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DEVELOPMENT OF SEQUENCING TECHNOLOGY AND ROLE OF NEXT GENERATION SEQUENCING TECHNOLOGIES IN WHEAT RESEARCH: A REVIEW

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Abstract

Sequencing is a fundamental component in the life science research. Sanger method known as first-generation sequencing technologies (FGSTs) was the first successful sequencing method introduced in 1977. Considering time and financial advantage, second-generation technology was introduced in 2005 which is also called as next-generation sequencing technologies (NGSTs). These technologies have significantly high throughput compared with FGSTs, and make break through revolution in the study of genomics and molecular biology. To overcome the mandatory sample amplification regarding the read length and the bias of the NGSTs, third-generation sequencing technologies (TGSTs) were introduced. Third-generation long-range sequencing and mapping approaches are making a renaissance in high-quality genome sequencing. The recent developments of the fourth generation sequencing methods hold great promises and expect to offer most important contribution in these key areas. Currently, NGSTs use most of the genomics field, third-, and fourth-generation approaches make a significant solution in the genomics era. Wheat is one the major crop species which is closely related with the development of agriculture and straighten of societies. The demands of wheat are increasing day by day which necessitate improvement of wheat genomics and functional genomics. Wheat yield has been accelerated by the advances of NGSTs that focused genome sequencing, genomic polymorphism, genes cloning and development of technical platforms. This review discussed about the development of sequencing technologies, wheat development through NGSTs and future outlook. This review is mostly targeted for the beginners (new students/ researchers) who have intended to work with NGSTs.

Key words: Sequencing technology; Next generation; Renaissance; RNA-Seq; Wheat

Introduction

The declaration of "central dogma" about genes mechanism by Crick (1958), help us to understand potentiality of the Omic technologies to know about the extreme complexity of simplest living cell. This approach allowed great number of data that made understanding of genotype–phenotype interaction after completion of Human Genome Project (HGP) in 2003. Before the genomics emergence, gene mapping methods needed likelihood statistics to find out locations and identifications of the genes. Omic technology was used mostly for genome sequencing and development in molecular genetics technologies. Being development of algorithms helped to investigate the great amount of data. Bioinformatics have also played an important role for high throughput DNA sequencing. Next generation sequencing technologies (NGSTs) were introduced in 2005 after completion of a human genome project. These technologies not only reduced time and costs of sequencing but also enhanced data generation capacity. Each of NGST follows different protocols for DNA template, short DNAs, image capturing, alignment, assembly, and variant detection. Each of the sequencing technologies has specific advantages and disadvantages. NGSTs are widely used in whole genome sequencing, exome sequencing, transcriptome, methylome, metagenomics, small RNA, de novo, ChIP (chromatin immune precipitation sequencing) and resequencing (Egan *et al.*, 2012; Ari & Arikan, 2016). The success of these NGSTs depends on development in nanofluidics, signal detection and progress in computational power tools (Mardis, 2013). NGSTs have been applied for transcriptome analysis which is known as RNA sequencing (RNA-Seq), has instantly revolutionized in the field. Transcriptome sequencing is practically applicable to any organism and allows transcript discovery.

Wheat is an important cereal crop, widely cultivated in the world for being a staple food. The world population is increasing day by day and it will be nine billion by 2050. Wheat is not only important cereal crop but also a model for study of an allopolyploid plant with a large, highly repetitive genome, hexaploid composition, and low regeneration following genetic transformation which lagged behind other cereal crops in the progress of genetic engineering and biotechnology. Wheat yield needs significant increase which can be assisted by advances of next-generation sequencing technologies. NGSTs will rapidly accelerate wheat genomics and wheat functional genomics.

In this review, we briefly discussed about development of NGS technologies with their advantages and disadvantages, role of NGSTs for wheat improvement, and future look, respectively.

Development of sequencing technologies: DNA sequencing technologies have made remarkable progress in the last thirty five years, and have generated large number of genomic data which have been used wide range of newly research areas and various applications. Here we discussed about the history of development of sequencing technologies with their advantages and disadvantages (Table 1), and various applications of NGSTs as listed (Table 2). We also briefly described about the sequencing technologies in below:

Table 1. Historical development and characteristics of sequencing technologies.

