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Review

Wheat omics: Classical breeding to new breeding technologies

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ABSTRACT

Wheat is an important cereal crop, and its significance is more due to compete for dietary products in the world. Many constraints facing by the wheat crop due to environmental hazardous, biotic, abiotic stress and heavy matters factors, as a result, decrease the yield. Understanding the molecular mechanism related to these factors is significant to figure out genes regulate under specific conditions. Classical breeding using hybridization has been used to increase the yield but not prospered at the desired level. With the development of newly emerging technologies in biological sciences i.e., marker assisted breeding (MAB), QTLs mapping, mutation breeding, proteomics, metabolomics, next-generation sequencing (NGS), RNA_sequencing, transcriptomics, differential expression genes (DEGs), computational resources and genome editing techniques i.e. (CRISPR cas9; Cas13) advances in the field of omics. Application of new breeding technologies develops huge data; considerable development is needed in bioinformatics science to interpret the data. However, combined omics application to address physiological questions linked with genetics is still a challenge. Moreover, viroid discovery opens the new direction for research, economics, and target specification. Comparative genomics important to figure gene of interest processes are further discussed about considering the identification of genes, genomic loci, and biochemical pathways linked with stress resilience in wheat. Furthermore, this review extensively discussed the omics approaches and their effective use. Integrated plant omics technologies have been used viroid genomes associated with CRISPR and CRISPR-associated Cas13a proteins system used for engineering of viroid interference along with high-performance multidimensional phenotyping as a significant limiting factor for increasing stress resistance in wheat.

Contents

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1. Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop feeding one-fifth of the world (FAO, 2011). It is also predicted that wheat production should be doubled to fulfill the demand for increasing population by 2050 (Foresight, 2011). With increasing population and wheat demand, the cultivable land is decreasing; it is also important to increase the production of wheat per unit area (Araus et al., 2003). Wheat crop generally grows in the temperate regions with 12–25 °C and having 250–1750 mm annual precipitation. The average production of all crops, including wheat is affected by the abiotic stresses (Barlow et al., 2015). About 50% of the total land covered by wheat cultivation is affected by periodic drought (Pfeiffer et al., 2005). Lesk et al. (2016) stated that statistical model results predicted that a 10% yield was reduced due to drought or extreme weather conditions. However, the condition should be severe as climate change was persistent, which results in precipitation decrease as well as evaporation driven by global warming (Dai, 2011). Abiotic stresses significantly reduce wheat productivity due to drought (Anwaar et al., 2019), salinity stress, and heavy metals (Shah et al., 2018). To resolve the abiotic stress issue, local wild genotypes should be modified from classical breeding to more advanced molecular applications and getting desired genes expressed in a specific situation (Grainger & Rajcan, 2014). In addition, the multi-selection experiment has been commonly used for the direct selection of abiotic stress-sensitive genotypes and for the direct selection of resistant varieties strongly affected by environmental conditions and low heritability (Manavalan et al., 2009). However, the direct selection is discreetly laborious and time-consuming. The genetic diversity among different wheat cultivars based on yield leads to the tolerant cultivar production but still a gap between the effort to determine the molecular markers (Xu et al., 2012).

The molecular markers development based on the latest wheat genome sequenced under specific abiotic stress conditions could serve identifying and screening genotypes based on salinity and tolerance to drought (Tomar et al., 2014). Complete genome sequencing played a significant role in markers development and characterized the genes related to abiotic stress conditions in marker-assisted breeding (Song et al., 2010). The presence of high-density molecular markers helps to recognized different allelic combinations linked with agronomic traits as well as haplotype analysis (Tardivel et al., 2014). However, MAB has been proficient for simple traits that controlled single or multiple loci (Shi et al., 2009; Jun et al., 2012). Moreover, MAB also suffers due to unwanted genetic strains (Shi et al., 2009). The phenotypic expression of the newly discovered genes can be regulated by parent genetic makeup due to epistatic interaction (Palloix et al., 2009). For multiple complex traits, epistatic interaction is impulsive. For this purpose, solid evidence is necessary to understand the molecular mechanism for the development of new traits. The new advances in genomics development will help to determine the genetic diversity, trait developments, genotype, and environmental interaction. The application of genome sequencing, RNA sequencing and DNA sequencing (resequencing) offers significant insights into the creation and enhancement of wheat programs. Next-generation sequencing (NGS) and molecular techniques, i.e., phenotyping, genotyping, markers development, quantitative trait loci (QTLs), genetic linkage map, single nucleotide polymorphism (SNP) identification, expressed sequence tags (ESTs), transcriptional factors (TFs) identification and current breeding technology have made possible for sustainable and safe genotypes development under abiotic stress condition (Afzal et al., 2020).

Moreover, RNA sequencing and transcriptome analysis would also provide substantial perceptions for wheat development. Genome editing is an advanced technique used to determine the desired genes and inserted in the host plant, favorable for biotic and abiotic stresses genes identification (Sedeek et al., 2019). However, novel approaches are still needed for further improvement in crop plants. The new advancement in genome editing has opened a new avenue for research using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 (CRISPR-associated protein9)-based technique less expensive but quick and specific results to edit genomes at multiplex level (Cai & Yang, 2014). A Combination of all approaches should provide deep insight and important for the progress of the model plant, help to improve better yield in contrary climatic conditions. The review largely emphasis to enlighten the new achievements using different omics techniques, future outcomes for the enhancement of wheat development, and improvement under abiotic stresses tolerant genotypes.

