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CYTOGENETIC APPROACHES TO UNDERSTANDING DROUGHT TOLERANCE IN WHEAT: A REVIEW

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ABSTRACT

Drought tolerance in wheat is a genetically complex trait involving various genes and physiological adaptations. While conventional breeding has made progress, advances in cytogenetics have significantly enhanced our ability to identify and localize stress-responsive genes. Modern cytogenetic tools such as FISH, GISH, and chromosome engineering now allow precise mapping and transfer of desirable traits from wild relatives into cultivated wheat. This targeted approach helps breeders overcome challenges in improving drought resilience. The integration of cytogenetic techniques with physiological insights offers a promising strategy to enhance wheat performance under water-limited conditions. This review focuses on cytogenetic perspectives, highlighting methods like in situ hybridization and QTL mapping, while briefly touching upon genomics and gene introgression techniques relevant to drought tolerance improvement.

***Keywords* :** Drought tolerance, Cytogenetic techniques, FISH, GISH, QTL mapping

Introduction

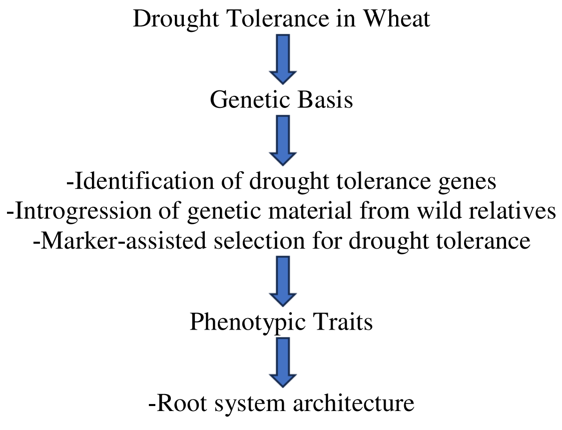
The development of drought-tolerant wheat cultivars requires a deep comprehension of the genetic basis of drought tolerance. Unfortunately, there hasn't been much development in this field because of a few wheat-specific traits (Bapela *et al.,* 2022). Firstly, wheat has three homoeologous genomes, each containing seven chromosomes, making it a complex genome polyploid. In terms of genetic analysis and the isolation of particular genes for breeding purposes, this creates unique challenges (Li and others, 2022). In order to produce a series of almost isogenic lines for each distinct drought-tolerant cultivar, backcrossing with a donor parent is a common method used in conventional Quantitative Trait Loci (QTL) study and gene mapping. It is no longer the most feasible method given the recent advancements, great promise displayed by transgenic crops, and the possibility of altering gene expression through marker assisted selection. Moreover, wheat has such a long and rich history of cultivation in so many different environments, that most work on drought tolerance has been confined to screening different cultivars rather than specific genetic studies with breeding outcomes in mind. This has rendered the obtaining of consistent and reliable results from generation to generation difficult (Mangrauthia *et al.*, 2024).

However, it is possible to view drought tolerance as a complex feature that combines a number of factors to allow a plant to live and complete its life cycle in the presence of restricted water. These elements consist of drought avoidance (maintaining tissue turgor and continuing growth at low water potential), tolerance (minimized yield loss per unit water lost), and escape (early flowering and seed set before to the commencement of severe drought). In the end, the expression of particular genes underlies all characteristics that provide drought resistance. Due primarily to the difficulty and intricacy of root systems in soil, the majority of studies on the genetic basis of crop drought tolerance have concentrated on water relations, leaf gas exchange, and, more recently, molecular markers linked to these characteristics (Shokat *et al.,* 2023).

Role of Cytogenetics in Studying Drought Tolerance

Whole chromosome transfers are often unsuccessful due to problems with chromosome pairing at meiosis between related species. When whole chromosomes are added to a chromosome complement, they often cannot pair with their homologues during metaphase and aneuploids will form (Song *et al.,* 2024). Euploid stocks may be produced if a whole genome doubling event is induced in a bridging genotype. High temperature treatments can double the chromosome number for a brief period, and plants treated at this stage can be regenerated in tissue culture to produce whole genome doubled plants. Whole genome doubling events often reduce chromosome number to a desired level for addition of chromosomes from another species (Chamara *et al.,* 2022). Techniques for genome in situ hybridization (GISH) using labeled chromosomes from a relative species to distinguish them from their homologues in a background of cultivated wheat have been developed and can be used to confirm whether the chromosomes have been successfully added into the wheat genome (Maurel & Nacry, 2020).

The physical mapping of genes on chromosomes, gene translocations between chromosomes, and the comprehension of the genetic basis of particular traits are all part of cytogenetic studies (Wight *et al.,* 2024). Genes for disease resistance and other features have been effectively introduced into farmed wheat through the use of translocations and gene mapping from wild relatives of the crop (Saini *et al.,* 2023). Cytogenetic techniques have been used to create wheat chromosome stocks which contain genes from wild relatives in the genetic background of cultivated wheats. These stocks have been extensively used for wheat improvement and have the potential to contain genes for drought tolerance (Farkas *et al.,* 2023).