Generation	Platform	Maximum read length (bp)	Technology	Accuracy (%)	Maximum output per run	Advantages	Disadvantages	Main applications	Launched	References
FGS	Sanger ABI 3730 X l	1000	chain-termination	99.99	100 Kb	Long read lengths, high single-pass accuracy	Low throughput, high cost sample preparation	DNA-Seq	Since 1995	Ari and Arikan, 2016; Liu *et al.*, 2012
SGS	454 GS 20	400	Pyrosequencing	-	20 Mb	Long read length	Homopolymeric	DNA-Seq	2005	Margulies, 2005.
	FLX 454+	700	Sequencing by synthesis	99.9	700 Mb	Long read length	Homopolymer errors and high cost	Resequencing, RNA-Seq	2008	Ari and Arikan, 2016; Liu *et al.*, 2012
	454 GS-FLX Titanium	500	pyrosequencing	98.93	100 Mb	Long read length	Homopolymers and high error rate	DNA-Seq	2008	Gilles *et al.*, 2011
	GS Junior	400	Sequencing by synthesis	99	35 Mb	Long read length	Homopolymer errors and high cost	Resequencing , RNA-Seq	2009	Ari and Arikan, 2016; Liu *et al.*, 2012
	MiSeq	2X300	Sequencing by recersible termination	99.9	15 Gb	Long read length, high accuracy and low cost	GC bais and Low output	Resequencing, RNA-Seq, Smal RNA-Seq	2011	Ari and Arikan, 2016; Liu *et al.*, 2012
	NextSeq 500	2x250	Sequencing by reversible termination	99.9	120 Gb	High accuracy, low cost and high throughput	GC bais	Exome seq, RNA-Seq, Resequencing	2014	Ari and Arikan, 2016
	HiSeq	2 × 125	Sequencing by reversible termination	99.9	1000 Gbp	High throughput High accuracy	GC bias	Short reads, whole genome seq, Exome seq, RNA-Seq	2010	Ari and Arikan, 2016; Liu *et al.*, 2012
	SOLiD	2 × 35	Sequencing by ligation	99.85	3 Gbp	High accuracy, Low error rate, Flexibility and scalability	Short reads, high cost,	Resequencing	2007	Ari and Arikan, 2016; Liu *et al.*, 2012
	SoLiD4	2×50	Sequencing by ligation	99.85	100 Gbp	High accuracy and high throughput	Short reads	Resequencing	2007	Seifi *et al.*, 2017
	SOLiD 5500xl	2×85	Sequencing by ligation	99.99	30 Gbp	High accuracy and high throughput	Short reads	Resequencing	2010	Seifi *et al.*,2017
TGS	Heli Scope	35	Single molecule fluorescent sequencing	97	35 Gbp	No amplification bias	High error rate Short reads	RNA-Seq, DNA-Seq	2010	Ari and Arikan, 2016
	PacBio RS II	10,000	Single molecule real-time sequencing	95	400 Mb	No amplification bias	High error rate	De novo seq	2011	Ari and Arikan, 2016
	Ion PGM	400	Semiconducter sequencing	99	2 Gb	Short run time	Homopolymer errors	Resequencing, RNA-Seq	2010	Rothberg *et al.*, 2011
	Ion Proton	200	Semiconducter sequencing	99	10 Gb	Short run time	Homopolymer errors	Exome seq, RNA-Seq, Resequencing	2012	Merriman *et al.*, 2012
	MinION	6000	Nanopore sequencing	98	500 Mb	No amplification bias and long read length	High error rate	DNA-Seq, RNA-Seq	2014	Mikheyev and Tin, 2014; Laver *et al.*, 2015
FGS	SOLiD	2 × 50	In situ sequencing	99.9	320 Gb	High accuracy	Short reads	RNA-Seq	2014	Mignardi and Nilsson, 2014

Note: FGT first-generation sequencing, SGS second-generation sequencing, TGS third-generation sequencing, FGS fourth-generation sequencing, bp base pair, Mb megabase, Kb kilobase, Gbgigabase

Table 2. Applications of next-generation sequencing technologies adapted from Mutz *et al.*, 2013.

Classification	Applications	Sequencing principle[a]	NGS technology	Basis for sequencing	Reference
Genome	de novo genome sequencing	Pyrosequencing	454	DNA	Margulies *et al.*, 2005.
	Whole-genome or targeted resequencing, detection of SNPs, indels, CNVs	Pyrosequencing	454	DNA	Wheeler *et al.*, 2008
Transcriptome	Gene expression profiling, SNP, alternative splicing	Sequencing-by-Ligation	SoLiD	RNA	Cloonan *et al.*,2008
	SNP discovery	Pyrosequencing	454	RNA	Barbazuk *et al.*,2007
	Mapping and quantification of transcriptomes	RNA-Seq	Genome analyzer	RNA	Mortazavi *et al.*, 2008
Epigenome	DNA methylation patterns	MeDiP-Seq	Genome analyzer	DNA	Li *et al.*,2010
	Histone modification	ChiP-Seq	Genome analyzer	DNA	Barbazuk *et al.*, 2007
Regulome	Nucleosome positioning	Sequencing-by-ligation	SOLiD	DNA	Valouev *et al.*,2008
	Transcription factor binding	ChiP-Seq	Genome analyzer	DNA	Jothi *et al.*, 2008
Metagenome	Microbial diversity	Sequencing-by-synthesis	Genome analyzer	DNA	Qin *et al.*, 2010
	Species classification	pyrosequencing	454	DNA	Nossa *et al.*, 2010
Diagnostics	Genetic diseases	Sequencing-by-ligation	SOLiD	DNA	Wheeler *et al.*, 2008
	Prenatal diagnostics	Sequencing-by-ligation	SOLiD	DNA	Chiu *et al.*,2010
	Cancer detection	Sequencing-by-ligation	SOLiD	RNA	Wu *et al.*,2011

Note: [a] Abbreviations: RNA-Seq (RNA sequencing), ChIP-Seq (chromatin immune precipitation sequencing), MeDIP-Seq (methylated DNA immune precipitation sequencing)

