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GENOMIC APPROACHES FOR IMPROVING DROUGHT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.): A COMPREHENSIVE REVIEW

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ABSTRACT

The uses of traditional methods and instruments have led to a great success in plant breeding through the creation of better cultivars. Currently, the accessibility of genomic data as well as techniques is fueling a novel paradigm in the breeding of plants since they make it easier to explore the relationship between the genotype and phenotype, solely for intricate traits. The mass sequencing of genomes, transcriptomes, is producing a multitude of genetic data and metabolomics made feasible by Next Generation Sequencing (NGS) technology. The analysis of NGS data utilizing advances in bioinformatics makes large sets of molecular markers available, fosters the discovery of new genes and regulatory sequences, and even provides accessibility to huge number of molecular markers. Breeders are able to comprehend molecular underpinnings of many complicated phenotypes, all as a result of genome-wide expression research. A complicated characteristic, drought tolerance (DT) is now a major danger to the world's food security since it limits productivity. Developing drought-tolerant wheat cultivars through traditional breeding has been hampered by itscomplexity. Hence, to increase wheat's resistance to drought, marker assisted selection (MAS) and genetic engineering have been employed to change some genes or transcription factors. With the evolution of new concept, "Omics," it has become easier for identification and characterization of genes related associated to drought tolerance. To recapitulate, breakthrough in genomics is giving breeders innovative tools and technologies, which enable a giant step ahead towards the advancement in plant breeding by developing drought tolerant wheat cultivars.

Key words : QTL, Molecular markers, Marker Assisted Selection, Transcriptomics, Metabolomics.

Introduction

Bread wheat (*Triticum aestivum*) is one of the most significant cereal crops in the world with a 756.7 million-tons of annual production (FAO, 2018) is consumed by nearly 40% of the population. It is an annual plant grown in more than 17% of the cultivable land and is a nutritionally rich grain enriched with carbohydrates, protein, and other dietary fiber (FAOSTAT, 2021). However, with increment in global population each year it has become a matter of challenge for fulfilling people's need that has been further accelerated by climatic patterns like irregular rainfall, water scarcity and extreme temperature

conditions which is anticipated to cause additional dry spells (IPCC, 2013). Since, water stress affects plants at all stages of growth, prolonged drought has had an impact on wheat production globally. (HongBo *et al*., 2005; Saeidi *et al*., 2015; Saeidi and Abdoli, 2015; Wang *et al*., 2015; Sarto *et al*., 2017; Ding *et al*., 2018).

Physio-morphological characteristics peculiar to each growth stage of wheat that are linked to drought resistance include early vigor (Rebetzke *et al*., 1999), coleoptile length (Rebetzke *et al*., 2007), leaf chlorophyll content (Khayatnezhad *et al*., 2011; Kira *et al*., 2015; Ramya *et al*., 2016), glaucousness (waxiness) for photo

protection (Merah *et al.*, 2000; Bi *et al.*, 2017), leaf rolling (Kadioglu and Terzi, 2007), carbon isotope discrimination (Kumar and Singh, 2009), flag leaf senescence (Verma *et al.*, 2004; Hafsi *et al.*, 2013) and plant height (Su *et al.*, 2019).All of these factors cumulatively affects the yield. As shortage of water becomes a limitation foryield, Plant breeders have been continuously working on new methods and technologies to address the situation or the problem. They have become successful in breeding varieties that perform in an outstanding way in water deficit environments.

Latest research and Progress in genomics, like genotype by sequencing has made it possible to investigate this genetic diversity and find markers and related quantitative trait loci (QTLs) that can be used in marker assisted selection and genomic selection to improve variety development (Huang and Han, 2014). Hence, the use of gene and genomic tools and technologies, markers such as SSR, SNPs and a novel approach like CRISPR/Cas9 have successfully led a pathway for identifying genes conferring resistance to drought. These genomic tools aid in exploring the genes for tolerating drought in wheat which identifies the varietythat can adapt and withstand such harsh condition (water stress) without affecting the yield of the crop. These technologies have become a great boon for Plant breeders.