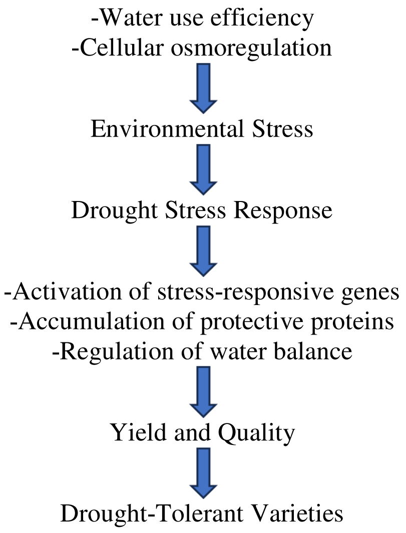


Fig. 1 : Drought tolerance in wheat

Genetic Mechanisms of Drought Tolerance

In wheat and its wild cousins, published investigations on candidate genes and gene expression have made a decent first step toward finding putative genetic elements governing drought resistance (Bapela *et al.,* 2022). Studies on candidate genes in wheat and barley have mostly concentrated on genes from other plant species that are known to play a role in stress response pathways. The QTLs identified for drought tolerance in wheat and barley have been found to significantly overlap with those found for genes involved in the metabolism of carbohydrates, osmotic control, and the scavenging of reactive oxygen species in rice and Arabidopsis (Elakhdar *et al.,* 2022). It is not unexpected that these genes are frequently discovered to be highly conserved between cereal species and other plant species, as these are essential mechanisms for plants' ability to withstand abiotic stress. An example is the HKT1;5 gene, which encodes a Na$^+$/K$^+$ symporter and was first identified as a QTL for leaf and root Na$^+$ accumulation and K$^+$ concentration in barley. This led to the cloning and sequencing of this gene for barley and wheat and development of a molecular marker to track this gene in wheat populations (Xu *et al.,* 2023). This marker proved useful in uncovering that an analogous Na$^+$ exclusion QTL and reduced leaf and stem Na$^+$ concentrations in durum wheat were due to a 1BL translocation from wild emmer, but in another study, the same mice this gene with underlying differences in Na$^+$ and K$^+$ selectivity. This highlights the need for functional studies on cloned genes and their products in determining effects on the phenotype and the potential for other linked genes in these QTL regions to also affect the trait of interest. This translocation will also be undemanding largely due to HKT1;5 being memory and potentially alter expression. Overall, these studies are making progress in linking phenotypic effects with specific genes and alleles, but the underlying functional and genetic complexity of these traits will require extensive further work.

The genetic analysis of this feature reflects the genetic complexity of drought tolerance in wheat. The genetics of wheat drought tolerance is a relatively little understood topic, and the genetics of wheat adaptation to a terminal drought is much less understood. But recent research is starting to clarify this complex characteristic (Li *et al.,* 2021). Understanding the genetic basis of this feature requires identifying the genetic components governing drought tolerance and how they are regulated. It is now feasible to comprehend the genetic regulation of drought tolerance more fully because to the development of molecular genetic techniques. This will help with marker-assisted selection in the creation of novel breeding tactics. Two recent reviews of this subject provide an overview of the knowledge that is currently emerging and will offer a framework to which we may add fresh data from wheat genomes and cytogenetic investigations (Khadka *et al.,* 2020).

Identification of Drought Tolerance Genes

One of the main goals of modern plant stress research is to unravel the genetic basis of drought tolerance. In wheat, the drought-tolerant cv. RAC875 has at least 40 up-regulated genes, according to representational difference analysis (RDA). There are several methods for mapping these genes, however isolating the genes directly is the most straightforward approach. Large amounts of pure DNA from the target gene are needed for this, which is challenging if the chromosome position and gene sequence are unknown (Tyagi & Pandey, 2022). Given the high gene content (80,000 genes) and >80% repetitive sequence of the wheat genome, the development of appropriate gene isolation and mapping tools is a work in progress. Due to its direct nature, isolating drought tolerance genes has been achieved in several cases (Khadka *et al.,* 2020). A barley NCED gene, key to ABA biosynthesis in drought conditions, was isolated via candidate gene approach and the rice salt and drought tolerance R2R3 MYB gene was found using a PCR-based method. With rapid advances in sequencing technologies, isolation of drought tolerance genes through identification of homologous genes in model species, mutation of known gene functions, and differential gene expression analysis is becoming more feasible (Nardino *et al.,* 2022). Mapping the location of gene to chromosome is an important step for cloning and functional analysis. Fluorescence in situ hybridization (FISH) is the most reliable cytogenetic method for gene mapping; however, it requires sequence information and chromosome-specific probes (Bapela *et al.,* 2022). Randomly amplified DNA markers can be used for comparative mapping across grasses and with sequence information, converted to chromosome-specific markers for map anchoring. When considering the large number of genes involved, a high-throughput method such as Eco tilling may be more practical. This reverse genetic approach uses tilled populations to identify single nucleotide polymorphisms (SNPs) in specific genes, by mismatch cleavage of gene PCR products. As an alternative to gene isolation, tilling can identify sequence polymorphisms in candidate genes from model species with known function in drought tolerance (Kandel, 2021).

Genetic Variation and Drought Adaptation

Development of wheat variants resistant to drought sometimes involves the introduction of genetic material from related species. Positional cloning of these QTLs, along with comparative genomic techniques, offers a chance to study the molecular genetic underpinnings of genotypes' varying responses to drought. Several QTLs for drought tolerance components have been found. Numerous wild cousins of wheat have adapted to grow in arid areas because drought is a major element in plant diversification. Considered the direct progenitor of hexaploid wheat, *Aegilops tauschii*, the D-genome donor, is native to an area with hot, dry summers and cold, rainy winters that stretches from NE Iran to NW China. Since the main wheat-growing regions of Australia have a similar environment, many D-genome-containing lines including cultivars, landraces, and wild accessions from this bread wheat progenitor are probably going to include helpful genes for drought tolerance. This offers a chance to take use of the genetic heterogeneity found in bread wheat and its wild cousins by employing a germplasm enrichment method that includes target QTL selection aided by markers, selection based on phenotypic performance under drought, and crossover to a contemporary elite genetic background. By using this method, popular wheat varieties that have been adapted for high rainfall and drought conditions may also be produced. Additionally, pre-breeding materials may be introduced into wheat breeding projects in the form of BIPs and/or candidate varieties.

Cytogenetic Techniques for Studying Drought Tolerance

Genomic in situ hybridization (GISH) is another helpful method in gene isolation. Using genomic DNA from a donor species that is permitted to hybridize in the metaphase chromosomes of a closely related recipient species is known as GISH. The difference in chromosome coloration or DNA sequence between both genomes allows the determination of gene loci and specific gene transfer from donor to recipient species. This would be very useful for the identification of genes which have been transferred from a wild relative species of wheat, which may aid drought tolerance and survival in harsh environmental conditions (Cabral-de-Mello & Marec, 2021).

With the recent advancements in DNA technology, one can assume that gene tagging and isolation will be the key to understanding drought tolerance in wheat. This can be achieved by locating specific genes and identifying the functions of each. This may then permit the creation of genetically modified wheat with higher drought tolerance without altering important agronomic traits (Nouraei *et al.,* 2022).

Compared to traditional karyotyping, fluorescence in situ hybridization (FISH) is a more advanced and novel method. This method makes use of a fluorescent DNA probe that can attach to a particular area of a cell's chromosome. The location of the probe is determined by the wavelength of light emitted. FISH has been widely used in human genetics, yet its application to plant studies has been limited (Takahashi *et al.,* 2023). By identifying variations in chromosomal homology and structural rearrangements between a drought-tolerant donor and a drought-sensitive recipient wheat genotype, this method has great potential for the study of drought tolerance. These differences may result in selective gene transfer by wide crossing. Any chromosomal differences found would indicate potential target areas for gene isolation or further investigation into gene expression (Sharma *et al.,* 2023).