1.1. Conventional breeding

Wheat genome has diploid, tetraploid, and hexaploid species, but currently, wheat varieties are mostly hexaploid called *Triticum aestivum* L., also known as bread wheat. Bread wheat is an allopolyploid of three gnomes A, B, and D (Schulman et al., 2004). For the wheat genome, the critical challenge is linkage mapping and characterization of genes for important traits, which is compulsory for the wheat enhancement program. The application of genome sequencing techniques credited to discover the polyploidy level, a large number of repetitive DNA, genome size, and low genetic diversity due to hexaploid wheat. On the other hand, the wheat genome has an advantage over other cereals, which is litheness for the application of cytogenetic and aneuploid stock. Moreover, these genetic resource materials are significant to discover essential yield-and disease-resistance genes (Rasheed et al., 2018). Traditional wheat improvement using hybridization, to some extent by mutation breeding, had played an important role during the last two decades. Moreover, transgressive segregation hybridization technique using superior traits with respect to genotype × environment (G × E) interaction for yield improvement. However, this selection was purely dependent upon traits selection without understanding molecular mechanisms of inheritance. The green revolution, an example of tremendous improvement in the wheat genome, in which the progress was slow but consistent. The application of *Triticeae* species provided new

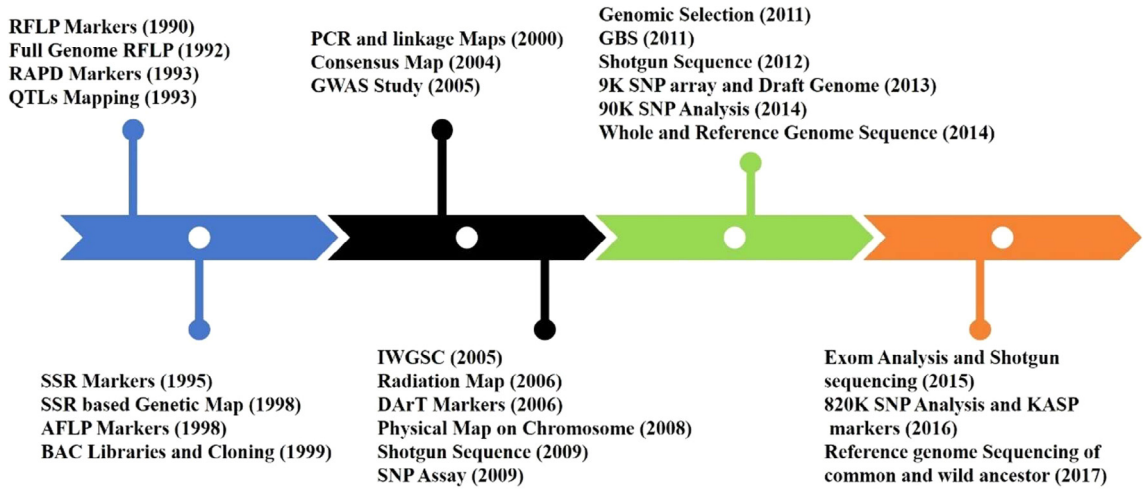

Fig. 1. The history timeline in the development of wheat genome.

information for genetic diversity to prevent it from biotic and abiotic stresses (Mujeeb-Kazi et al., 2013). Moreover, it also improves the quality and nutritional contents (Tabbita et al., 2017). Significant effect of different alleles of important genes, i.e., vernalization (Chen et al., 2013), size of the grain, and photoperiod response were selected during conventional breeding, but little improvement is attained during the process (Wurschum et al., 2018). Based on the facts, the wheat genome needs more evidence such as comparative genomic and functional analysis that will be important in characterizing and understanding the genetic basis of yield under specific environment and molecular breeding technique could serve the purpose (Li et al., 2018). The complete genome sequencing will contribute more to identify the gene for wheat improvement under abiotic stress conditions. Breeding new cultivars require gene or pair of genes and introduction of new genetic diversity by adding or deleting alleles, which led to the current yield increment under biotic and abiotic stress conditions.

1.2. Marker-assisted breeding (MAB)

Molecular markers are more appropriate because they resolve many of the shortcomings of morphological and biochemical methods as they are not influenced by the environmental or developmental stage and can detect DNA-level variations (Ashraf et al., 2014). The marker-based breeding was first started in the 1990s when fragment length polymorphism (RFLP) markers were used for mapping purpose, genetic diversity, wheat-rye identification, and homologous arms chromosome identification (Gupta et al., 1999). Genetic diversity knowledge regarding plant community is an essential step in the design and preservation of plant breeding and extinction programmes using molecular markers (Alawhibi et al., 2020). While the genetic diversity analysis reported that the polymorphism was low for D genome until the international Triticeae mapping initiative (ITMI) population was coordinated to map linkages (Deynze et al., 1995). The history of the primers developments in wheat genome was presented in the Fig. 1. The success was due to synthetic hexaploid wheat and subsequently used for each molecular marker class for mapping linkage group and facilitate for QTLs mapping (Sorrells et al., 2011). There is no doubt for success using RFLP for the development of linkage groups in wheat, but it was not of interest due to low frequency, time consuming, high cost, and laborious. Later, the researchers focused on PCR-Based markers i.e., randomly amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR), which were cost effective, mapping friendly (Marwal et al., 2014). However, the RAPD application was reduced due to the lack of reproducibility and less information about the location in the genome (Devos and Gale, 1992). While SSR markers were considered more reproducible and abundant for genome specific studies (Röder et al., 1995). The first SSR report in wheat was published in 1998, which open a new avenue for identification of new loci and better resolution for yield traits (Röder et al., 1998).

Some examples were also found for RAPD and sequence characterized amplified regions (SCAR) to map different QTLs as well as sequenced tagged sites (STS) (Naik et al., 1998). Functional markers are important for gene pyramiding, introgression, genomic selection, and accuracy. About 97 functional markers were used to determine the 30 loci from 93 alleles in bread wheat. However, this number was increased due to the tremendous advancement in genome-based studies. There are 157 markers data reported from 100 loci for disease resistance, grain yield, quality, and abiotic stress tolerance (Liu et al., 2012). Moreover, the development of cloning due to functional genomics was helpful but showed slow progress in wheat. Some genes were successfully incorporated with the help of conventional positional cloning in wheat *Lr21* (Huang et al., 2003), *VRN1* (Yan et al., 2003), and *Gpc-B1* (Uauy et al., 2006). Functional genomics mainly depends on the comparative genomics technique with other family members (grasses) due to high collinearity and genetic association in wheat (Valluru et al., 2014). Many genes *TaGS5* (Ma et al., 2016), *TaGS3* (Zhang et al., 2014) and *TaCwi-A1* (Ma et al., 2012) related to grain size, *Psy1* (He et al., 2009) phytoene synthase gene and *Zds1* (Zhang et al., 2011) gene related to zeta-carotene desaturase are reported using competitive genomics methods. Moreover, *Cre3, Cre1,* and other *Cre* resistance genes are now used in our marker-assisted selection (MAS) programs to identify cereal cyst nematode-resistant wheat genotypes (Moustafa et al., 2015). Studies showed that cloning of some genes with conserved motifs for disease resistance in wheat had been accelerated (Thind et al., 2017).

