nucleotide polymorphisms (SNPs) distributed over A, B and D genomes. The random shotgun libraries of total genomic DNA and cDNA from *T. aestivum* cv. Chinese Spring (CS42) have been sequenced using the next-generation Roche 454 pyrosequencer and generated ~85 Gb of sequence data (~220 million reads), which is equivalent to 5X coverage of estimated whole wheat genome (17 Gb). The whole genome next-generation sequence (NGS) evidenced the presence of 94,000 and 96,000 genes in bread wheat genome. Aligning the NGS data to the genome sequence of wheat progenitors (*T. monococcum*, *Aegilops speltoides*, *Ae. Tauschii*) showed that two-thirds of these genes belong to A, B or D genomes (Brenchley *et al.*, 2012). The assignment of larger set of functionally annotated genes to the individual chromosomes of A, B or D genome is crucial resource for wheat researchers, as it allows them to differentiate and select genes and DNA markers from each of the three genomes chromosome-wise, which is a complex and tedious process (Brenchley *et al.*, 2012). Besides, the conserved orthologous relationships of genes localized on wheat A, B and D genomes with members of grass family (*Brachypodium distachyon*, sorghum, rice and barley) could be an amenable comparative genomics resource

for map-based isolation of genes useful in larger complex wheat genome, utilizing the gene-based sequence information of small diploid crop species. The high-resolution synteny maps showed many small interruptions to conserved gene order with maximum synteny towards *B. distachyon*. However, the complex evolutionary processes like polyploidization and significant loss of gene segments as compared to its diploid progenitors and other highly complex genomes like maize in recently domesticated hexaploid wheat were evident (Brenchley *et al.*, 2012). Overall, the structural and comparative genomics resources including genomic and transcript sequences, orthologous genes and SNP markers generated through wheat whole genome next-generation sequencing could act as reference for sequencing of other wheat cultivars (Muthamilarasan and Prasad, 2013a). As anticipated, the genome sequences of two wild wheat progenitors, viz., *T. urartu* (A-genome progenitor) and *Ae. Tauschii* (D-genome progenitor) were recently reported (Ling *et al.*, 2013; Jia *et al.*, 2013). This would also facilitate the examination of genetic and evolutionary factors controlling desirable traits such as disease resistance, drought tolerance and yield, and thus appears promising in accelerating large-scale molecular breeding and transgenic research, particularly towards producing elite cultivars capable of tolerating adverse environmental conditions. The strategies for the applications and utility of currently available whole genome NGS data for wheat improvement in changing climate is discussed below and also illustrated in Fig. 1.

### Transcription Factors – Key Factors for Stress Tolerance in Changing Climate

Transcription factors (TFs) and its corresponding *cis*-regulatory sequences operate as molecular controls for gene expression, regulating their temporal and spatial expression, particularly during stress (Lata and Prasad, 2011). The functional annotation of genes predicted from wheat whole genome NGS data (Brenchley *et al.*, 2012) and their ontology have shown that about 2% sequences of each A, B and D genome encodes for TF families. Interestingly, these TF families demonstrated an apparent ability to be



**Fig. 1:** Overview on application of wheat NGS data in crop genetics and breeding. The possible strategies to be followed for developing stress tolerant high yielding wheat genotypes using the recently released whole genome NGS data. (eQTL: expressed Quantitative Trait Loci; InDels: Insertion Deletions)

retained as functional genes in all three genomes without gene loss, which was evident in most of the functional gene classes in the three genomes (Brenchley *et al.*, 2012). Jia *et al.* (2013) have identified 1,489 TFs (grouped into 56 families) in *Ae. Tauschii*, of which the MYB-related TFs constituted a major proportion. MYB-related TFs are involved in stress-tolerance (Garg *et al.*, 2012) and in activating the expression of stress-tolerance genes. It is noteworthy that the number of MYB TFs in *Ae. Tauschii* (103) was higher than that of *Brachypodium* (66) and maize (96), which suggests the enhanced abiotic stress tolerance behaviour of this wild cultivar. Moreover, the ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks) co-expression analysis using the RNA-seq data of *Ae. Tauschii* showed an expression system of 1283 interactions, in which 13 TFs were associated with the regulation of expression of genes involved in

drought tolerance (Jia *et al.*, 2013). Further, imparting stress tolerance using TFs is best explained through the role of *TaASR1* (abscisic acid-, stress-, and ripening-induced), a TF in wheat which conferred drought stress tolerance in transgenic tobacco (Hu *et al.*, 2013). The *TaASR1* was found to play a major role in preventing the plants from oxidative damage by regulating the expression of stress- and defense-related genes and augmenting the antioxidant system (Hu *et al.*, 2013).