First generation: Sanger sequencing: After discovering DNA double helix shape by Watson and Crick (1953), scientists had to face various difficulties in DNA sequencing till introduction of the Sanger method (Sanger *et al.,* 1977). Short sequences were the main constraint for today's sequencing technologies. Some approaches were already improved to obtain short sequences though having limitation of finding them in chains (Sanger, 1988). To overcome difficulties of DNA Sequencing, a new method was introduced by Sanger & Coulson, known as "plus and minus method" (Sanger & Coulson, 1975).This approach assembled some developments to DNA sequencing. In mid 1980s, automated DNA sequencing system was starteded. These new techniques of DNA sequencing also made a significant progress in quality and data generation. ABI Prism 310 was the first commercial automated DNA Sequencer declared by PE Biosystems in 1996. After two years later in 1998, GE Healthcare MegaBACE 1000 and PE Biosystems ABI Prism 3700 entered the sequencing market as commercial platforms. Sanger sequencing gradually decreased total costs as new technologies and modifications has been done. The HGP were successfully completed through the Automated DNA Sequencer which decreased both time and cost of the project. Sanger sequencing (known as a first generation technology) allowed significant role in development of biological science and advanced the understanding about nucleic acids. This technology improved our knowledge about molecular and cellular mechanism. Limitations of these methods such as data production, speed, sequence quality, laborious and difficulties in answering about the application genomics were basic problem of the first generation technologies (FGSTs).Innovation of technology and concept about biological science, sequencing technology is new possibility in each branch of biological science to solve their numerous problems.

Second generation: Next Generation Sequencing Technologies: The Roche/454 FLX, the Illumina/ Solexa Genome Analyzer, the Applied Biosystem (ABI) Solid Analyzer and HeliScope sequencing platform of the NGSTs are commercially available in the market. New technologies ideally are low cost, high speed and time saving. The methodologies of NGSTs are discussed briefly below:

Roche GS-FLX 454 analyzer: The Roche GS-FLX 454 sequencer is known as the analyzer, was first developed method launched in 2005 and completed the second genome sequencing of an individual. This analyzer applies sequencing-by-synthesis (SBS) principle, recognized as pyrosequencing that is capable to identify of sequence variation rapidly and accurately. Emulsion PCR is the basic process in this platform. Firstly the 454 platform can produce 100 bp read length, but now it can generate 400 bp read length. The maximum capacity of 454 analyzer has~600 bp. Among the NGS platforms, 454 analyzer can produce longest short reads (600 bp). This platform can produce ~400–600 Mb reads/run and base accuracy of raw is over 99 % (Wheeler *et al.*, 2008).

Illumina/Solexa sequencer: Currently, Illumina/Solexa sequencer is the most widely used platform applies sequencing-by-synthesis principle on an eight channel flow cell to generate 10 million reads per flow cell with read length 100 bp. This sequencer is the most congenial and has a simple approach and has been applied in this sequencing platform. This platform is capable to produce super quality data and proper read lengths which made the platform a choice for many project. Illumina HiSeq 2000, a recent sequencer can produce 2 × 100 bp read length (pair-end reads) and generate about 200 Gb sequence per run, up to 25 Gb per day, two billion paired-end reads per run. The accuracy of the raw bases is greater than 99.5%. Illumina has already developed several systems such as the HiSeq 2500, HiSeq 1500, HiSeq 2000, HiSeq 3000, or HiSeq 4000 (Ari & Arikan, 2016).

ABI SOLiD platform: The ABI SOLiD analyzer is a platform of next-generation sequencing technology developed by Life technology (now Thermo fisher) and introduced in 2006. It uses unique sequencing-by-ligation principle and applies an emulsion PCR approach with small magnetic beads to amplify DNA fragments for sequencing. A 5500 Solid analyzer produce short reads up to 90 Gb per run, 2 x 60 bp reads, up to 10-15 Gb per day at 1.4 billion paired-end reads (700 million beads)/run. The 5500×l is latest model as the solid system, produce up to 180 Gb per run, 2 x 60 bp reads, up to 20-30 Gb per day at 2.8 billion paired-end reads (1.4 billion beads)/run and base accuracy is 99.94% (Liu *et al.*, 2012; Ari & Arikan, 2016).

Heli scope sequencing platform: Heli Scope platform is the first method to use the principle of single molecule fluorescent sequencing that uses high sensitive fluorescence detection system to directly detect each nucleotide. It is also known as Single-Molecule Real Time (SMRT) DNA sequencing. Library preparation is known as a crucial drawback for NGSTs. It eliminates difficulties for library preparation. Heli Scope sequencer can generate 35 Gb data per run, includes 35 bp short reads with base accuracy 99%. This platform decreased higher error rates but repetitive sequencing increased cost per application. The main disadvantage of this platform is shot reads. Though, it has some benefits to use as a single-molecule DNA sequencing technology but Heli Scope platform could not be much utilized for sequencing and was produced no longer (Ari & Arikan, 2016).

Third generation: Next Generation Sequencing Technologies: The demands of low-cost sequencing platforms are increasing day by day. The third generation sequencing platform is differentiated by new chemistry, less operation time, desktop design, and lower operation cost. We discussed briefly about the technologies in below:

SMRT sequencing: Single molecule real time sequencing (SMRT) was launched by Pacific Biosciences (PacBio) in 2009 as a third generation sequencing technologies (Eid *et al*., 2009). SMRT sequencing applies sequencing by synthesis approach and real-time detection. In this method, there is no need of library preparation step until single DNA molecules are available (Schadt *et al.*, 2010). SMRT technology differs in many features with some NGSTs. It can generate considerably longer and highly accurate DNA sequences of an individual. PacBio RS II is the latest technology of Pacific Bioscience which makes over 14,000 bp read length and 400 Mb per run. Base modification and RNA-based research are the main benefit of this technology. It also allows of assemble method of de novo genomes sequencing. As a result, SMRT having some benefit to its features which make it a substitute to NGST (Schadt *et al.*, 2010).