Drought resistance in wheat is a crucial trait that researchers and breeders aim to enhance using genomic tools. These tools leverage the advancements in genomics and molecular biology to identify and manipulate specific genes and genetic markers associated with drought tolerance. Some of the genomic tools which are commonly used to improve drought resistance in wheat are highlighted below:

Genomic tools

Genome sequencing : The complete sequencing of the wheat genome has made it possible to conduct research and identify genes linked to drought resistance. Taking advantage of high throughput technologies such as Next Generation sequencing (NGS), researchers can now identify potential candidate genes for drought-related behaviors and understand their genetic basis.

QTL mapping : It facilitates the identification of the particular regions of the wheat genome associated with drought resistance. By comparing the genetic variation and drought tolerance levels across multiple wheat lines, one can pinpoint the areas responsible for this trait. This information is useful for the breeding and marker-assisted selection procedures for wheat cultivars resistant to drought.

Marker assisted selection (MAS) : It uses DNA markers linked to QTLs or genes that are known to withstand drought. Plant breeders can discover plants with favorable markers and a higher probability of displaying the desired drought resistance feature by using population genotyping might be possible. This reduces the time required for genotypic selection, which speeds up the breeding process.

Transcriptomics : It comprises analyzing the way in which drought stress affects the expression of genes. Through high throughput methods like RNA sequencing, genes that exhibit differential expressions under drought can be found. Gaining more knowledge about the genes and regulatory network involved in drought response facilitates the development of targeted breeding strategies to enhance wheat's tolerance to drought.

Genome editing : With the help of recent developments in genome editing technologies like CRISPR-Cas9, it is now possible to precisely alter particular wheat genome genes. This may improve the wheat plant's resistance to drought conditions by directly introducing or changing genes linked to drought tolerance.

Genomic selection : It is a method that utilizes genomic information from multiplemarkers spread across the entire genome. By training statistical models, it enables the prediction of an individual's breeding value for drought resistance without the need for individual phenotyping. Genomic selection has the potential to enhance the efficiency and accuracy of selecting drought tolerant wheat varieties. Furthermore, novel pathways for effectively introducing targeted alterations in the genome have been made possible by genome editing technologies (Puchta, 2016). It is possible to modify the plant genome by changing the particular genomic region by inserting a foreign DNA segment or by performing nucleotide substitution, insertion, and deletion. Sequence specific nucleases (SSNs) like Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR Associated Protein) systems are responsible for this type of modification at the targeted genetic locations. Due to its simplicity of use and capacity for precise editing of numerous genes at once, the CRISPR/Cas system has drawn a lot of attention (Bilichak *et al.,* 2020). According to Jain (2015), these editing methods have a tremendous deal of potential for understanding functional genomics in stressed environments.

Genomic applications

In general, genomic tools provide information and

understanding of individual genes, gene networks or pathways and the structure and function of genomes where the specific genes governing the desired characteristics are known. Gene information, when it is available, can be utilized to create and implement molecular markers in order to track the target alleles in breeding programs as well as to identify, locate, and tag particular alleles (Gupta *et al*., 2010). Equipped with genetic knowledge, one can search various germplasm pools for novel alleles, including wild relatives; one can study expression variation and one can produce new alleles, both structural and expression, using new genome editing techniques or genetic engineering.

Breeders can utilize new breeding techniques like genomic selection and optimal recombination procedures with the aid of genomics and whole genome analysis at the genome structure level (Heslot *et al*., 2014).

Genomic techniques are used in situations when the target trait's genetic regulation is well-defined and therefore straightforward, or when genome structural and predictive models may be employed in place of individual gene information (Jarquin *et al*., 2014). Plant breeders have always shown keen interest in improving resistance to stress conditions. There have been initiatives in recent decades to increase wheat productivity in a variety of settings, particularly during a drought. This paper discusses the various methods, such as the application of "omics," and the advancements made to enhance wheat's resistance to drought.