1.3. Double haploid

Double-haploid (DH) populations have a wide array of plant breeding applications, including crop and germplasm growth, wild-life transference, quantitative genetics research and the mapping of whole genomes. Anther cultivation and *Hordeum bulbosum* have stronger genotypic specificities among different techniques of development, which provide a solution for large hybridisation (Patial et al., 2019). While DHs have long been recognized for their value, they were renewed with the advent of biotechnology. The

scientists employed various methods to produce DHs in wheat (Mahato & Chaudhary, 2015), including ovule culture, anther culture, the removal of chromosome using hybridization, chemicals, and haploid gene inducers. Synthesize DH (SynDH) meiotic restitution genes was used for wheat (Zhang et al., 2011). There must be a meiotic restitution inducer line (s) and a foreign line traversed without embryo rescue. This is a 3-step hybridization system which results in recombination, interspecific hybridizations to extract haploids, and spontaneous chromosomal duplication by the F1 interspecies with no special machinery or double haploid processes. Arabidopsis suggested a technology-driven solution involving a DH-development centromere-mediated genome removal process (Maheshwari et al., 2015). This method uses centromere-specific histone CENH3 to create haploids from plant seeds. If the abnormal CENH3-mutant is crossed to the wild form, elimination of mutant chromosomes and ultimately the development of haploids. This approach is applicable to any plant because CENH3 is common in eukaryotes. This method was taken in various crops, but in wheat success was not described (Dwivedi et al., 2015). The accuracy and effectiveness of all these methods are different. Among all of the techniques of induction for DH, anther culture (Patel et al., 2004) and widespread hybridisation of barley with wheat (Barclay, 1975), maize (Laurie & Bennett, 1986) and *imperata cylindrica* (Chaudhary et al., 2005) have been commonly used in wheat breeding programmes and because of their high efficiency, seem to be most effective for DH production.

1.4. Somaclonal variation

Plant tissues culture is now one of the main techniques in plant science. It is widely used for plant resource production, conservation, and improvement. The existence of somaclonal variation in tissue culture populations has a negative impact on tissue culture and remains a foremost obstacle. It is also a source of new clones with enhanced agronomic characteristics (Bairu et al., 2011). Somaclonal variation (SV) is characterized as a cell and tissue culture variation (Larkin & Scowcroft, 1981). There has been a phenotypic difference between cultivated wheat plants. the diversity among the traits i.e. fertility, ear morphology, plant height and flowering were recorded (Maddock, 1986). The mutations are known as inherited changes in the genetic segregation or recombination DNA sequence (Van Harten 1998). Mutants can be caused by genetic variability by physical and chemical mutagens or tissue culture (Predieri, 2001). SV regenerated from tissue culture as mutations in plants (Anastassopoulos and Keil, 1996). It was also noted that in soma-clonal variation frequency and expression of dominance is greater than in other mutation forms (Yang et al., 2010). The growing evidence that mutagenesis along with tissue culture in ornamental plants and other plants has resulted in positive outcomes, thus demonstrating the good potentials (Wang et al., 2007). In vitro cultures mutagenic conditions may be used; plants regenerated from organ cultivation can be phenotyped, with DNA variants, calli, protoplasmic and somatic embryogenesis (Orbović et al., 2008). Studies have found that SV in long-term crop regenerated plants is particularly apparent and more evident (Petolino et al., 2003). In the long period culture, for example, the number of soma-clonal variants used in the regeneration of wheat has increased (Hartmann et al., 1989).

1.5. Somatic cell hybridization

Somatic cell genetic evolution has allowed the transfer of alien genes by somatic hybridization over broad taxonomic distances. Where the genes are interesting and isolated, can be transformed, however, the genes were not known for most of the features and somatic hybridity may then be the alternative. In addition to being important for the transmission of unknown genes, somatic hybridisation is a means to modifying and improving polygenic characteristics (Waara and Glimelius, 1995). Over time, techniques for sexual hybridization have been used to grow and improved new crops i.e. triticale, a crop developed by the cross among rye and wheat plant in 1875 (Macintyre and Campbell, 1974). In addition, heterotic impacts of wheat and rye have been seen on yields as the spike density and biomass are increased (Owuoche et al., 2003). Despite its high potential, the various drawbacks of some hybrid discontinuities are still limited, including infertility, isolation, and sex distortions, high-frequency mutations, chromosome rearrangements, non-disjointment and chromosome rearranging and differences in stems and leaves. The large hybridization techniques need to be further developed as a possible substitute to strategies for genetically modified crop enhancement (Mwangangi et al., 2019).