Semiconductor sequencing: Toumazou and his colleagues developed semiconductor sequencing platform in 2006 which was based on sequencing by synthesis approach. This platform is time saving technologies which directly compute pH changes in the microenvironment, and save time by using a special camera through removing the time-consuming imaging step. This technology also consists of amplification step before sequencing. It is known as third-generation sequencing technology due to its exceptional and new sequencing protocol (Ari & Arikan, 2016). This sequencer follows similar approach as like as FLX 454 system. The error rate of semiconductor sequencing methods is more or less 1 % and average read length is 400 bp. Ion Torrent systems Inc. (Life Technology) launched Ion PGM and Ion Torrent Proton in 2010 and 2012, respectively, and average read length were 400 bp and 200 bp, respectively. Both of them uses semiconductor sequencing technology (Rothberg *et al.*, 2011).

Nanopore sequencing: Nanopore sequencing is known as a third generation sequencing technology which uses the sequencing of biopolymer particularly, polynucleotide in the form of DNA or RNA (Niedringhaus *et al.*, 2011). This technology can sequence a single molecule of DNA or RNA without PCR amplification or any chemical labeling. This sequencing technology provides us the potential offer to comparatively low cost and time. This technology is used for identification of viral pathogen (Greninger *et al.*, 2015), monitoring ebola (Nick Loman, 2015), human genome sequencing, plant genome sequencing, environmental monitoring, food safety monitoring, monitoring of antibiotic resistance (Cao *et al.*, 2016), haplotyping (Ammar *et al.*, 2015) and other applications. Oxford Nanopore Technologies are electronics-based DNA/RNA sequencing technology, is being used in more than 80 countries, for a range of biological research applications including human genomics, cancer, microbiology, plant science and environmental research. Minion was first product and introduced in 2014 and made commercially available in 2015. The scaled-up GridION was commercially launched in 2017 and PromethION in 2018. Flongle is scheduled to release in 2018. SmidgIon, a mobile-phone-compatible, low cost, portable sample preparation Ubikis underdeveloped, and can be used by any user and anywhere.

Fourth generation sequencing technology: In situ sequencing (ISS) is known as the fourth generation sequencing technology, hold the great opportunities to perform trnascriptomics by sequencing nucleic acid directly in cell and tissue (Ke *et al.*, 2013; Lee *et al.*, 2014). This technology follows the previously described NGSTs chemistries and allows detection even of single-nucleotide. This technology would become a standard method for the sequencing of tissue but needs further development of the technology to overcome the obstacles (Crosetto *et al.*, 2015).

New sequencing and assembling technologies: BioNano genome mapping and linked reads sequencing-10×Genomics were developed for new sequencing and assembling. These two sequencing technologies are being used for genome sequencing and high quality assemblies (Moll *et al.*, 2017; Chen *et al.*, 2017; Coombe *et al.*, 2017; Rasekh *et al.*, 2017). Moreover, an approach known as Hi-C is able to find out three-dimensional of architecture of chromosome, provided genome assemble and scaffold order on chromosomes (Lieberman-Aiden *et al.*, 2009). In China, the combined new technologies have been applied to generate high quality assemblies of *Triticum urartu* using BAC-by-BAC strategy combined with the SMRT sequencing technology and BioNano genome mapping and linked reads sequencing-10x Genomics technologies. This combined technology completed the genome sequencing which enhanced the scaffold length and accuracy as evaluate to the first version of the A genome sequence. The earlier determined a genome size was 4.94 Gb, allowed 98.4% assemblies of the *Triticum urartu* genome by the combined new technology (Shi & Ling, 2018).

As mentioned above, the DenovoMAGIC2 assembler was developed by the NRGene (NesZiona, Isreal) company for huge, repetitive and complicated genome such as wheat. Illumina short reads able to enlarge N50 up to several Mb scaffolds (Avni *et al.*, 2017). Wheat cultivar 9204 was sequenced and assembled by using this software. Prof. Jizeng Jia and his colleagues of CAAS produced simultaneously high qualities and large amount of assemblies comprising scaffold with N50 size of 14.1 Mb in *Aegilops tauschii* genome (Zhao *et al.*, 2017; Shi & Ling, 2018).