Phenomenon of stress from drought

The repercussions of drought stress are not steady; they can happen at any point in a crop's growth cycle, they might differ in severity, and they often occur in tandem with other environmental stresses such salinity and heat (Suzuki *et al*., 2014). Different plant tissues or organs may react contrastingly to stress, and the level changes throughout the day, elevating during peak photosynthetic hours and dropping downat night (Tardieu *et al*., 2011). The way that a plant reacts will also differ significantly based on if this is the first time it has ever experienced stress or after being exposed to it multiple times, and whether or not they are regaining their composure after a stressful event or irrigation activity (Vadez *et al*., 2013).

Drought tolerance exists in several distinct forms comprising

- A plant's ability to acquire water is determined greatly by the structure and health of the   root structure, which together affect aplant's ability to absorb water (Pinto *et al*., 2010, 2015; Courtois

et al., 2013).

- The capacity to obtain CO_2 for photosynthesis while reducing the loss of water, which is typically the basis of a series of compromises that determine how efficiently a plant uses water (Condon *et al*., 2004).

- The distribution of resources (growth against carbon storage, roots versus shoots, etc.) (Lopes *et al*., 2010).

- Protection from heat-related, dehydration-related, and oxidative stress-related harm, especially to reproductive growth (Rang *et al*., 2011; Barnabas *et al*., 2008).

Strategy for investigating the genetics of drought resilience in low-yielding environments

Since many years three main strategies are being employed to boost drought tolerance in wheat.

1. The empirical yield selection in conditions of water scarcity, this has been employed extensively and contemporary cultivars' strong performance attests to the effectiveness of this methodology. Tester and Langridge (2010) have noted that there are indications that the rates of gain are decreasing and will not be adequate to fulfill the demand.

2. Determine physiological ideotypes for higher yield in water constrained settings, identify the underlying factors causing variance in these attributes and incorporate these characteristics onto better cultivars (Reynolds *et al*., 2009; Richards *et al*., 2010). Despite being used for several decades, this strategy has had very patchy effectiveness. Carbon isotope discrimination is likely the sole feature that can produce novel cultivars when used to screen for WUE.

3. Marker assisted selection centered on identifying favorable alleles at drought tolerance QTL, there was not even a single case of drought or droughtrelated marker being employed, as per recent analysis of molecular markers applied to wheat breeding projects, despite numerous publications of QTL linked to drought tolerance (Table 1) (Gupta *et al*., 2010). Nearly 50 loci were found to be tracked using genetic markers at the time of the survey, but the only loci associated with tolerance to elevated soil boron (Bo1), aluminum toxicity (Almt-1), nematode resistance (cereal cyst and root lesion nematodes) and stature of plant were the factors

that performed best in low-yielding settings. This suggests clearly that loci useful for wheat breeding programs have not been found in the prior drought QTL research.

Table 1 : QTLS detected for wheat in response to tolerance to various abiotic stresses.

Stress	Chromosome location	Reference
Cold	5A,1D	Baga *et al.* (2007)
Salinity	47 QTLs	Ma *et al.* (2007)
Aluminum toxicity	4DL, 3BL, 2A, 5AS and 2DL	Cai *et al.* (2008), Ma *et al.* (2006)
Heat	1B, 5B and 7B	Mohammadi *et al.* (2008b)
Copper toxicity	1AL, 2DS, 3DS, 4AL, 5AL,5DL, 5BL and 7DS	Balint *et al.* (2007), Balint *et al.* (2009)
Nitrogen deficiency	2D, 4B and 5A	Laperche *et al.* (2008)
Drought	20 QTLs	Kirigwi *et al.* (2007), Mathews *et al.* (2008), Salem *et al.* (2007)

The poor efficacyof physiological and molecular breeding techniques up to this point indicates that our approaches need to be carefully rethought in order to gain deeper insight and produce drought resistant offspring. The problem of increasing wheat's resistance to drought should be approached by a multidisciplinary research program that integrates genetic and genomic tools like transgenic crops, quantitative trait loci (QTL) and microarrays with physiological analysis of drought-tolerance traits. Teams of molecular biologists, physiologists and breeders should be involved in performing the tasks. This strategy is used by significant businesses who have effectively shown how to apply intellectual property for field-based drought resistance (Passioura, 2007).