In addition, wheat TF databases such as ‘PlantTFDB’ (see: [http://plantfdb\\_v1.cbi.pku.edu.cn:9010/web/index.php?sp=ta](http://plantfdb_v1.cbi.pku.edu.cn:9010/web/index.php?sp=ta)) and ‘Database of Wheat Transcription Factor’ (see: <http://www.appli.nantes.inra.fr:8180/wDBFT/>) would serve as resources for the research community to study the involvement of TFs in stress response. The role of TFs towards diverse environmental stresses in various model crops, based on conserved gene orthology in the present whole wheat genome sequencing, has also been demonstrated (Riechmann *et al.*, 2000; Lata *et al.*, 2011; Brenchley *et al.*, 2012; Ling *et al.*, 2013; Jia *et al.*, 2013). This knowledge should be applied in deciphering the role and activity of wheat TFs. An added advantage with TFs is that, they provide multiple stress adaptation and hence it would stand as the hottest topic of research in biotic as well as abiotic stress tolerance. Therefore, the wheat TF genes which are showing non-additive gene expression would enable the researchers to identify stress-responsive TFs. By combining ‘omics’ approaches, the role of these TFs in stress tolerance towards changing climate can also be elucidated.

#### ***Use of Whole Genome Sequence in Marker-Assisted and Transgene-Based Genetic Improvement of Wheat***

Genic markers are useful in analysing practical diversity in natural populations or germplasm sets and serve as crucial anchor markers for comparative mapping, evolutionary studies and marker-assisted selection. Genome-wide generation of these genic markers in wheat by utilizing the NGS data and their validation and high-throughput genotyping in a larger set of natural and mapping population could lead to the rapid identification of major quantitative trait loci

(QTLs) possessing consistent positive effects on wheat yields across diverse environmental conditions. Moreover, whole genome sequence (WGS)-based genetic and association mapping approaches also assist in plotting the genes/QTLs responsible for stress tolerance, where the population surveys for haplotypes investigated based on WGS exploits the past recombination events to decipher trait–marker relationships on the basis of linkage disequilibrium (LD). Trait mapping and use of genic markers in heat and drought tolerance is demonstrated in many crop plants (Ramu *et al.*, 2009; Mbanjo *et al.*, 2012). Resequencing the genomes of many wheat cultivars and aligning it to the presently published reference genome will aid in the discovery of more SNPs in addition to 132,000 SNPs reported in bread wheat (Brenchley *et al.*, 2012), 2,989,540 SNPs in *T. urartu* (Ling *et al.*, 2013) and 711,907 SNPs in *Ae. Tauschii* (Jia *et al.*, 2013). This would also enable the generation of trait-specific SNP markers, which serve as a diagnostic for specific alleles at important genes for stress tolerance. For example, in one of our recent studies we had identified a synonymous SNP-associated with drought tolerance at 458th bp (an A/G transition) in the *TaMYB2* gene of bread wheat and developed an allele-specific marker for dehydration tolerance for the same which could now expedite marker-assisted selection for dehydration tolerance in wheat breeding programmes (Garg *et al.*, 2012). Hence, these efforts will ultimately enable in performing high-resolution genetic and association mapping studies in wheat, which would assist in investigating the diverse accessions quarantined in germplasm collections for superior alleles at key genetic loci. Thus, the new wealth of DNA sequence data and advances in high-throughput SNP genotyping techniques will offer breeders with innovative tools and resources to hasten the analysis of complex traits and enable novel traditional and next-generation marker-assisted selection approaches. Among the various abiotic stresses induced by climate change, heat stress coupled with drought is the most predominant environmental stress hampering the agriculture worldwide (Witcombe *et al.* 2008; Knox *et al.* 2012). Prior to the release of genome sequence, numerous efforts have been