Wheat development through NGSTs: Common wheat (*Triticum aestivum* L.) is the most important cereal crop, used by more than 30 % people in the world (IWGSC, 2014). Wheat annual production is more than 620 million metric tons. The largest wheat producer and consumer is China where produced 100 million tons of annual wheat (Ling, 2016). To meet the food demand of the peoples, wheat production needs to be continuously increased. Next-generation sequencing (NSG) technologies have made enormous progressed in wheat genomics and functional genomics. Wheat is a huge complex, repetitive, polyploid genome and large genome about to 17.0 Gband was unassailable in the past. Due to progress of NGST platforms, is now catching up wheat. In China, the National High-tech R&D Program (863 Program) was introduced the wheat functional genomics program in the era of NGSTs in 2005. Wheat researcher made multitudinous development and progress in wheat functional genomics. Number of main successes getting in wheat genomics and functional genomics is discussed briefly here:

Wheat genome sequencing: In 2005, the International Wheat Genome Sequencing Consortium (IWGSC) initiated with a group of scientist and breeders to overcome the wheat genome complexities to simplify for wheat molecular breeding. Currently, IWGSC projects were selected for all chromosomes of Chinese Spring and built their physical maps (http://www.wheatgenome.org/Projects/IWGSC-Bread-Wheat-Projects), have made available sequence of many chromosomes such as 1AS, 1BS, 3DS, 5DS, 7DS, 1AL, 1BL, 4A, 5A, 6A, 6B, and 7B (Holusova *et al.*, 2017; Shi and Ling, 2018). In 2012, Hall and his colleagues also made sequencing of Chinese Spring wheat using whole genome shotgun (WGS) approach with 454 pyro-sequencing (Synthesis by sequencing principle) methods, and coverage expanded five-fold of Chinese Spring genome sequence. They produced 5.42 Gb assemblies, expected 94,000 to 96,000 genes, and allocated two-thirds of genes to the wheat subgenomes (A, B, and D).Whole genome shotgun was less cost and time effective as compared with clone to clone approaches. In 2017, Clavijo *et al.*, (2017) used mate-pair libraries and an optimized algorithm to develop an improved Chinese Spring wheat genome sequence. They created a new assembly which represented more than 78% of the genome, much higher than the scaffold proportion (~49%) produced by IWGSC previously. It was a more accurate assembly of wheat genome (Zimin *et al.*, 2017). The final Chinese Spring wheat genome sequencing was done with a combination of next-generation (short reads, Illumina) and third-generation (long reads, Pacific Bioscience) approaches. This genome size was greater than 15 Gb representing assembly more than 90% of the Chinese Spring wheat genome was performed by mixing two set of sequences assembled using the MaSuRCA (Zimin *et al.*, 2013) and FALCON (Chin *et al.*, 2016) assemblers. It will be most completed genome of Chinese Spring wheat and will be published very soon (Shi & Ling, 2018). However, recently, IWGSC declared the genome sequence of Chinese Spring wheat (IWGSC v1.0) and open for publicly accessed (http://www.wheatgenome. org/News/Latest-news/RefSeq-v1.0-URGI). In July 2017, the tetraploid wheat wild emmer was sequenced besides of Chinese spring wheat and chromosome arms (Avni *et al.*, 2017). DenovoMAGIC2 (NRGene, NesZiona), a software package was used to assembly of the Illumina short reads, is capable to complete difficult assemblies within days. Wild emmer 10 Gb genome sequence was obtained by using whole genome shotgun sequencing. The assembly of the wild emmer should be further validated by genetic data and three-dimensional architecture of chromosome (Hi-C) data (Shi & Ling, 2018). The wild emmer genome sequence will help us for development of common wheat.

Wheat genome editing for transformation: Genome editing is a key tool for crop improvement (Khurshid *et al.*, 2018; Shinwari *et al.*, 2018). In addition, it is a new discovery and promising tools for transformation in wheat biotechnology. Common wheat is a major food crop for human beingswhich is very large, complex, repetitive, hexaploid genome and low generation in genetic transformation. Wheat transformation made this crop lagged behind to other cereal crops. Transgenic wheat is not still commercialized in the market where traditional breeding is costly and time consuming (Wang *et al.*, 2018). Several approaches were used for wheat transformation previously such biolistic particle bombardment and Agrobacterium species. But both of the approaches were low transformation efficiency. Japanese scientists, Japan Tobacco Company, (Ishida *et al.*, 2014) used recently a new approach known as Pure Wheat, which made distinguished improvement to wheat transformation. This technology permits genome editing technology for application and development to common wheat. Wang *et al.*, (2018) reported that genome editing might be great ascension for the progress in wheat transformation and a breakthrough in genetic engineering of wheat. It will be also helpful to other platforms such as exon sequenced TILLING libraries to identify the important agronomic traits in future. It is also speculated that Genome editing technology will be enabled to modify more genes through wheat genetic engineering and also provided transgenic-free wheat varieties for commercialization.

Understanding molecular mechanism of wheat response to abiotic stresses: NGSTs have made major breakthrough improvements to sequence and dissect the plant genomes including bread wheat and its progenitors and also revealed their differential expression patterns during development stages and influence to stress conditions (Budak *et al.*, 2014). The improvement of next-generation sequencing technologies (NGSTs) have made progress in the discovery and functional characterization of microRNA (miRNA). It has been identified to play essential roles in various stresses conditions in wheat (Alptekin *et al.*, 2016) including abiotic stresses such as salt (Lu *et al.*, 2011: Pandey *et al.*, 2014), drought (Pandey *et al.*, 2014:Akpinar *et al.*, 2015), dehydration (Ma *et al.*, 2015), phosphorus (Zhao *et al.*, 2013), heat (Xin *et al.*, 2010). Ni *et al.*, (2015) reviewed the mechanism of the heat tolerance and related genetic improvement of wheat. Heat-tolerance QTLs were identified on different chromosomes and using genome-wide analysis to find out heat responsive genes/proteins in wheat. Salinity is increasing day by day due to climate change and expanding saline land all over the world (Narusaka *et al.*, 2003; Jan *et al.*, 2016; Jan *et al.*, 2017). Studies about molecular mechanism of wheat salt tolerance and breeding will be able to develop wheat salt tolerant varieties for the use of saline prone areas. Wang & Xia (2018) reviewed salt tolerance physiological process and associated genes and reported that high-affinity potassium transporter (HKT) genes enhanced salt tolerance in wheat. They made a link with reactive oxygen species (ROS) homeostasis and salt tolerance in their introgression line of wheat.