1.6. QTLs mapping to improve wheat genome

Quantitative trait loci (QTL) mapping is an approach used to determine the genetic construction of traits. The greater developments have been made to determine major QTLs and isolating genes related to quality, yield, seed size, and kernel weight in different cereals crops (Li et al., 2010). Many genes responsible for kernel size and weight in rice i.e., *GS3* (Fan et al., 2006), *GW2* (Song et al., 2007), *GS5* (Li et al., 2011), *qGL3* (Zhang et al., 2012), and GS8 (Wang et al., 2012) has been reported until now. Another study reported that *OsMKKK10-OsMKK4-OsMAPK6* genes related to the signal pathway significantly control the size and weight of the kernel in rice (Xu et al., 2018). Similar results were reported by Su et al. (2018) showed a high-density linkage map was developed for QTLs mapping in wheat. The linkage map was consisting of 6312 SNP and SSR markers for the identification of QTLs related to the size and weight of kernel using a recombinant inbred line (RIL). About 78 QTLs were discovered related to kernel diameter ratio, 1000 kernel weight, kernel width, and kernel length from 5 environments using inclusive composite interval mapping (ICIM). Moreover, six stable QTLs were detected from 4 environments for kernel weight, kernel length, and kernel width. However, cluster analysis results predicted that chromosome position on 2D and 5B stable for kernel traits and selected markers were tightly linked with these QTL and consider best gene discovery and fine mapping (Su et al., 2018). Wu et al. (2015) reported that QTLs Yanda1817 for kernel length, total kernel weight was found in Chinese wheat. Similar results were reported by Kumar et al. (2016) recognized QTL-15 was harbor an ortholog of GS3 gene in rice. QTLs related to heat stress in wheat were identified using 251 RILs derived from HD2808, and HUW510 represents heat tolerant and susceptible, respectively with composite interval mapping technique. Their position was located on 2A, 2B, and 6D chromosome number. The important QTLs were identified are grain weight/spike (6), grain number/spike (6), grain filling rate (4), 1000 grain weight (3), grain yield (3), and grain filling duration (2). Moreover, these hot spot regions with consistent QTLs could be used to improve heat stress tolerance after validation (Bhusal et al., 2017). A recent study suggested that RIL linkage map consisted of 21 linkage groups covering the genomes A, B and D for tagging and mapped a total of 60 QTLs with major and minor effect. Moreover, it was also reported that genomes B and D had more potentially active genes that conferred salinity tolerance that could be exploited for MAS of high yielding wheat genotypes under saline condition (Ilyas et al., 2020). A List of important QTLs related to protein content, grain Zn, Fe, and yellow pigments was presented in S1-Table.

1.7. Proteomics

Wheat (bread and durum) is a significant crop due to its dietary, nutritional, and economical value. plants. Molecular processes that reinforce the anatomy and physiology of wheat, which enable better varieties to grow with increased yields or stress tolerance. Proteomics is an interesting feature for researchers to determine the molecular events that reinforce the agricultural relevant traits in crop plants (Roy et al., 2011). Moreover, the ongoing challenges for researchers, limitation in analytical technologies employed (103–104) compared to a large number of abundances across the cellular proteome (Schwanhausser et al., 2011). Low-abundance protein is more significant biologist interest; therefore, researchers engage procedure to limit sample intricacy or deplete high abundance proteins of the sample before starting proteomic analyses. Physiochemical separation of protein was done from whole-cell lysates for decreasing sample complexity. Though these techniques have a disadvantage, the resultant protein fractioned were categorized based on arbitrary parameters i.e., size, charge, or chemical affinity. These fractions were based on target enrichment of specific organellar proteomes and grouped in subcellular location. The significant organellar fractionation methods based on subcellular proteomes are highly dependent on the set of biochemical processes. List of proteomics techniques for evaluating the differential proteins expressed in wheat under abiotic stress conditions was presented in S2-Table. Cellular functions are compartmentalized into discrete subcellular locations (Millar et al., 2009). As a result, protein base profiling for functional proteins allows researchers to deeper insight using a narrow set of biological pathways. Whole-cell proteomics provide tools to gather narrow information over broadened biological processes. Integrated approaches for whole-cell proteomics provide information with the biochemical process to examine subcellular proteomics and collective information for a specific process. The mitochondria have the biological system used to explain a large array of mitochondrial phenomena ranging from cytoplasmic male sterility (CMS), RNA editing, configuration and function of the electron transport chain, metabolite transmission, and stress response. Analysis of the mitochondrial protein was used to demonstrate major molecular changes intentionally causing cytoplasmic male sterility (CMS) on hybrid lines in bread wheat (Gray et al., 1999). However, initial biochemical characterizations of C-U RNA editing (Bégu et al., 1990). In addition, bread wheat mitochondria were used to describe the synthesis of complex III mitochondrial precursor proteins (Braun et al., 1995) to describe complex composition I subunit (Combettes & Grienenberger, 1999). However, durum wheat showed malate (MAL)/oxaloacetate activity with oxaloacetate (OAA) junction and intercede by MAL/OAA antiporter (Pastore et al., 2003). Proline transport into mitochondria using carrier antiporter (proline/glutamate) was initially characterized by the biochemical study in durum wheat (Di Martino et al., 2006). Trono et al. (2013) suggested that mitochondria isolated from stress wheat plants had an activity with phospholipase A2 in plant mitochondria, as well as reactive oxygen species (ROS) activity reduced in succinate but not NADP- dependent membrane potential and oxygen uptake (Pastore et al., 2002). The significance of proteomics moves upward from model plant to other crop plants i.e., wheat using mass spectrometry technique, which helps the researchers for characterization of proteomics. Moreover, it also helps to determine the amino acid sequences of protein which execute functions in mitochondria and junk with genetic loci (Jacoby et al., 2016).

Moreover, the development in functional proteomics and subcellular proteomics in wheat, as it provides more profound information on the intrinsic mechanism of abiotic stress and responds against specific abiotic stress conditions. Separation of the specific organelle of total tissue is a limiting factor and challenging condition to determine the subcellular proteomics. However, traditional proteomic techniques involved subcellular fractionation, and density gradient configuration follow up by centrifugation step to isolate different cellular compartments based on mass or density (Wiederhold et al., 2010). Furthermore, the purification of immunoaffinity has been successfully used to isolate sub-cellular with high specificity and yield. Many subcellular proteins, including stresses and housekeeping proteins, remain unclassified. The future consensus for proteomics with the aim of identification and characterization of organelle and subcellular level protein help the scientists for profiling under stressed plants. The integrated proteomics approaches should facilitate to isolate specific regulatory proteins and help to use in molecular cloning to address a basic question about plant physiology under abiotic stress conditions. As a result, the combination of wheat omics approaches, and interactive databases are important to develop the bioinformatics model to provide deeper insight about the underlying mechanism under abiotic conditions (Komatsu et al., 2014).