Fig. 1 clearly depicts how various "omics" technologies, population development, phenotyping, and physiological analysis promote a gene identification pathway. Determining the kind of drought regime/regimes that are under investigation is the first step, given the complexity and variety of the ecosystem that is experiencing drought. Choosing germplasm that is both appropriate for the intended conditions and likely to disclose important loci linked to tolerance is the second problem. The segregating populations which serve as the foundation for the genetic study are developed using these lines. Physiological traits, mathematical models and the omics of parental and chosen recombinant lines offer useful information for choosing potential QTL genes.
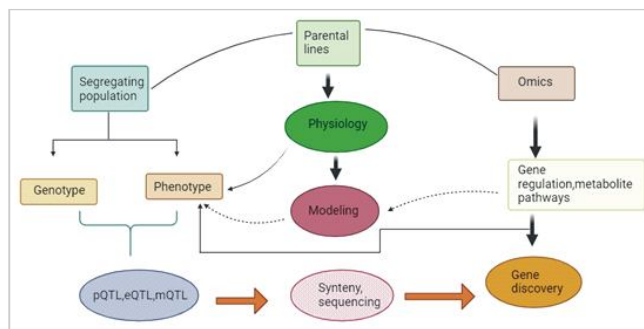

Fig. 1 : Diagrammatic representation of pathway from selection of parental lines to gene discovery.

Omics as a resource to assist other methods for discovery of gene

The prevailing view in drought genetics and physiology for a long time has been that we may improve drought response by developing physiological or biochemical models and then employ trials and cross-strategy to assess these approaches. The success of this technique has led many wheat breeders throughout the world to adopt it; in Australia, for instance, transpiration efficiency was selected for using carbon isotope discrimination (Condon *et al.*, 2004) and canopy temperature to choose deeper rooting (Reynolds *et al.,* 2009; Pinto *et al.,* 2010; Pinto *et al.*, 2015). This approach has even led to the widespread adoption of physiological breeding techniques (Reynolds *et al.,* 2009; Pask *et al.,* 2014).

The notion of whole genome sequencing, proteome, metabolome, and associated technologies has emerged, offering a helpful instrument for advancing and analyzing established characters that are adaptable in addition to a novel, non-hypothesis driven approach. It was widely anticipated that numerous genes or pathways that may be linked to improved drought response would surface if several large "omics" datasets were produced using drought-adapted versus unadapted lines in stressed and non-stressed environments (Langridge *et al.*, 2011). To date, these investigations have primarily served to validate the intricacy of drought response while offering minimal new perspectives. These databases do, however, now offer a resource that can be connected to alternative methods. For instance, if a significant yield-related QTL is found, gene expression databases can be used to look for the underlying genes and determine which ones become active or dormant in response to drought stress.; changed expression of a gene associated with stress pathway might be especially important, alternatively a germplasm panel may exhibit noticeable allelic variation (Courtois *et al.*, 2013; Thudi *et al.*, 2014). Additionally, presence or lack of polymorphisms for genes associated to stress adaptation, which we commonly observe, can

identify potential possibilities (Sutton *et al.*, 2007; James *et al.*, 2011; Gamuyao *et al.*, 2012).

Population composition for the genetic investigation of drought resilience

It is essential to carefully consider the size and makeup of population employed for genetic study. The selection of germplasm ought to be predicted on the probability of the lines yielding novel genetic combinations that are directly and immediately relevant to breeding programs aimed at producing cultivars suitable for the intended environment. Drought resistance selection shouldn't have a massiveadverse impact on other breeding program selection targets, including height, disease resistance, maturity and grain quality. There are certain advantages to using exceptional varieties in the intended setting, such as the ability to employ the lines straight into a breeding program. Furthermore, because they were previouslyselected during the production of elite wheat cultivars, alleles found in non-elite germplasm may not result in improvements (Collins *et al.*, 2008).