Chromosome analysis and karyotyping, the study of the number and appearance of an organism's complete set of chromosomes, often demands a prolonged period of stress for the observation of induced chromosomal changes. The results often indicate various cytogenetic changes which are associated with the expression of a drought response. Using this information, it is possible to select for plants which have altered certain chromosomes that may confer a more favorable response to water deficit (Liu *et al.,* 2024).

Chromosome Analysis and Karyotyping

Karyotype analysis has been used to study chromosome structure and variation in wheat. It has provided useful information regarding the karyotype evolution within the genus and has assisted in the characterization of certain chromosome mutations associated with improved stress resistance (Mou *et al.,* 2022). *Aegilops tauschii* chromosomes from the D genome have been identified in lines carrying enhanced stress tolerance through mutation breeding of the CIMMYT bread wheat line. Phenotypic observation of these lines has revealed the possible presence of genes located on the 3D chromosome, which confer an improved drought response compared to lines with the normal 3B (Sun *et al.,* 2024). 3D translocation lines identified using cytogenetic techniques may be crossed with 3B deletion lines to identify genes existing on the 3DS chromosomal arm related to drought response (Adel & Carels, 2023). With the recent announcement by the International Wheat Genome Sequencing Consortium (IWGSC) to comprehensively sequence the wheat genome, the applicability of this information has been limited thus far. Research on the characterization and isolation of genes linked to drought and other stress responses in wheat is likely to increase. Karyotyping will provide an essential tool for sorting and identifying the chromosomes of interest and their relative genes for further analysis (Mou *et al.,* 2022).

Fluorescence In Situ Hybridization (FISH)

A technique called DNA fluorescence in situ hybridization (FISH) makes use of a DNA probe that is unique to a certain chromosomal region in an individual's genome. It is a very efficient technique because it allows visualization of crosses from the DNA to RNA with very few side effects to the RNA (Adel & Carels, 2023). Utilizing FISH, plant breeders can assist in introducing important genes into wheat cultivars in the wild cousins of wheat. This is due to the fact that the genome configurations and chromosomal morphologies of wheat's wild cousins frequently differ greatly. (Adel & Carels, 2023) FISH can be used to help plant breeders in the wild relatives of wheat to introgress valuable genes into wheat cultivars. This is because wild relatives of wheat often have very different genome arrangements and chromosome morphologies (Mou *et al.,* 2022). If a breeder has isolated a particular chromosome or chromosomal region which has been found to confer a favorable trait such as disease or pest resistance, drought or salt tolerance, it is desirable to keep as much of the wild relative chromosome as possible to maintain as many of the favorable wild relative gene linkages as possible (Adel & Carels, 2023). If a DNA probe has already been isolated for a certain chromosome of a wild relative using FISH, it may be possible to identify the chromosome in the wheat-wild relative introgression and isolate the chromosome in a backcross generation (Zhang *et al.,* 2022).

Genomic *In Situ* Hybridization (GISH)

The intriguing but effective method of studying the genetic links between distinct species within a genus or between genera is called genomic in situ hybridization, or GISH. The importance of GISH has been realized after getting success in various crops, including wheat, for which this technique was first developed (Adel & Carels, 2023). It makes use of genomic DNA from one species as a probe on the chromosomes of other species of the same genus, in a solution of blocking DNA from the species which is being used as the source of the probe (Mou *et al.,* 2022). The extent of DNA sequence conservation can be evaluated by the intensity of probe hybridization using the material of the source species, compared to the material of the species being examined. This way, it is possible to introduce the desired genes of the source species to the target species through marker-assisted selection without incorporating unwanted genes (Mou *et al.,* 2022; Georgieva & Vassileva, 2023). Although target gene introgression through wide hybridization results in a chromosomal linkage drag in the segregating progeny, GISH is still effective in discriminating the chromosomes of two related species. At the beginning of wide hybridization of *T. dicoccoides* and *T. durum*, with the aim of transferring valuable genes for stem reserve mobilization from Aegilops species into durum wheat, we used the orthodox GISH protocol to compare chromosomes of wild species and durum wheat (Georgieva & Vassileva, 2023). The degree of hybridization was insufficient to distinguish between the durum wheat genome chromosomes A and B, hence we were unable to obtain accurate results. Later on, we modified the protocol by making the probe DNA into a mixture of A, B, and S genome DNA, and it worked best for discriminating A and B genomes from the S genome of the donor species *T. dicoccoides* (Sun *et al.,* 2024). This modification proved to be a landmark for discriminating wheat chromosomes, and it was extensively utilized by various workers who were working on wide hybridization of wheat. However, in order to obtain the necessary results, GISH needs a highly developed technique and highly skilled labor, and the lack of these resources has remained a barrier to its wider deployment in order to investigate the genetic basis of drought tolerance in crop species (Mou *et al.,* 2022; Zhang *et al.,* 2022).

Mapping Drought Tolerance Loci in Wheat

In order to analyze the genetic basis of wheat's drought tolerance, genetic mapping and linkage analysis have been widely used. Genetic mapping cannot occur without the creation of molecular markers and genetic maps. Because there were mapping populations available and well-characterized marker polymorphisms, RFLP markers were the first to be mapped (Georgieva & Vassileva, 2023). Using a population of recombinant inbred lines (RILs) from the cross, a genetic linkage map based on an RFLP map of Seri and Babax, created in our lab, was built. With rice maps and the rice genome sequence at their disposal, plant biologists and the rice research community have an incredible opportunity to advance our knowledge of rice biology and use it to improve a staple grain that feeds over half of the world's population (Georgieva & Vassileva, 2023). To identify the rice genes corresponding to the Seri-Babax QTL for root penetration ability, a two-step diploid wheat-wild emmer wheat-rice method of gene transfer was developed (Sharma *et al.,* 2022). Genome-wide association studies (GWAS), which use natural populations to test the genetic link to complex traits, have replaced genetic mapping using bi-parental populations thanks to advancements in genotyping and phenotyping technologies. GWAS offers several advantages over bi-parental linkage mapping, including higher mapping resolution, avoidance of the population-specific effects, and lower research costs (Kui *et al.,* 2023). In a fruitful pilot investigation, loci linked to drought-resistant root characteristics in hexaploid wheat produced from synthetic sources were found using a heterogeneous population of landraces. Seminal root angle, number, and length of a panel of 384 different synthetic-derived lines were phenotyped at two locations in Mexico and Morocco. The panel's genetic diversity was assessed using a set of 1,536 SNP markers, and many QTL linked to root angle were found due to the landrace populations' high linkage disequilibrium (Xie *et al.,* 2024). A subsequent study used the same panel to identify loci associated with the stay-green phenotype, an indirect marker of terminal drought tolerance, at multiple environments in Mexico, Morocco, and Chile. A major advantage of these GWAS approaches is that the genetic resources, marker platforms, and datasets developed can be shared and used by the wider wheat research community (Xie *et al.,* 2024).