1.8. Metabolites

To develop new varieties working fine under stress conditions, there is a need for genetic improvement in wheat cultivars for stress tolerance regarding physiological mechanisms along with biochemical processes and their affiliation with different yield traits. The strength regarding metabolic changes and their interaction with different phenotypes, purifies these metabolites as possible biomarkers for genetic improvement. Different types of anthropogenic resources i.e., land usage and fossil fuels, produced carbon dioxide and green-house gases (GHG); as a result, climate change and global warming issues initiated (IPCC, 2013). Since wheat is a crop of higher latitude, due to high temperature in spring, early summer, and terminal heat stress affect the wheat plants (Farooq et al., 2011). Advanced metabolomics application to characterize biotic and abiotic stress tolerance in wheat was presented in (S3-Table). The wheat plant is more delicate during heat stress at the reproductive stage than other stages, cause pollen sterility, anthesis and as a result reduced the grain number (Porter & Gawith, 1999). Moreover, heat stress also decreases assimilation, reserve metabolites can become an important source of grain yield (Blum, 1998), as well as plant senescence resulting decline in growth and yield (Al-Khatib & Paulsen, 1984). Though recording morphological and physiological traits could be productive, but it often not provides a clear depiction of the underlying mechanism with changes of metabolites status in plants. Advanced omics technique i.e., mass spectrometry, allows the scientists and researcher to provide comprehensive and comparative metabolic profiles on crop genotypes (Khakimov et al., 2014; Zhao et al., 2014; Obata et al., 2015). A wide range of metabolites produced by the plants, which can be differentiated under specific stress conditions. The change in metabolites and their expression linked with different genotypic and phenotypic traits (Schilmiller et al., 2012). Experimental results predicted that metabolites could be a powerful technique to determine the relationship between genotypes and their appearance. It also offers a more in-depth insight into the genetic basis of plant responses to stress. However, it also differentiates drought and well water situation differences in plants (Servillo et al., 2012; Witt et al., 2012). The metabolites expression results predicted that branched-chain amino acids were recorded high intolerant wheat cultivars under drought conditions (Rontein et al., 2002; Krugman et al., 2011). Tryptophan, beta-alanine, threonine, serine, proline, glutamate, myo-inositol, and urea metabolites showed high in concentration in maize plants (Obata et al., 2015). Moreover, many other metabolites recorded have a negative correlation with grain yield under greenhouse and field conditions (Obata et al., 2015). Also, Al-Doss et al.

(2010) reported that three groups of peroxidase genes (*TaPrx111-A, TaPrx112-D*, and *TaPrx113-F*) were found to be greatly induced by cereal cyst nematode in the resistance wheat line. In particular, parenchyma cells, where the nematode starts its feeding, were hyper-reactive to some probes belonging to (*TaPrx112-D* and *TaPrx113-F*) groups. There is a gap between metabolite expression and biochemical mechanisms intricate stress tolerance mechanism for the identification of desired metabolites working well under stress conditions. Filling this gap might help to develop a strong breeding plan and collect information for the identification of cultivars perform well under abiotic stress condition. Metabolomics has achieved a protuberant place in wheat research. Wide variety of applications in plant sciences exploring different abiotic stresses, searching the underlying mechanism and identifications of novel genes to analyses the whole biological process in cells which help to aid phenotype and genotype interaction (Razzaq et al., 2019). Furthermore, plant sciences need more extensive research and special attention to data mining, data annotation, and valuation. The cumulative bioinformatics techniques competently separate novel metabolic networks for crop improvement. Metabolomics data with post-genomics approaches has presented an escaping way to determine the genetic regulations of plants with respect to metabolism.

1.9. Next-generation sequencing (NGS)

The genomics-assisted selection has not yet contributed much to the development of wheat drought resistance (Berkman et al., 2012). Most improvement is based on conventional breeding approaches. Private companies and public entities continue to seek to increase drought tolerance through their breeding programs or by using transgenic approaches. Many efforts have been undertaken worldwide using conventional or molecular breeding approaches for improving drought stress in wheat (Mwadzingeni et al., 2016; Saleem et al., 2016). Whole transcriptome analysis enables researchers to better understand changes in gene expression-level with respect to environmental stress. Transcriptomes of non-model organisms have been reported for many plants, including wheat (Davidson & Oshlack, 2014; Singh et al., 2016; Wan et al., 2008). Furthermore, the study of drought resistance and drought tolerance by transcriptomic analysis is becoming more widespread within the scientific community (Zhou et al., 2016). The introduction of sequencing techniques for next generation greatly expanded our capabilities by allowing for massively parallel sequencing efforts at a greatly reduced cost. In NGS technology and its advances, RNA sequencing (RNA-seq) was commonly used in crop breeding, especially in those plants that have lack complete genomic information. Similarly, efforts to sequence the proteome in wheat treated with salinity and drought, salt (Guo et al., 2015; Peng et al., 2009) have provided translational insights into drought-responsive mechanisms. Moreover, results coming from RNA-seq may facilitate the identification of new and interesting biochemical traits (Wang et al., 2009).

1.10. RNA sequencing

The RNA-seq technologies generate large amounts of transcriptomic data in real-time, which requires investments and expertise in bioinformatics for data management. Furthermore, genes involved in drought tolerance can be functionally characterized by transgenic incorporation and analysis. This is most easily performed in model species, for example, Arabidopsis, rice, or brachypodium, where functional information is available. Hexaploid wheat has one of the largest genomes of any crop species (17 Gb in size) encoding for more than 124,000 gene loci. So far, only 76% total genome has been sequenced (International Wheat Genome Sequencing Consortium [IWGSC], 2014). The near sequencing of the wheat genome functional annotation by homology is becoming increasingly useful but far from completing in comparison to model organisms. The size and complexity of the wheat genome face challenges in transcriptomics. Additionally, RNA-seq and proteomic analysis will lead to marker production for a variety of traits to advance cultivar growth more effectively in breeding programs. There are many approaches for RNA-seq. The Illumina company has created a wide range of kits and instrumentation to identify differentially expressed genes, targeted DNA sequencing, whole-genome sequencing, targeted RNA-seq, and whole-transcriptome sequencing. Other platforms have been used for RNA-seq analysis, including Roche/454 pyrosequencing (the first commercial platforms for NGS), SOLiD (developed by Life Technologies), MinION (Oxford Nanopore Technologies), and PacBio (Pacific Biosciences of California, Inc.). The latter two are currently referred to as third-generation sequencing technologies and are capable of sequencing much larger fragments than the Illumina sequencers achieving of wider de novo genome sequencing and gene expression analysis (Berkman et al., 2012; Elshire et al., 2011; Mwadzingeni et al., 2016).