An overview of the traits for the parental lines covered below is shown in Table 2. The two lines Excalibur and RAC875 are important sources of drought tolerance in the climate of Southern Australia. Furthermore, compared to all other materials employed in the breeding programs, RAC875 was believed to show superior resistance to heat stress during grain packing. During 2006 season, when there was a severe drought and average yield at field locations was only 0.8t/ha, which when compared to other kinds, Excalibur; RAC875 consistently produced larger yields (116% and 122% of the site means, respectively). Under extreme water stress, Excalibur and RAC875 out yielded the variety Kukri, which was selected as the drought sensitive parent, by 10–40%.

Table 2 : Traits of Parental lines of wheat selected for drought mapping populations.

Traits	Excalibur	Gladius	Drysdale	Kukri	RAC 875
Heat tolerance	Intermediate	High	Unknown	Low	High
Drought tolerance	High	High	High	Low	High

Methods to escalate productivity under drought conditions

Genomics perspective

Next Generation Sequencing

Development of targeted crop enhancement tactics has been accelerated by technological breakthroughs in Next-generation sequencing (NGS) and effectively improved our ability to analyze the candidate gene(s) for desired features (Edwards *et al.*, 2013). Key crops like rice (Yu *et al.*, 2002), soybeans (Schmutz *et al.*, 2010) and maize (Schnable *et al.*, 2009) have all had their genomes sequenced. The International Wheat Genome Sequencing Consortium (IWGSC), 2018 released the completely annotated reference genome of wheat, which is noteworthy for offering a great platform for investigating new genes linked to desirable features. The environment has a significant impact on a plant's phenotypic appearance, which further leads to inconsistent selection of desired features. Regarding this, molecular markers have highly aided us in achieving the desired characteristics, such as drought tolerance with increased efficiency and dependability (Budak *et al.*, 2015). A new avenue for creating distinct molecular markers has been made possible by the advent of genomics. Moreover, the development of sequence-based Simple sequence repeats (SSRs) and Single nucleotide polymorphisms (SNPs) markers has been greatly aided by advances in genotyping and DNA sequencing methods in recent years (Lucas *et al.*, 2013; Pascual *et al.,* 2020). SNPs in particular are a valuable tool for genetics and genomics research due to their abundance and greater compatibility with high-throughput genotyping techniques. (Winfield *et al.,* 2016). The accessibility of sophisticated molecular markers has sped up the creation of the high-density linkage map and facilitated the discovery of stress-responsive genes and wheat QTLs (Budak *et al.*, 2015; Masoudi *et al.*, 2015; Gupta *et al.*, 2017).

Recombinant DNA technology

This has been shown to be an effective and trustworthy method for comprehending wheat's drought-adaptive responses. It has been demonstrated that the barley HVA1 gene, which codes for late embryogenesis abundant proteins (LEA), increases biomass buildup and water consumption efficiency during drought stress, leading to increased drought tolerance in transgenic wheat lines (Sivamani *et al.*, 2000). Furthermore, studies conducted on transgenic wheat containing proline-inducing gene (P5CS) revealed that the resistance against drought stress involves oxidative stress pathways. (Vendruscolo *et al.*, 2007). Osmo-protectant mediated improvement in drought tolerantis demonstrated by a comparable transgenic investigation by mannitol biosynthesis gene (mtlD) from Escherichia coli in wheat (Abebe *et al.*, 2003). In addition, it has been observed that transgenic wheat plants' DT under field conditions is enhanced by over expressing the soybean DRE-binding transcription factor (GmDREB1) (Zhou *et al.*, 2020).