Understanding mechanisms of wheat underlying disease resistance: Fusarium head blight (FHB) is known as scab, caused by *Fusarium graminearum*, is one of the most devastating fungal diseases of wheat hampering to wheat production in China and all over the world. The NGS technology was used to discover the responsive genes, pathways and QTLs to FHB for resistance breeding by using high-throughput RNA-Seq in wheat (Xiao *et al.*, 2013). NGSTs have increased the traceability of the expression and co-expression of genes regulating FHB with the coupled advanced of the tools of gene expression analysis such as transcriptome, proteome and metabolomics approaches (Dweba *et al.*, 2017). Dr. Zhengqiang Ma's laboratory at Nanjing Agricultural University worked for twenty years to understand the mechanism of FHB and resistance to FHB in Wangshuibai wheat variety. They progressed in resistant QTLs identification, discover candidate genes and resistant FHB varieties, and also cloned one of a FHB resistance gene (Rawat *et al.*, 2016). The molecular mechanism of FHB disease is still argumentative and need further research to entirely understand (Jia *et al.*, 2018).

Biotrophic pathogens in wheat: Wheat is a major cereal crop whose production is hampered by fungal diseases. Two groups of biotrophic fungi belonging to Basidiomycetes and Ascomycetes cause rust and powdery mildew disease in wheat, respectively. The major rust pathogen disease such as stripe rust caused by *Puccinia striiformis* f. sp. tritici (Pst), stem rust caused by *Puccinia graminis* f. sp. tritici (Pgt), and leaf rust caused by *Puccinia triticina* (Pt), and powdery mildew caused by *Blumeria graminis* f. sp. tritici (Bgt) in wheat. In China as well as the world, the rust and powdery mildew diseases of wheat are major biotic limitations for the production of wheat. Genomic sequences of wheat biotrophic fungi Pst, Pgt, Pt, and Bgt were made available through the high throughput next-generation technology and also have made progressed in cloning of avirulence gene, discovery of pathogen effectors, and pathogenomics. Professor Zhengshen and his team at Northwest Agriculture and Forestry University have made great contributions to develop pathogenomics studies to wheat biotrophic fungal disease improvement in the world (Tang *et al.*, 2018). The development of wheat biotrophic pathogenomics will accelerate in wheat resistant breeding and help to control of the cereal rusts and powdery mildew with sustainable manners.

Wheat grain qualities development: Wheat grain quality (WGQ) is most important condition to accept by the consumer and added values to wheat cultivars. Wheat genomic analysis and genome editing might be future effort to develop wheat grain quality (WGQ) traits. In China, scientists have made enormous effort to develop the wheat grain quality and their results on end-use properties. Genomic analysis will help to find out the genes as related to WGQ and genome editing

allows preciously transformations for wheat breeding (Zong *et al.*, 2017). These will help to improve functional genes into appropriate varietal background. This will make elite cultivars with good adaptability, high yield potential and desirable WGQ traits (Wang *et al.*, 2018). The combination effort of the scientist will be achieved with the rich genetic resources. China has made considerable progress in WGQ research due to having available genomics information and genome engineering tools.

Development of wheat SNP microarrays: Wheat genome size is very large and complex is still working with this genome very costly and tough in data processing though decreasing NGSTs price. Thus, SNP discovery was very important factor for wheat researcher to achieve genome-wide knowledge for different cultivars of wheat. Several powerful tools for SNPs discovery have been developed which reveal genomic diversity. SNP discovery will be helpful to identify genetic variation between individuals and marker-traits association mapping. Firstly, wheat SNP microarray was constructed using nine cultivars and 3000 world-wide cultivars to develop improved landraces and common wheat (Allen *et al.*, 2013). The 9K I Select SNP chip was used for genotypic and phenotypic characterization of 262 accessions of Chinese wheat. Total 2420 SNPs from A genome chromosome and 2396 SNPs from B genome chromosome were found. Secondly, high density (90K) wheat SNP chip was built from 19 bread wheat from different origins (Wang *et al.*, 2014).This chip produces huge amount of SNPs allocated to common wheat genome. Presently, 52,607 markers are developed and mapped (Wen *et al.*, 2017). Recently, 660K chip contained about 630,517 SNPs were generated by 192 common wheat genres. This chip was included 60worldwide modern wheat cultivars, 72 wheat landraces, 30 wild emmer accessions, and 30 *Ae. tauschii* accessions (Cui *et al.*, 2017). SNPs of Chinese spring wheat were available (https://urgi.versailles.inra.fr/download/iwgsc/IWGSCWGASequences/). 90K and 820 K array were used to construct high quality genetic map of wheat (unpublished).These arrays are unique which have more diversity from wheat donor accessions and wheat cultivar. Increasing numbers of SNPs have been discovered in wheat with the development of new sequencing technologies. Accurate and reliable methods have been developed to perform high-throughput genotyping based on SNPs.