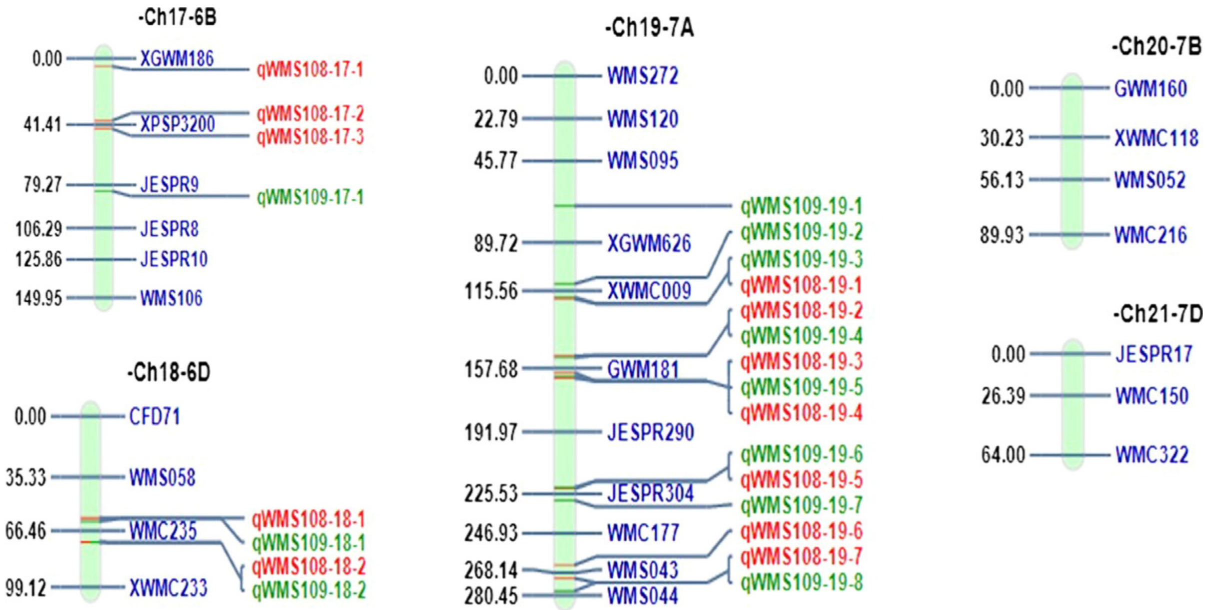


Fig. 2 : Identification and Mapping QTLs linked to drought tolerance traits in wheat via SSR markers.

Quantitative Trait Locus (QTL) Analysis and Genetic Mapping

Proof of concept for marker-assisted selection of a drought tolerance trait involved a study to select for improved root characteristics in barley (Xie *et al.,* 2024). A hybrid between a drought-tolerant variety (Horn) and a drought-sensitive variety (Gairdner) produced two doubled haploid populations, based on the observation that seminal root number was substantially linked with final shoot biomass under drought-stressed circumstances (Xie *et al.,* 2024). SEM observation of the root systems in the two populations allowed for detailed phenotyping of seminal root quantity in both control and drought conditions. Analysis of variance showed a significant genotypic effect, and greater than expected similarities in the seedling biomass data and the root number were further used to justify a QTL study to map root number and direction in the ND2405 x Herrman and CM72 x Harrington populations (Zwyrtková *et al.,* 2022). A more detailed and 'higher throughput' marker analysis technique was employed using the Herrman X Gairdner population by employing AFLP with the intent of using results to select for better water use efficiency in the Horn/Gairdner cross (Xie *et al.,* 2024).

Genetic mapping is a process by which a genotypic factor (usually a phenotype) is associated with a specific set of molecular markers or a genomic region by studying inheritance patterns in segregating populations. If the trait is under genetic control, then the marker/trait association will persist over generations in a predictable manner (Xie *et al.,* 2024). In the specific case of QTL analysis, the trait's heritability is compared to that of known markers distributed across the genome to determine if any co-segregation is occurring between the trait and marker in question. In the event marker and QTL heritability are significantly associated, the marker can be used as a predictor for the trait in breeding programs due to the fact that trait expression should occur in a similar fashion to the observed co-segregation. This allows for more efficient and accurate trait selection than phenotype screening in field environments. During the past 20 years, there have been many trials to map drought tolerance traits in wheat using this approach (Xie *et al.,* 2024).

Table 1 : Genetic Categories and Instances Associated with Drought Tolerance in Wheat

Category	Description	Example Genes
Signal Transduction	Genes involved in sensing and responding to drought stress	Protein kinases, transcription factors
Functional Factors	Genes responsible for cellular processes that improve drought tolerance	LEA proteins (involved in protecting cells), enzymes for osmoregulation (maintaining water balance), ROS (reactive oxygen species) scavengers
QTLs (Quantitative Trait Loci)	Chromosome regions associated with features related to drought tolerance	Drought-related grain yield, root system architecture, and water use efficiency

Genome-Wide Association Studies (GWAS)

GWAS has been effectively applied to rice and other diploid crops to uncover potential genes linked to complex characteristics (Zwyrtková *et al.,* 2022). However, due to its complex genome and absence of widespread linkage disequilibrium (LD), wheat poses a special difficulty for association mapping, especially when it comes to landraces and breeding material from different geographic origins (Rauf *et al.,* 2021). Despite this, successful association mapping of the vernalization loci in tetraploid and hexaploid wheats and the Ppd photoperiod response genes have provided proof of concept that GWAS can be applied to dissect complex traits in wheat using ploidy-specific marker platforms (Heslop-Harrison *et al.,* 2023). These vernalization and photoperiod investigations failed to find connections between several candidate genes and phenotypic variance, which emphasizes the necessity to strike a balance between statistical stringency and phenotypic resolution, especially when traits are influenced by many genes with tiny effects (Parade *et al.,* 2021). The ability to do high-density GWAS on vast and diverse germplasm panels has increased because to the significant advancements in SNP arrays and the affordability of genotyping-by-sequencing techniques for wheat. This has increased the ability to analyze the genetic basis of complex characteristics. Drought tolerance needs to be narrowly defined with precisely quantified phenotypes that are measured across multiple environmental conditions (Burridge *et al.,* 2023). By expanding on earlier developments in physiological and agronomic trait phenotyping for drought association mapping, it will be possible to mine the germplasm for alleles that are particularly adapted to drought and decreased water availability (Srivastava *et al.,* 2022).