Furthermore, a transgenic method was used to produce the first drought-tolerant cultivar (MON 87460) in maize by expressing bacterial cold shock protein B (Csp B) (Nemali *et al.*, 2015). Transgenic lines expressing the bacterial Csp genes (CspA and CspB) in wheat show better salt and drought tolerance when compared to the equivalent controls (Yu *et al.*, 2017). The study on the overexpression of the wheat TaPUB1 gene, which codes for U-box E3 ligase, in *Nicotiana benthamiana* shows that transgenic plants under drought stress had better antioxidant and survival capacities (Zhang *et al.*, 2017). Better drought tolerance during germination and seedling stage is also demonstrated by an analogous overexpression research of the wheat TaMYB30-B gene, which expresses R2R3-type MYB protein, in Arabidopsis (Zhang *et al.*, 2012). These studies imply that additional research and utilization of the putative candidate genes that are already present in wheat, could result in the development of an enhanced DT cultivar. Evaluation of the transgenic lines under field conditions is still necessary, despite the fact that the identification of genetically modified droughttolerant crops has made huge progress. On the other hand, comprehensive transgenic line phenotyping in a controlled environment might be useful for predicting the performance of cropsin field (Deikman *et al.*, 2012).

QLT$_s$ and genes governing Drought Tolerance

Crop output is eventually decreased by drought stress because it prevents photosynthetic and other vital metabolic processes. To enhance drought tolerance, techniques such as marker assisted breeding, quantitative trait loci mapping (QTL mapping) and introgression of a gene from the wild gene pool have been used (Merchuk-Ovnat *et al.*, 2016; Mwadzingeni *et al.*, 2016). According to Pour Aboughadareh *et al.* (2017), wild relatives of wheat may offer a source for enhancing stress tolerance. Recent advancements in high-throughput genotyping and phenotyping procedures have made it possible to examine complicated traits using more sophisticated methods, such as genomic selection. Molecular marker techniques, particularly Single nucleotide polymorphisms (SNPs), have demonstrated efficacy in deciphering intricate architecture of this feature. Additionally, QTL identification of drought tolerance continues to be difficult because of the impact of the vast majority and number of genes in wheat (Kadam *et al.*, 2012; Kumar *et al.*, 2012; Shukla *et al.*, 2015). Moreover, phenotyping data precision is crucial for identifying the genes or QTLs linked to drought tolerance (Tuberosa, 2012).

In addition, a number of phenotypic drought responsive characteristics in wheat have been connected to molecular markers, which permits accurate chromosomal mapping of these QTLs (Ibrahim *et al.*, 2012). Furthermore, high-through put phenotyping, molecular approaches, and biochemical analysis combine to derive information on the impact of some genes' expression on complex features as well as the presence and expression of other genes affecting particular characteristics. The discovery of relevant QTLs linked to desired quantitative traits, such as drought tolerance have revolutionized marker-assisted selection in genomics (Tuberosa *et al.*, 2006; Mir *et al.*, 2012). Specific QTLs for drought tolerant related features have been foundin wheat genomes A and B. It is noteworthy that the majority of the pertinent QTLs appear to be located on chromosomes 2B, 3A, 4A, 4B, 7A and 7B. Using varied mapping populations, several QTLs for drought tolerance related characteristics in wheat have been found. Some of the lists of the QTLs for drought tolerance and associated characteristics are shown in Table 3.

Reducing water loss requires an understanding of the mechanical underpinnings of mobility and concentration of stomata. It is interesting to note that QTL associated with the yield and quality enhancement attributes was discovered to colocalize with other varied QTLs on the 7A chromosome (Goel *et al.*, 2019). Wheat QTLs were found to be associated with nine different stress-induced hormones when applied exogenously (Castro *et al.,* 2008). Moreover, chromosomes 3B, 4A, and 5A had the most crucial QTLs for Abscisic Acid (Barakat *et al.,* 2015). Qyld.csdh.7AL, a key yield QTL, was recently successfully introgressed into four top notch Indian wheat cultivars to create genotypes that are resistant to drought and have high yielding characteristics under stressed environments (Gautam *et al.,* 2020). Of the nine key characteristics in the ultimate stages of drought stress, a QTL hotspot region on wheat chromosome 1B was discovered in a different study. QTLs for biological yield, grain number per spike, grain yield, spike length, spike weight, thousand grain weight, stem weight, and plant height are present in this hotspot region (Zandipour *et al.,* 2020). Although, a number of significant QTLs were found for droughtrelated features in wheat, drought tolerance is a complicated quantitative trait which is even influenced by a large number of minor QTLs. Thus, during investigations connected to drought, identification of minor QTLs associated with drought tolerance needs to be prioritized in order to produce wheat cultivars that are drought tolerant.