It is clear; RNA next-generation sequencing (RNA-seq) provides many minute details concerning the transcriptional landscape under considerable treatment conditions. Gene ontology provides a biological approach system that is simple and widely used to understand and highlight functional processes that are affected in response to treatment. Differentially expressed transcripts are grouped into preformatted functional categories, including biological processes, cellular processes, and molecular processes (Young et al., 2010; Glass and Girvan, 2014). The transcriptional activities in the flag leaf during grain filling was conducted in rice with a focus on stem remobilization of carbohydrates but not the transcriptional activities of the flag leaf itself (Wang et al., 2017), or in the flag leaf but not during grain filling (Xu et al., 2012). The typical integration events in wheat may involve transposition events, given that 68% of the wheat genome has its origin with transposition (Li et al., 2004). Moreover, transposition activities appear to be upregulated under drought stress (Alzohairy et al., 2014). Though, the transposition is predominately down-regulated during the grain filling stages of wheat development under stress, whereas in the vegetative stages, it was up-regulated (Alotaibi, 2018). This indicates that transposition activities are likely associated with pre compared to post-anthesis processes. A recent study about drought tolerance wheat genomics and data analysis using the RNA-seq technique was developed from root tissue. The results predicted differential expressed genes (DEGs) 45139, transcription factor (TF) 13820, pathways 640, markers development 435829, and micro RNA (miRNAs) 288 were available. Also, it was suggested that about 18 DEGs with one hundred ninety sequence variants, detected from two different wheat verities. However, 11 SSR diverse markers were selected, and the study was validated using 18 diverse wheat verities (Iquebal et al., 2019). Another study reported that RNA-seq based approach is significant to develop a wide range of molecular markers development and used for population genetic studies in wild relatives of wheat, i.e., *Ae. Umbellulata* with less genomic data. The development of SNPs forms high throughput sequencing, covered all the chromosomes, and help to provide a wide range of molecular markers for *Ae. umbellulata* germplasm. The genes or alleles with infrequent incidences provide more deep information about the genetic diversity in a wild relative of wheat genome (Okada et al., 2018). Alotaibi (2018) studied the drought tolerance mechanisms of two wheat cultivars at flag leaf and seed head stage using RNA-seq profiling tool under well water and stress condition. The analysis reported that flag leaf weights averaged across durations were 91%, 62%, 68%, and 41% of well-watered under mild stress and sever stress (MS and SS)

respectively, in Alpowa and Idaho, respectively, when compared with the well-watered control. Furthermore, the differential expressed genes were 2.32 and 3.9-fold more up and down-regulated genes in Alpowa compared to Idaho, respectively. Shared transcripts between stress intensities MS and SS constituted only 3 to 17% of the overall differentially expressed transcripts in both cultivars. RNA-seq technique also significant for determine the wide genome polymorphism based on coding regions of the chromosome. For this purpose, 19 sitopsis wheat species were analyzed using RNA sequencing technique with reference to tetra and hexaploid wheat B genome. The wide genome exon sequencing and phylogenetic results suggested the *Ae. Speltoids* tend to be the primary donor of all wheat B-genome chromosomes (Miki et al., 2019). Similarly, another study was conducted for sequencing the transcriptome of tetraploid *A. cristatum* with common wheat. The analysis was done using phylogenetic relationships and interspecific diversity among wheat cultivars. About 214 and 854 transcript sequences and 3457 orthologous genes were selected to generate the phylogenetic relationship among common wheat, *A. cristatum,* and related genome. Additionally, the similarity between the sequence of many genes was greater than 95% among *A. cristatum* and wheat. Mismatch analysis was also done, and results suggested that the diversity among common wheat and *A. cristatum* 862,340 high-quality diversity sequence reads were recognizable (Zhou et al., 2017). Li et al. (2018) suggested genome wide expression profiling used to determine the regulatory mechanisms of six developmental stages. High gene expression relation among two stages and suggested that early spike development controlled by a subset of genes. Furthermore, auxin signaling increases while cytokinin signaling is limited. However, 375 transcription factor (TF) genes, like Arabidopsis and rice meristem function, flowering time, transition, floral organ development, and many other stress related genes, were identified. Some online transcriptome resources were presented in S4-Table.

1.11. Computational resources

The presence of the genome sequencing facility from restricted resources is not enough to apprehension the entire diversity range within a gene pool accountable for phenotypic assortment, plasticity, and ecological variation. Functional and structural diversity, i.e., presence or absence variation (PAV) and copy number variation (CNV) major adaptation genes and essential agronomic characteristics responsible for diversity, such as *Vrn-A1*, *PpD-D1* and *CBF* (Zhu et al., 2014) and this differences might be recognized by de novo sequencing of diverse members among species. After the sequence of the reference genome, the next step is the construction of de novo pan-genome. Pan-genome consists of core genome, dispensable genome, and unique genome. It is also important to the identified gene of interest from the genome and success story was reported in *Zea mays* (Hirsch et al., 2014), *Brassica rapa* and *Brassica oleracea* (Cheng et al., 2016) and *Glycine soja* (Li et al., 2014). Data regarding wheat cultivar for whole-genome shotgun sequencing is less, and less sequencing depths limit the use of the pan-genome tool (Montenegro et al., 2017). For the generation of high-quality pan-genome, there is a limitation of the availability of these data set. IWGSC took the step for developing high-quality wheat pan-genome, including de novo sequencing assemblies of wheat cultivars (http://www.10wheatgenomes.com/). Moreover, the development was made on sequencing of more accessions of wheat progenitors that will expose loci experienced domestication and selection during modern breeding. Borrill et al. (2016) also reported that Grain Genes have 16 diploids, 27 tetraploid, and 68 tetraploid wheat maps were constructed using different molecular markers. Wheat IS database and bioinformatics software used for wheat genome information. Moreover, (http://www.wheat-expression.com/) expression browser also a powerful database for transcriptome analysis and visualization in wheat. Some important computational sources and tools were presented in the S5-Table.