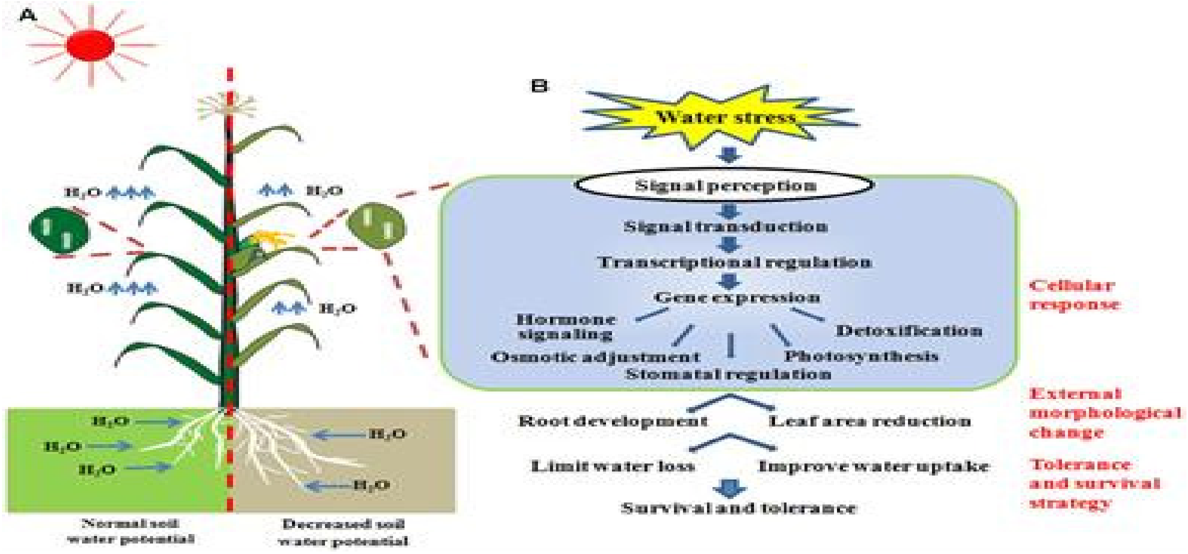


Fig. 3 : Natural Variations Affecting Crop Resistance to Drought: A Genome-Wide Association Study

Comparative Genomics and Synteny Analysis

The beginning of the comparative genomics era in wheat has been significantly enhanced by the release of the full sequence of the rice genome. The B-trifoliate complex, which includes rice and wheat, is a group of grasses that are thought to have diverged from a common ancestor between 50 and 70 million years ago. This level of divergence is considered minimal, and it is expected that vast areas of the genome are still conserved between the two species. This conservation of genome structure and gene content is exemplified by the extensive collinearity between rice and wheat chromosome 3A (Walkowiak *et al.,* 2020). created an ideogram that illustrates the sections of rice 3, rice 1, and rice 5 that are preserved in 3A using a sequence homology-based method. This type of analysis allows QTL from rice chromosome regions to be quickly linked to wheat QTL, and candidate genes can be directly identified in the rice genome with full knowledge that they are looking at a genetic source that has an equivalent in wheat (Bharti *et al.,* 2023).

Comparative genomics is the comparison of two genomes to discover the genes and the regions in these genes that are similar and dissimilar. Synteny is the conservation of blocks of order within two sets of chromosomes that are being compared to each other (Wang *et al.,* 2024). Comparisons between model species (such as Brachypodium, rice, barley, etc.) and wheat can give clues to gene function that can be exploited in wheat improvement. Much of the genetic and QTL mapping work in wheat has focused towards making it more drought tolerant, so this essay will focus on the synteny between regions of chromosomes that contain genes for drought response/tolerance in wheat and other cereals. This subject is very detailed, and this review is using just a few key examples to illustrate the potential of this approach (Tian *et al.,* 2023).

Genetic Engineering to Improve Wheat Drought Tolerance

The development of genetically modified (GM) wheat varieties has the potential to greatly increase drought tolerance. Significant progress has been achieved in the last few years in creating effective genetically modified wheat. These include Agrobacterium-mediated gene gun transformation and more recently, the use of CRISPR/CAS9 (Bapela *et al.,* 2022). GM strategies for improving wheat osmoregulation have received much attention given its importance for maintaining grain filling under terminal drought. Numerous enzymes, such as fructan:fructan 1-fructosyltransferase (1-SST), fructose exohydrolase (FEH), and sucrose:sucrose 1-fructosyltransferase (1-SST), regulate the buildup of osmolytes (Sharma *et al.,* 2023) (Bian *et al.,* 2023). Transgenic wheat plants overexpressing various osmoregulation enzymes have shown improved tolerance in moderate to severe water deficit, albeit abnormalities in vegetative growth and development were for some lines an impediment to agronomic superiority (Mondal *et al.,* 2023). Optimizing this strategy requires an understanding of the genetic underpinnings of various osmoregulation networks and the integration of transgenic expression within them. Once the genetic basis for a given strategy is understood, standard GM techniques can be employed for introgression into elite varieties. More recent advances in GM and non-GM based introgression methods are discussed by (Kim *et al.,* 2023).

The potential of genetic manipulation to improve drought tolerance in wheat is immense. While substantial advances in knowledge have been made using the model plant rice, much remains to be done in wheat. The current lack of drought tolerant commercial wheat varieties reflects the genetic complexity underlying this trait (Bapela *et al.,* 2022). Development of improved varieties has been impeded by the often-poor correlation between the drought tolerance of donor wheat lines and the agronomically superior but drought susceptible elite varieties. Understanding the genetics of drought tolerance in detail is therefore necessary to enable targeted breeding or genetic engineering approaches based on certain genetic origins (Kumar *et al.,* 2023).