RNA-Seq in wheat development: Researchers are working on numerous ways to find out new methods and applications of NGST in plant science. The next-generation sequencing technologies (NGSTs) have also been successfully applied for research and development in wheat and its closely related species for several years. Next-generation transcriptome analyses have been applied to understand the biological basis of agronomic characteristics in other plant species. Therefore, it is expected that the application of these technologies in wheat can accelerate wheat crop improvement. Some extensive applications and studies about transcriptome sequencing have been listed in last 5 years since 2013 to 2018 (Table 3). Among them, more than half (53%) studies were conducted from China for improvement of wheat. We understeand that China is the most dominant country using RNA-Seq for wheat improvement. Different RNA-Seq platforms have been used for the wheat transcripts analysis. Among RNA-Seq, Illumina (63%) were the most applied platform using for improvement of wheat. Cuticular waxes are also very important components for the wheat establishment. RNA-Seq might be used to identify cuticular waxes genes for further research. The molecular mechanisms of wax biosynthesis and export are still unknown.

Future outlook: The global NGSTs market size is increasing day by day, was valued at USD 4.62 billion in 2015. It will be expected to significantly progress over the next decades. More capable and fast genomic sequencing technologies shall be expected to further make adoption of NGS platforms. Automation in the pre-sequencing protocols is expected to make improvement in the years to come. Development of NGSTs for the personalized medicine by medical analyses at genetic level is expected to enhance demand for NGSTs over the forecast period. Moreover, the researchers and drug developers are raising interest about the NGSTs to achieve knowledge into the genetic level of a large number of organisms. These will raise demands to the NGSTs through to 2025.

Conclusion

Several platforms have been developed for sequencing within very short time, and new approaches are continuously being developed to generate long reads and more reads per run. NGSTs are created to appear as the dominant genomics technology due to their much-recovered cost-effectiveness, assessed by others sequencing methods and their many different utilizes. Several computer tools and software have improved to analysis the data from NGS technologies. NGSTs have made significant progress in wheat genomics and functional genomics including large-scale genomic resources, transcripts and sequence data, molecular markers, genetic and physical maps, cloning of genes on agronomic importance and development of technical platforms. NGS technologies are particularly used to accelerate for the development of wheat. But still have a research gap about cuticular wax studies. Cuticular waxes are very important element for crop establishment specially wheat. Using NGSTs and bioinformatics combined will provide the drive ways for wheat functional genomics. This is the time for genomics-assisted wheat breeding.

Table 3. A comprehensive list of publications using transcriptome sequencing approaches to study for wheat research and development.