Table 3 : QTLS associated with drought tolerance and related traits in wheat.

Traits	Population analysis	No. of QTLs identified	Linked group to the chromosome	Marker linked	Reference
Drought adaptation related Meta QTLs	Meta analysis	502	All	SNPs	Acuna-Galindo *et al.* (2015)
Root length under drought stress	Core collection of 91 diverse genotypes	2	2B,3B	DArT	Ayalew *et al.* (2018)
Yield and related traits under drought and heat stress	RILs	5	2A,3D,6D,7B	SNPs	Liu *et al.* (2019)
Nine drought responsive agronomic traits	Doubled Haploid (DH)	98	20 different chromosomes except 4D	DArT, SSR, Gene based marker (MARS)	Gahlaut *et al.* (2017)
Grain yield under multiple environments	DH and RILs	1	3B	SSRs, ISBP	Bonneau *et al.* (2013)
Senescence related traits under high temperature	RILs	16	2A, 6A, 6B, 3A, 3B, 7A	SSRs, AFLP	Vijayalakshmi *et al.* (2010)
Terminal heat tolerance	RILs	3	2B, 7B, 7D	SSRs	Bonneau *et al.* (2013)

Proteomics and metabolomics

Proteomics

It is a highly effective approach for investigating the relationships between certain proteins, protein levels, and characteristics like Downs syndrome. Plant proteome responses are significantly impacted by environmental stress. Ye *et al.* (2013) performed proteomics analysis on wheat using matrix-assisted laser desorption/ionization (MALDI-TOF/TOF)-mass spectrometry (MS) and two-dimensional polyacrylamide gel electrophoresis (2DPAGE). Several drought-responsive proteins that are responsible for photosynthesis, energy production, protein biosynthesis, cell defense, carbon metabolism, oxidation-reduction and signal transduction were found to express differently. Furthermore, their investigation of proteomics revealed that the most significant impact of drought stress is onphotosynthesis of wheat. An analogous proteome investigation in Chinese Spring wheat- *Aegilops longissimi*, chromosomal substitution line CS-1Sl (1B) revealed the inclusion of 1Sl chromosome as potential source of gene for DT improvement in wheat and further emphasizedon involvement of albumins and globulins in drought stress. According to Zhou *et al.* (2016), there are five possible gene resources for wheat DT enhancement. 98 and 85 differentially expressed proteins in leaves and roots, respectively, of drought tolerant wild wheat (*Triticum boeoticum*) were found using comparative proteomics analysis.

Furthermore, in a drought-prone environment, various reactions specific to tissues were seen at protein level (Liu *et al.*, 2015b). Conversely, a comparative proteomic study of wheat grains for drought tolerant and sensitive varieties revealed that the bulk of differentially expressed proteins in droughttolerant (Kauz) and sensitive (Janz) cultivars are related to the metabolism of carbohydrates (26%), detoxification/defense (23%) and storage proteins (17%) (Jiang *et al.*, 2012). Moreover, a leaf proteomics analysis of two wheat cultivars; droughttolerant Ningchun 47 and drought sensitive Chinese Springshowed differential protein accumulation. (Cheng *et al.*, 2015). A distinct comparative proteome profiling study between durum wheat and wild emmer wheat revealed 75 proteins that were differently expressed in response to drought stress (Budak *et al.*, 2013). Using liquid chromatography (LC)- based quantitative proteomics (iTRAQ), researchers examined the impact of ABA in root proteome of wheat cultivars that are sensitive (Opata) and drought-tolerant (Nesser) and further indicated that ABA regulates differently and depends on variety during drought stress (Alvarez *et al.*, 2014). However, three bread wheat cultivars with different drought toleranceare found to have 1,299 proteins identified by a shotgun proteomics analysis (Ford *et al.*, 2011).