Overexpression of Drought Tolerance Genes

We have also had promising results from transgenic wheat plants overexpressing enzymes involved in trehalose synthesis. Trehalose is a non-reducing disaccharide with a strong desiccation protectant function, and there is evidence that its presence in transgenic plants can provide osmotic protection and increase stress tolerance. In ongoing work, the group is involved with genome-wide studies of cis-acting regulatory elements, aimed at obtaining expression data for both endogenous dried genes and those introduced from other species. Should it become possible to achieve tissue-specific or inducible expression patterns for any given transgene, the chances of success in obtaining a drought-tolerant phenotype will be greatly enhanced (Jinhua *et al.,* 2022).

mRNA targeted to the plastid of cells from the resurrection grass *Sporobolus stapfianus* gave encouraging results in transgenic rice. Transgenic plants demonstrated much greater growth, yield, and drought recovery than control plants when the gene was expressed under the control of the maize ubiquitin promoter. These data indicate a clear potential of this gene in improving drought tolerance, and success in similar work with wheat would be of great benefit given the known water-wasting proclivities of C3 cereals (Xie *et al.,* 2023).

The foregoing description of drought-related gene identification provides a strong foundation for biotechnological methods aimed at enhancing wheat's resistance to drought (Adel & Carels, 2023). Creating transgenic wheat plants that overexpress genes for drought tolerance is the easiest place to start. Early research used the 35S promoter of the cauliflower mosaic virus (CaMV) to control the introduction of two genes encoding the late-embryogenesis abundant (LEA) proteins into wheat. Unfortunately, despite the promise of the promoter in driving wide-ranging gene expression in monocots, the transgenic lines showed no improvement in drought tolerance over null segregants, and neither expression of the transgene nor the endogenous wheat LEA genes were found to be drought-inducible (Zhou *et al.,* 2022).

CRISPR/Cas9-Mediated Editing of Genomes

The greater problem with the earlier genetic alteration technique is target selectivity. Consequently, it would be more economical to change endogenous genes using CRISPR/Cas9 systems. It has recently been demonstrated that CRISPR/Cas9 functions in wheat (Zhou *et al.,* 2022). An effective procedure was developed using an RNA-guided Cas9 nuclease system for the targeted mutagenesis of the TaMATE1 gene, which controls wheat's tolerance to aluminum. The editing was observed in T0 transgenic plants with mutation rates at the targeted site in the TaMATE1 loci of 30-100%. Five mutated lines transmitted the edited TaMATE1 gene to the T1 generation, and these plants were not phenotypically different from null segregants. The wheat genome has three homoeologous copies of TaMATE1, which makes the mutant gene transmissible and prevents phenotypic change. To achieve the desired phenotype, a greater mutation rate and multiple mutation selection are required. In spite of this, the findings show that CRISPR/Cas9 is a more accurate and technically straightforward technique for producing targeted mutations than other approaches like TILLING (Targeting Induced Local Lesions IN Genomes), which necessitates an expensive screening procedure in order to identify the plant containing the desired mutations (Hossain *et al.,* 2023). The isolation and identification of null segregants in T0 plants can be achieved through progeny screening and genotypic analysis, representing an additional cost effectiveness to this genome editing technique (You *et al.,* 2023).

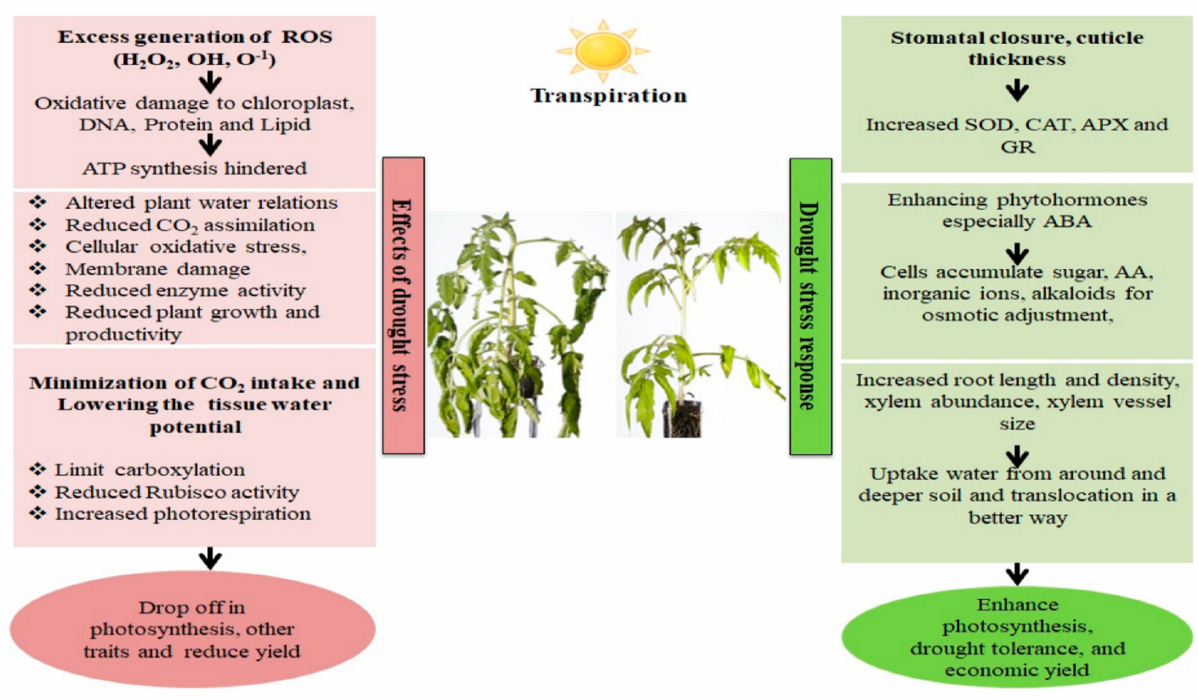


Fig. 4 : Increasing Crop Resilience to Drought Stress with CRISPR-Cas9 Genome Editing

Breeding Strategies for Enhancing Drought Tolerance

Comparing MAS to the alternative transgenic method of changing the genetic makeup of plants, it is also possible to achieve greater efficacy at lower implementation costs. Due to social and regulatory restrictions, this method of gene transfer has not yet been very effective in genetically enhancing drought tolerance. However, the extremely quick progress in plant genetic engineering and the proof of some transgenic breakthroughs for drought tolerance indicate that the role and effects of transgenics may be modifiable in the future (Anwar & Kim, 2020).