1.12. Gene editing CRISPR/Cas9 and Cas13

There is a need to use modern agriculture technology to enhance agriculture production through breeding technology worldwide. The recent advances in genome editing technology, i.e., Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) gene-editing help scientists for desired modification in many crop plants including wheat; as a result, to speed up the crop development process. Moreover, genome base editing techniques also facilitate to alter target-based nucleotide sequence modification and delivery system in crop plants (Chen et al., 2019). The currently significant methodologies are mutation breeding, cross-breeding, and transgenic breeding used for crop improvement. The only limitation is taking to many years to get the desired results through cross-breeding and genetic recombination (Scheben et al., 2017). Moreover, mutation breeding has wider genetic diversity due to random mutation using chemical mutagens (Pacher & Puchta, 2017). Such processes are controlled by stochastic and need large screening mutant population stimulation. But these processes are time consuming, untargeted, and laborious and do not produce desired results to increase crop production, even when molecular breeding are implemented for selection (Scheben et al., 2017). Conversely, transgenic technology help to identified desired traits and transfer through exogenous genes into elite genotypes. On the other hand, the commercial release of GMO crops is limited due to public apprehensions (Prado et al., 2014). Target gene study was first done in tobacco (Nicotiana tabacum) in 1988 (Paszkowski et al., 1988), and later DNA double-strand breaks (DSBs) was reported in 1993 enhanced target efficiency (Rees et al., 2017). With more advancement in technologies, the invention of zinc finger nuclease in tobacco during 2005 was reported (Wright et al., 2005) and transcription activator-like effector nucleases (TALENs) in 2010 for traits improvement (Christian et al., 2010). Alternatively, the homology-directed repair (HDR) gene alteration technique was used in crop plants but faces some technical issues like low efficiency of HDR and limitation in donor template delivery in plants. Many strategies are used to improve the HDR base editing and CRISPR/Cas9 based positive and negative selection, which gives good results in rice (Nishizawa-Yokoi et al., 2014). Other methods to increase the efficiency of the HDR system are use of Geminivirus replicons and wheat dwarf virus to enhance donor copies, as well as enhanced the frequency of the gene insertion (Čermák et al., 2015; Gil-Humanes et al., 2017). The use of chimeric sgRNA and repair template sequences also enhance the HDR effectiveness in rice (Butt et al., 2017). The different research groups researched the CRISPR system during 2013 in wheat, rice, Arabidopsis, and tobacco (Li et al., 2013; Nekrasov et al., 2013; Shan et al., 2013). A different transport method called transient gene expression for achieving transgene-free editing was introduced in wheat. In this method, the canonical herbicide gene was eliminated, and the resulting plant was free from foreign DNA in the genome (Zhang et al., 2016). Furthermore, in vitro transcript of Cas9 and sgRNA delivered in immature embryos using particle bombardment and the resulting plant was DNA free (Zhang et al., 2016). Later studies suggested that Cpf1 to RNP toolbox used as an editing tool in plants, which was first used in wild tobacco and soybean proto-

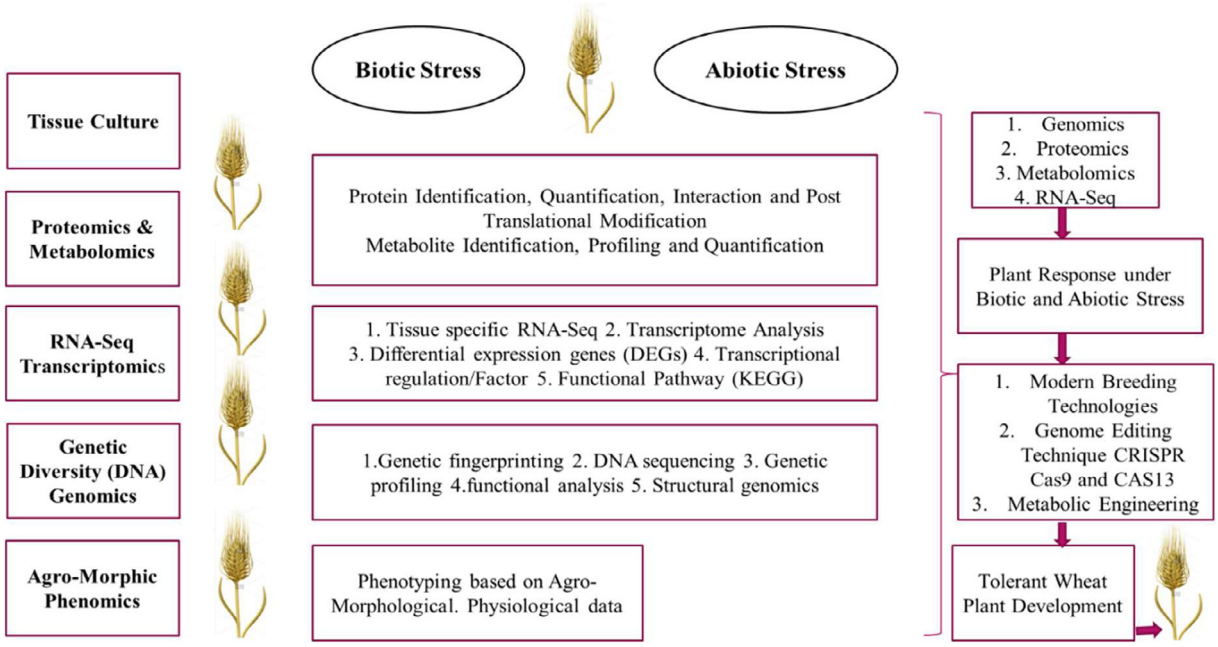

Fig. 2. Integrated approaches for the understanding of plant stress mechanism. Application of genomics, proteomics, metabolomics, and transcriptome analysis results enable researchers and scientists to figure out the gene of interest at a specific stage of plant growth and development. Moreover, genome editing techniques also facilitate the knockout desired gene of interest and transformation for the successful development of wheat cultivars under stresses and ultimately yield.