Research target	Country	Sequencing platform	Conclusions	References
Separating homeologs by phasing (*T. urartu cv.*G1812)	USA	llumina HiSeq2000	98.7% of SNPs analyzed are separated correctly by phasing	Krasileva *et al.*, 2013
Sequencing and composition Chromosome 6B (*cv.* dDt6B)	Japan	Roche 454 GS-FLX	4798 non-repetitive gene loci were identified	Tanaka *et al.*, 2013
The discovery of phosphate starvation-responsive genes (*cv.* Chinese Spring)	Japan	Illumina HiSeq 2000	892–2,833 responsive transcripts in roots and shoots were identified	Oono *et al*., 2013.
Stripe Rust pathogenicity identified (*cv.* Morocco)	Aus	Roche 454	400 genes encoding secreted proteins were identified	Garnica *et al*., 2013
Gene Expression during Photomorphogenesis (*cv.* DV92;G3116)	USA	Illumina HiSeq 2000	500,000 SNPs and, 22,000 SSRs were identified	Fox *et al.*, 2014
Chromosome 5D reveals lineage-specific translocations (cv. Chinese Spring)	Turkey	Roche 454 GS-FLX	518 markers were identified at chromosome 5D	Lucas *et al.* 2014
Transcriptome analysis (*cv.* Nongda211)	China	Roche/454	7355 DEGs were upregulated in the grain library	Wei *et al.*, 2014
Genome interplay in the grain (*cv.* Chinese Spring)	German	Illumina HiSeq. 2000	Observed no global but cell type–and stage-dependent genome dominance	Pfeifer *et al*., 2014
Cold-responsive genes in young spikes (*cv.* Jimai22)	China	Illumina HiSeq 2000	526 up-regulated and 489 down-regulated genes were identified	Zhang *et al.*, 2014
Comparative Transcriptome Analysis (*cv.* Neimai 8; line II469)	China	Illumina HiSeq2000	Total of 1300 DEGs were identified	Zhang *et al.*, 2014
Genome-wide marker development (cv. *Ae. tauschii*)	Japan	Roche 454 GS-FLX	13,347 high-confidence SNPs were discovered	Iehisa *et al.*, 2014.
Genes contributing to heat and drought (*cv.* TAM107)	China	Illumina HiSeq2000	1,328 DEGs were responsive to stress responsive	Liu *et al*., 2015
Structural and functional organization (*cv.* Chinese Spring)	France	Illumina HiSeq2000	8,800 transcription sites are identified	Pingault *et al.*, 2015
Mutation Scanning by Exon Capture (*cv.* Cadenza)	UK	Illumina GAII	464 high-confidence SNPs were detected	King *et al.*, 2015
Male sterility induced by the chemical hybridizing agent (*cv.* Xinong 1376)	China	Illumina HiSeq 2500	1,088 unigenes were significantly differentially expressed	Zhu *et al.*, 2015
Identify available gene resources (*cv. Agropyron cristatum*)	China	Illumina GAII	73,664 unigenes were identified	Zhang *et al.*, 2015
Common wheat genome annotation and grain transcriptome research (cv. Xiaoyan 81)	China	Illumina HiSeq 2000	Identification of 6030 genes differentially regulated during caryopsis development	Dong *et al.*,2015
Genes related to resistance against powdery mildew (*cv.* SN6306 and YN15)	China	Illumina	39 unigenes were identified and validated for Bgt resistance	Li *et al*., 2016
Transcriptome analysis (*cv.* Yunong 201 and Yunong 3114)	China	Roche 454	1363 DEGs were identified	Zhang *et al.*, 2016
Transcriptome asymmetry in synthetic and natural (*cv.* AT2, TD, TTR13 and ETW)	China	Illumina Hiseq 2000	9 304 SNPs representing 9409 genes were recognized	Wang *et al.*, 2016
Comparative temporal transcriptome Profiling (*cv.* T756 and WL711)	India	Illumina HiSeq2000	Total of 3020 transcripts were differentially expressed	Yadav *et al.*, 2016
Construction as well as characterization (*cv.* Dwarf polish)	China	Illumina Hiseq 2000	5,531 SSR sequences were observed from 4,531 unigenes	Wang *et al.*, 2016
Identification of vernalization responsive genes (*cv.* Jing 841)	China	Illumina HiSeq 2000	636 DEGs were identified as vernalization responsive genes	Feng *et al.*, 2016
Analysis of Purple Pericarps (*cv.*Opata and Gaoyuan 115)	China	Illumina HiSeq 2000	23,642 DEGs were identified in the purple and white pericarps	Liu *et al*., 2016
Single Nucleotide Variants identified (*cv.* Yunong 201)	China	Illumina Hiseq 2000	4021 genes with SNVs were obtained	Chen F. *et al.*, 2016
Identification of salt-induced differential genes (*cv.* Punong 365*)*	China	Illumina/ Solexa	Five differential genes were used	Ma *et al*., 2016
Microspore embryogenesis induction (*cv.* Svilena)	German	Illumina Hiseq 2000	20,224 putative transcripts were identified	Seifert *et al.*, 2016
Elevated CO_2 in comparison with ambient CO_2 (*cv.* Norin10)	China	Illumina HiSeq 2000	The gene expression of Norin 10 was regulated in response to elevated CO_2	Yue-bing *et al*., 2016
Transcriptome Analysis for Abnormal Spike Development	China	Illumina HiSeq 2000	Identified 419 genes highly expressed in spikes	Zhu *et al.*, 2016
Regulators of Wheat Spike Architecture (*cv.*90 cultivers)	China	Illumina HiSeq 2500	1538 genes were significantly correlated	Wang *et al.*, 2017
SNP discovery (*cv.*HD2329)	India	SOLiD3	83 SNPs were validated by Kompetitive Allele Specific PCR	Chandra *et al.*, 2017
Long-term salinity stress tolerance (*cv.* Kharchia)	India	Illumina HiSeq 2500	Fourteen differentially expressed genes were identified	Mahajan *et al.*, 2017
Lr28 mediated leaf rust resistance (*cv.* S:HD2329, and R:HD2329 + Lr28)	Aus	SOLiD sequencing	Validated 28 key genes using qRT-PCR	Singh *et al.*, 2017
Unintended effects in transgenic wheat (*cv.* D3)	China	Illumina HiSeq 2500	Seven genes were differentially expressed in D3	Jiang *et al*., 2017
Comparative Transcriptome Analyses (*cv.* Chancellor; NIL L031)	China	Illumina HiSeq 2000	1028 DEGs dominant and 2214 DEGs recessive were identified	Xing *et al.*, 2017
The transcriptome of the developing grain (35 genotypes)	Aus	Illumina	26,477 transcripts were common	Rangan *et al*., 2017
Transcriptome of wheat inflorescence development (*cv.* Chinese Spring)	China	Illumina HiSeq 2000	Identified 19,060 DEGs	Feng *et al.*,2017
Powdery Mildew Resistance Gene *Pm4b* by Combining SNP Discovery from Transcriptome Sequencing Data (*cv.* Bainong 3217)	China	Illumina HiSeq 4000	Identified 283,866 raw single nucleotide polymorphisms (SNPs)	Wu *et al*., 2018
Transcriptome analysis of wheat seedling and spike tissues (*cv.* Jingmai 8)	China	Illumina HiSeq 2000	40,938 SNPs were obtained in the genes expressed in hybrid JM8	Liu *et al*., 2018
Transcriptome responses in wheat roots to colonization (*cv.* Chinese spring)	China	Illumina HiSeq PE150	11,746 DEGs were identified	Li *et al*., 2018

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