During the breeding process, particular genes or genomic areas that underlie a target trait are chosen using a technique known as marker-assisted selection (MAS). This requires that there is a known genomic region or gene variation that is linked to phenotypic variation in the target trait, and that this can be assayed with a molecular marker. Given the polygenic and complex trait of drought tolerance, MAS could theoretically be applied to several aspects of this target trait, although finding markers and the utility of this approach will vary between different aspects of drought tolerance (Rayaprolu *et al.,* 2022) (Shende & Shinde, 2023). With the exponential fall in cost and increase in throughput of marker data owing to ongoing advancements in DNA sequencing technologies, MAS is now a feasible option for selection in breeding projects, especially in self-pollinating crops. When juxtaposed with indirect selection of these traits via phenotypic data, this could significantly facilitate the collection of genetic variation for drought tolerance in elite and modern germplasm (Budhlakoti *et al.,* 2022).

The fruition of functional markers for drought resistance genes, allied with developments in molecular and genomic techniques, will likely elucidate the basis of remarkable progress in genetic enhancement of drought tolerance. Essentially, there are currently three alternative breeding strategies that can benefit from the genetic information and resources being gathered (Raza *et al.,* 2023). Conventional breeding tactics aimed at enhancing drought tolerance will have a stronger basis thanks to our diligent study of the physiological and molecular mechanisms of drought tolerance and the genetic variation underlying them. However, the introduction of this genetic knowledge into breeding programs also presents chances for more drastic systemic modifications that might completely reimagine the approach and effectiveness of drought tolerance breeding (Shokat *et al.,* 2023).

Marker-Assisted Selection (MAS)

The process of finding and choosing desirable features based on the existence of a gene or DNA marker that is directly linked to that trait is known as marker-assisted selection (MAS). If the DNA marker is known for a specific gene conferring drought tolerance, MAS can be a powerful tool for selecting the positive trait (Sunil kumar *et al.,* 2023). In comparison to phenotype selection, MAS can be faster, more efficient, less costly, and can enable selection of several loci using multiplexing methods. For these reasons, MAS has been the subject of much research and interest in plant breeding including wheat. Although the technique is simple in theory, there are now a number of barriers that make it impossible to use MAS to increase wheat's resistance to drought. These include the intricacy of features related to drought tolerance and the absence of known genes and molecular markers (Pradhan, 2023; Kumari *et al.,* 2024). With the speed at which technology is developing in this area, it might not be long before MAS is utilized as a helpful tool for wheat drought tolerance breeding (Heredia *et al.,* 2022). The capacity to produce a high-density genetic map for a species is the result of significant advancements in genomics technology. Effective MAS requires a genetic map of this kind, and in the past ten years, genetic maps comprising thousands of DNA markers have been created for wheat. Numerous agronomically significant wheat features have loci identified thanks to a high-density genetic map, which has also made MAS for quality and disease resistance traits easier to implement. MAS will become more and more possible as QTL mapping research advances and candidate genes for drought tolerance in wheat are found (Berraies *et al.,* 2023). The study of genotype by environment interactions can potentially benefit from the identification of genes or DNA markers linked to drought resistance. With this knowledge, genes that are particularly successful in drought-prone locations could be chosen, enabling the creation of drought-tolerant cultivars that are suited to their intended settings. Though there isn't much use of MAS for drought tolerance in wheat right now, more study and genetic understanding will make MAS a more potent tool in the future (Pandey *et al.,* 2021).

Genomic Selection (GS)

The estimated breeding value (EBV) of an individual or a line is used in genomic selection (GS), a type of marker-assisted selection, based on whole-genome marker profiles and phenotypic data. Genomic selection is an appealing method for enhancing complicated traits like drought tolerance since it can accelerate the pace of genetic gain in breeding cycles as compared to MAS (Beena *et al.,* 2021). The success of GS depends on the assumption that every QTL influences the trait; in other words, GS fits every QTL by creating GEBVs by fitting a large number of markers using regression models, RDF, or BLUP. Another major benefit of GS is that it is capable of predicting the performance of untested lines or crosses, allowing breeders to discard poor-performing lines before phenotyping. This can save significant time and costs, particularly in the creation of near-isogenic lines for QTL validation and transfer to breeding lines. Even though GS has a lot of potential, its high genotyping costs have prevented it from being widely used in plant breeding. However, as genotyping prices decline, this possibility is growing (Mishra *et al.,* 2021). The use of GS in plant breeding to address drought tolerance has the potential to significantly accelerate the development of novel drought-tolerant cultivars and assist meet the growing demand for food in water-limited environments (Kumar *et al.,* 2020).

Hybridization and Selection for Drought Tolerance

Hybridization is the process of breeding and introducing genes from a donor parent to a recipient parent depending on the phenotypic selection of the segregating generations. As exterior genes from a donor parent are usually present in a wild species and may carry unfavorable alleles, the primary aim is to substitute these genes with the original genes of the recipient parent (Mishra *et al.,* 2021). Phenotypic selection applied at each generation can be effective if germplasm is available in the hybrid generations and if the trait is under simple genetic control. Success of the selection will depend on the strength of the phenotypic expression of the drought tolerance in the segregating populations, the heritability of the trait, and the volume of gene substitution. Since Simmonds first proposed this method in his models (1977), little progress has been made in practical terms of selection. Phenotypic selection is the most commonly used method of gene introgression into cultivars/regional gene pools with a known trait target. This has been a successful method for improving drought tolerance into landraces and cultivars with specific traits that can be phenotypically screened under drought, i.e. stay-green into sorghum (Verma & Sarma, 2021). The CWR method is a form gene substitution using backcrossing (avoiding mutations of the species) and selection of the portion of wild genomic and variation in a modified or synthetic hybrid to improve trait performance (Chakraborty *et al.,* 2022). Selection success will depend on the proportion and frequency of favorable alleles and also the maintained variability so that wild alleles are not fixed. This can be difficult if the wild species is interfertile with the crop, as the first-generation wild hybrids are usually sterile or have fertility barriers. This is largely due to their reduced adaptability and/or lowered induced fitness with a change in ecology from natural to agro-ecosystem. The introduction of transgenes by biotechnology can be employed to increase the GM (Li *et al.,* 2021). This model seeks to develop a cost-effective breeding strategy to increase performance of maize hybrids in low input areas of SSAP's using qualitative traits, i.e. drought tolerance. These are expected to be more cost-effective and realistic than setting a transgenic target for hybrid performance in poor populations (Chakraborty *et al.,* 2022). Drought stress treatment environments are simulated to find the gene frequency and marker for a given trait under these defined conditions. The frequency of genes with markers is then assessed in a subsequent generation using marker-assisted or phenotypic selection in correlation to trait performance. Although the later sections of the model are yet to be developed, the methodology can be tested as the gene frequency and trait performance is known. This is therefore a realistic method of simulating the potential outcome of a breeding strategy and can be an excellent tool for comparing the expenditure and trait improvement of any breeding strategy for drought tolerance (Mishra *et al.,* 2021).