invested in generating wheat cultivars tolerant to these stresses through conventional breeding and indirect examination of the genome. One such attempt is the genetic dissection of wild wheat germplasms (*Aegilops* sp.) for heat tolerance, which provided an insight in breeding bread wheat (Pradhan *et al.*, 2012). Garg *et al.* (2013) had performed suppression subtractive hybridization in samples derived at different time points during drought and salinity stress in 28 bread wheat cultivars and identified about 107 differentially expressed transcripts. But, now the whole genome NGS data immediately allows the researchers to identify and select a set of candidate/known stress-responsive genes (or TFs), informative markers (SNPs) and utilize them in marker-assisted genetic enhancement studies in wheat (Muthamilarasan *et al.*, 2013). More importantly, the stress tolerant trait-specific novel genes and allelic variants could be functionally validated through wheat transgenics using over expression and knock-out analysis methods and thus, it would expedite the transgene-based genetic enhancement studies in wheat for stress tolerance. This facilitates in selection and use of novel natural allelic variants well adapted to adverse climatic conditions and their understanding for drought tolerance mechanisms in wheat. For such genetic improvement studies, the novel major and minor allelic variants regulating stress tolerance could be transferred to desirable genetic background of high yielding wheat varieties through introgression breeding, traditional foreground and background selection and modern next-generation marker-assisted breeding methods including genomic/haplotype selection for developing high yielding stress tolerant wheat varieties.

#### ***Role of Climate Change in Imposing Biotic Stress – A Case Study and Possible Remedy Through NGS Data***

In addition to abiotic stress, climate change also imposes direct effects on plant disease epidemics. A case study on *Fusarium* head blight (FHB) in wheat reported by Chakraborty and Newton (2011) outlines the key influences of climate change on FHB and its effects on production and quality of wheat. The report shows that the changes in both physical climate

(temperature, rainfall and relative humidity) and atmospheric composition (CO<sub>2</sub> concentration) manipulate the severity of FHB, resulting in increased mycotoxin accumulation in wheat, which is toxic to both plants and humans (Chakraborty and Newton, 2011). Though the most significant influences occur during the production phase, the impacts can affect the entire wheat value chain (Vasil, 2007; Juroszek and von Tiedemann, 2013). Keeping in view that the climate change and its related impact on pathogens are dynamic processes, immediate attention of researchers is solicited towards this issue. Examining the biotic stress tolerant wild wheat cultivars at sequence level, and comparing the information with the present WGS data of hexaploid wheat will help in identification of genes/QTLs for FHB resistance and also assist in understanding its role in biotic stress responses and eventually, it will aid in imparting biotic stress tolerance in wheat. Further, the recent release of a 4-gigabase physical map of *Ae. Tauschii* provides a roadmap for the mapping of genes that confers tolerance to diseases, heat and drought (Luo *et al.*, 2013). Strikingly, Jia *et al.* (2013) had reported a significantly higher number of R genes (Resistance genes) in *Ae. Tauschii*. These R genes play an important role in the defence mechanism (Muthamilarasan and Prasad, 2013b). Nucleotide-binding-site Leucine-rich-repeat (NBS-LRRs), a class of R genes were found to be two-fold higher (1219) than that reported in rice (623) and six folds than in maize (216) which could be positively correlated with the biotic stress tolerance of *Ae. Tauschii* (Jia *et al.*, 2013). Moreover, about 878 NBS-LRRs mapped onto the wheat chromosomes at specific positions by using molecular marker-genome sequence alignment provides a huge number of potential disease resistance loci for further investigation. Similarly in *T. urartu*, Ling *et al.* (2013) had reported a significantly higher number (593) of another class of R genes called NB-ARC (nucleotide-binding APAF-1, R proteins, and CED-4), which is more abundant than in *Brachypodium* (197), rice (460), maize (106) and sorghum (211).

In addition, genetic factors like orthologous markers and genes responsible for biotic stress tolerance from other diploid plant genomes showing gene-level conserved orthology with wheat genome can also be transferred to wheat through traditional and modern introgression breeding including ILs (Introgression Lines) and MAGIC (Multiparent Advanced Generation Inter-Cross). Though no reports has yet been available on the above-mentioned strategies in wheat, the release of WGS should encourage such projects leading towards the generation of disease-tolerant cultivars.

### Concluding Remarks and Future Prospects

Thus, the present article highlights the applications of wheat genome sequence in cereal genetics and breeding, including the investigation of genome variation (SNPs), genetic/association mapping, comparative genome mapping and identification of genes/QTLs for stress tolerance traits. The identified novel genes/QTLs and alleles (haplotypes) regulating stress tolerance can be eventually transferred to cultivated genetic background by marker-assisted genetic enhancement studies and transgenics. The three landmark genome sequence papers in wheat (Brenchley *et al.*, 2012; Ling *et al.*, 2013; Jia *et al.*, 2013) had also reported diverse classes of genes involved in energy harvesting, metabolism and growth that could be possibly associated with crop productivity which can now be exploited for the development of transgenic wheat capable of withstanding climate change. By 2050, the world population is projected to be 9 billion and realizing this massive burden on food security, researchers and breeders should take the moral responsibility to utilize the wheat whole genome sequence and thousands of SNPs information thus generated into a valid research.

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