plast, but still challenging for cereal especially monocots (Kim et al., 2017). The RNP based CRISPR system was used through particle bombardment into an immature wheat embryo with a low off-target editing rate (Liang et al., 2017). With the advancement in CRISPR/Cas9, a Chinese institute introduces fragrance traits in about 30 elite rice cultivars (unpublished data). The proteins (gluten) in cereals activate celiac disease in western countries, and about 7% of individuals were affected by gluten protein. CRISPR/Cas9 system offers a new way to alter the α-gliadin gene family contains more than 100 genes or pseudogenes. Furthermore, immediate knocking out of the most preserved realms of the α-gliadin gene family by reducing or limiting low gluten wheat (Sánchez-León et al., 2018). The gene-editing system also provides important to overcome the abiotic stresses issue and quality in crop plants, including cereals. Both biotic and abiotic stress resistance has been attained using CRISPR/CAS9 by addition or deletion gene. Powdery mildew is an overwhelming fungal disease in crops. Combine activity of TALEN and CRISPR/Cas9 knocked out six alleles (TaMLO) in wheat, and resultant plants were resistant against powdery mildew (Wang et al., 2014). Hybridization is important to enhance crop productivity, but the precondition for high-quality hybrid development is the male sterility maternal line. CRISPR/Cas9 system help to knockout gene and develop male sterile line including (tm5 line) important for thermosensitive male sterility (Zhou et al., 2016), *ms45* in wheat (Singh et al., 2018) and csg in rice (photosensitive genic male sterile) (Li et al., 2016). Single nucleotide polymorphism (SNP) is an important for trait introgression (agronomic). Base editing is significant for plant breeding and improvement program. The application of base editing techniques is successful in wheat (Zong et al., 2018) for confirmation of herbicide resistance. Nitrogen as inorganic fertilizer is important for crop growth, seed development, and ultimately yield. Many nitrogen-fixing gene and their expression have been identified (Temme et al., 2012). CRISPR/Cas 9 system could be used to decrease the dependence of inorganic fertilizer by transferring a nitrogen-fixing gene from legume to cereals, i.e., wheat, let cereal crops to fix atmospheric nitrogen and hence manipulate plant behavior and enhance crop productivity (Jusiak et al., 2016).

1.13. CAS13

Viroid discovery opens the new direction for research, economic, and target specification. Integrated plant omics technologies have been used viroid genomes composed on 21–24 vd-sgRNAs made by silencing the RNA defense of the host. The CRISPR and CRISPR-associated Cas13a proteins system used for engineering of viroid interference in plant biology. The system is also significant for specific detection of human RNA viruses by graphic reads in ninety minutes. Multitarget RNA tests recorded good potential for known viroids that were faster and specific (Hadidi, 2019). The guided RNA nuclease activities of Cas9 is functionally different from other nucleases like Cas13a, Cas12a, and Cas13b which recently been exploited to develop sensitive detection methods for human viruses (Myhrvold et al., 2018; Chen et al., 2018). The system Cas12a was used for DNA virus detection (Gootenberg et al., 2018; Chen et al., 2018), while Cas13a and Cas13b used for RNA viruses (Gootenberg et al., 2018; Myhrvold et al., 2018). Moreover, Cas13 SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) system can integrate pre-amplified input materials to change into a paper-based analysis with modified sensitivity (Gootenberg et al., 2018). At the DNA level, transcriptional regulation affects the isoforms splicing, while Cas13 enables specific isoforms targeting. As a result, it could eliminate abnormal isoforms splicing or pathogen isoforms in wildtype transcripts (Mahas et al., 2018). Additionally, the Cas13 system also facilitates quick downregulation of the expression of the gene by cutting away the cytoplasmic mRNA pool, while regulation on DNA level limit the mRNA production and trusting on the ordinary squalor rate of mRNA (Wolter and Puchta, 2018).

2. Conclusion

Wheat is sensitive to abiotic stresses at different growth stages but especially at flowering and grain filling levels. Production of different reactive oxygen species (ROS) cause oxidative stress condition, and as a result, the yield of the wheat is severely affected.

Overproduction of many ROS macromolecules affects protein and nucleic, which results in cell death. Combinations of these stresses disturbed the photosynthesis and enhance photorespiration by modifying the homeostasis system in the plant cell. Moreover, advanced application of proteomics techniques enables to identify organelle proteins i.e., housekeeping as well as stress induced that were unidentified before. However, characterization and identification of unidentified organellar protein could help for proteomic profiling under stress condition. Integrated protein technologies will allow target protein to be identified, which helps to answer basic questions about plant physiology in specific circumstances in molecular cloning. Metabolic study and its application also helpful to identified markers significant for plant metabolism and determined the nature of stress.

Metabolic based breeding help in crop improvement programs to develop high yielding stress tolerance cultivars and enhance crop production under a smart climate. Application of probiotic bacteria enhances the soil organic matter, macro and micronutrients, nitrogen-fixing capacity, and the yield but reduces biotic and abiotic stress. RNA-sequencing and transcriptomics analysis open a new venue for plant biology, identification of differentially expressed genes (DEGs), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis, transcription factor (condition-specific), functional genomics and structural analysis which help the scientists and researchers to enhance the wheat improvement program (Fig. 2). Lastly, combine approaches for wheat omics, i.e., genomics, proteomics, transcriptomics, metabolomics, and interaction omics with bioinformatics software, are significant to develop mathematical models that will offer a comprehensive picture about the causal mechanism of the plant under stress condition. No doubt, wheat genomics enhance the development of wheat genetics and assist breeding deliberately along with omics approaches, i.e., especially gene-editing techniques CRISPR/Cas9 and Cas13 could enhance the pace of the development. CRISPR/Cas9 is the technology that should be applied in the 21st century. It is effective; scientists may use it to remove attach genes inside the plant to produce varieties with adequate zinc, iron, and vitamin A content suitable for the human body, disease, and resistance to pests and varieties resistant to drought for increased yield. Health researchers used the CRISPR to create vaccines, and scientists focus on using this evolutionary application of technology for modern plant breeding. Furthermore, introgression of new genes and their functional characterization would help to get unique homozygous progressive lines with high throughput genotyping systems. Application of the wheat resource for sequencing will enhance the effectiveness of the prevalent genomic tools to promote hybrid and inbred linebreeding based on genomes.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.11.083.

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