Metabolomics

Along with proteomics metabolomics is also one of the potent tool for examining alterations at the metabolite level in stressful environments. Nuclear magnetic resonance (NMR), Gas chromatography mass spectrometry, Liquid chromatography mass spectrometry (LC–MS), Gas chromatography mass spectrometryand Capillary electrophoresis mass spectrometry (CE-MS) are primary analytical instruments utilized for the metabolite characterization process. Bowne *et al.* (2012) investigated the metabolite reactions of 3 wheat cultivars under drought stress using a targeted GC–MS based metabolomics approach. Elevated levels of amino acids, specifically proline, leucine, isoleucine, tryptophan and valine were found in all of these cultivars. Likewise, elevated levels of proline, glutathione, methionine, cysteine and antioxidant defense system were discovered to be among the key factors contributing to wheat's ability to produce DT (Islam *et al.*, 2015). In wheat leaves under stress from drought, Hill *et al.* (2013) estimated the number of metabolites to be 205. They also noted a link between a few agronomic parameters and certain primary metabolites.
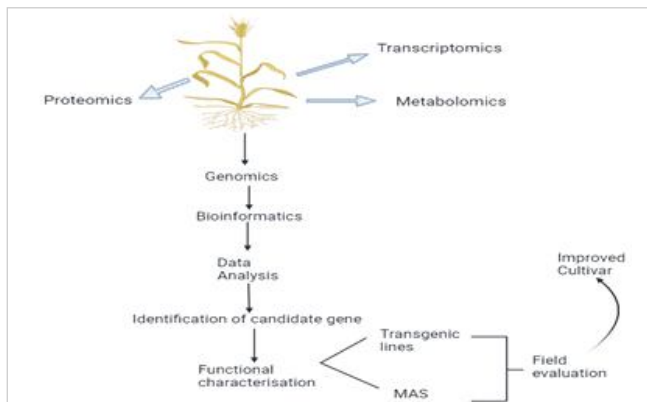

Fig. 2 : An outline of the several methods for improving drought tolerance in wheat.

According to their findings, particular combinations of metabolite are associated with a particular genomic area of the wheat genome. Augmented levels of proline, glycine, glucose, trehalose, and tricarboxylic acid cycle intermediates were foundon various wheat cultivar under drought stress (Krugman *et al.,* 2011). Using Ultra-performance liquid chromatography mass spectrometry (UPLC-MS), Guo *et al.* (2020) recently identified the metabolome of drought-tolerant (HX10) and sensitive wheat genotypes with the YN211 gene. When these genotypes were subjected to drought stress, differences in the levels of 56 metabolites were noted. The drought-tolerant genotype's robust DT capacity may be explained, in part, by its elevated levels of phenolics, thymine, alkaloids, amino acids, flavonoids and organic acids.

Conclusion

Drought-induced wheat losses significantly impact farmers' livelihoods, food availability and global economies, jeopardizing food security worldwide. Despite challenges, leveraging genomic tools offers promise in enhancing wheat's drought tolerance. Exploration of wheat diversity in gene banks and identification of genome-wide quantitative trait loci (QTLs) have facilitated the identification of potential drought-tolerant genes. However, few drought-resistant wheat cultivars have emerged, largely due to breeders prioritizing morphological traits over physiological drought resistance. Breeding for drought tolerance remains challenging due to the complex wheat genome. Integrating physiology, 'omics, and quantitative genetics is essential for developing drought-tolerant wheat cultivars, yet research initiatives embracing this approach are limited. Advances in genetic evaluation and trait breeding, enabled by novel genomic techniques, offer potential for producing superior wheat cultivars. Functional genomics has unveiled drought-signaling molecules, facilitating the identification of crucial genes for future breeding efforts. Additionally, CRISPR/ Casgenome-editing presents opportunities for deeper insights into drought tolerance pathways. Integrating various 'omics data with morpho-physiological responses accelerates the development of drought-tolerant wheat cultivars.

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