Future Directions and Challenges in Drought Tolerance Research

Future research in wheat drought tolerance should prioritize cytogenetic approaches that enable the precise introgression of stress-tolerant genes from wild relatives. Emphasis should be placed on refining techniques such as GISH and FISH for better chromosome tracking and gene localization. With advances in wheat genome sequencing and high-throughput marker systems, integrating cytogenetics with genomics will accelerate the identification of candidate genes. Multi-environment trials and high-resolution mapping will be essential for validating these genes and ensuring their effective use in breeding programs.

Conclusion

Cytogenetics plays a pivotal role in enhancing wheat drought tolerance by enabling targeted transfer of beneficial genes. Tools like GISH, FISH, and karyotyping support the identification and introgression of chromosomal segments linked to stress adaptation. With improved molecular markers and genome data, marker-assisted selection and chromosome engineering can be more effectively used to develop resilient cultivars. A strategic combination of classical breeding, cytogenetics, and molecular tools holds great promise for sustainable wheat improvement under drought-prone conditions.

References

Adel, S. & Carels, N. (2023). Plant tolerance to drought stress with emphasis on wheat. *Plants*. **12**(11), 2170.

Anwar, A. & Kim, J. K. (2020). Transgenic breeding approaches for improving abiotic stress tolerance: recent progress and future perspectives. *International Journal of Molecular Sciences*, **21**(8), 2695

Bapela, T., Shimelis, H., Tsilo, T. J., & Mathew, I. (2022). Genetic improvement of wheat for drought tolerance: Progress, challenges and opportunities. *Plants*, **11**(10), 1331.

Beena, R., Kirubakaran, S., Nithya, N., Manickavelu, A., Sah, R. P., Abida, P. S., ... & Siddique, K. H. (2021). Association mapping of drought tolerance and agronomic traits in rice (*Oryza sativa* L.) landraces. *BMC Plant Biology*, **21**, 1-21.

Berraies, S., Cuthbert, R., Knox, R., Singh, A., DePauw, R., Ruan, Y., ... & Meyer, B. (2023). High-density genetic mapping of Fusarium head blight resistance and agronomic traits in spring wheat. *Frontiers in Plant Science*, **14**, 1134132.

Bharti, J., Verma, R., Gupta, I., Chakraborty, P., Eashwaran, M., Sony, S. K., ... & Kaul, T. (2023). Functional characterization of novel mutations in the conserved region of EPSPS for herbicide resistance in pigeonpea: structure-based coherent design. Journal of Biomolecular Structure and Dynamics, 1-16.

Bian, H., Zhou, Q., Du, Z., Zhang, G., Han, R., Chen, L., ... & Li, Y. (2023). Integrated Transcriptomics and Metabolomics Analysis of the Fructan Metabolism Response to Low-Temperature Stress in Garlic. *Genes*, **14**(6), 1290.

Budhlakoti, N., Kushwaha, A. K., Rai, A., Chaturvedi, K. K., Kumar, A., Pradhan, A. K., ... & Kumar, S. (2022). Genomic selection: A tool for accelerating the efficiency of molecular breeding for development of climate-resilient crops. *Frontiers in Genetics*, **13**, 832153.

Burridge, A. J., Winfield, M., Przewieslik-Allen, A., Edwards, K. J., Siddique, I., Barral-Arca, R., ... & Barker, G. (2023). Development of a next generation SNP genotyping array for wheat. *bioRxiv*, 2023-11.

Cabral-de-Mello, D. C. & Marec, F. (2021). Universal fluorescence in situ hybridization (FISH) protocol for mapping repetitive DNAs in insects and other arthropods. *Molecular Genetics and Genomics*, **296**(3), 513-526.

Chakraborty, A., Viswanath, A., Malipatil, R., Semalaiyappan, J., Shah, P., Ronanki, S., ... & Thirunavukkarasu, N. (2022). Identification of candidate genes regulating drought tolerance in pearl millet. *International Journal of Molecular Sciences*, **23**(13), 6907.

Chamara, N., Islam, M. D., Bai, G. F., Shi, Y., & Ge, Y. (2022). Ag-IoT for crop and environment monitoring: Past, present, and future. *Agricultural systems*, **203**, 103497

Elakhdar, A., Solanki, S., Kubo, T., Abed, A., Elakhdar, I., Khedr, R., ... & Qualset, C. O. (2022). Barley with improved drought tolerance: Challenges and perspectives. *Environmental and Experimental Botany*, **201**, 104965.

Farkas, A., Gaál, E., Ivanizs, L., Blavet, N., Said, M., Holušová, K., ... & Molnár, I. (2023). Chromosome genomics facilitates the marker development and selection of wheat-Aegilops biuncialis addition, substitution and translocation lines. *Scientific Reports,* **13**(1), 20499.

Georgieva, M. & Vassileva, V. (2023). Stress Management in Plants: Examining Provisional and Unique Dose-Dependent Responses. *International Journal of Molecular Sciences,* **24**(6), 5105.

Heredia, M. C., Kant, J., Prodhan, M. A., Dixit, S., & Wissuwa, M. (2022). Breeding rice for a changing climate by improving adaptations to water saving technologies. *Theoretical and Applied Genetics*, **135**(1), 17-33.

Heslop-Harrison, J. S., Schwarzacher, T., & Liu, Q. (2023). Polyploidy: its consequences and enabling role in plant diversification and evolution. *Annals of Botany,* **131**(1), 1-10.

Hossain, A., Maitra, S., Sarker, S., Al Mahmud, A., Ahmad, Z., Emon, R. M., ... & Brestic, M. (2023). Aluminium stress in plants: consequences and mitigation mechanisms. Beneficial Chemical Elements of Plants: *Recent Developments and Future Prospects*, 123-168.

Jinhua, S. H. A. O., Weixiong, W. U., Rasul, F., Munir, H., Huang, K., ALBISHI, T. S., ... & AAMER, M. (2022). Trehalose induced drought tolerance in plants: Physiological and molecular responses. Notulae Botanicae Horti Agrobotanici *Cluj-Napoca*, **50**(1), 12584-12584.

Kandel, S. (2021). Wheat responses, defence mechanisms and tolerance to drought stress: a review article. *International Journal for Research in Applied Sciences and Biotechnology,* **8**(5), 99-109.

Kim, C., Cnaani, A., & Kültz, D. (2023). Removal of evolutionarily conserved functional MYC domains in a tilapia cell line using a vector-based CRISPR/Cas9 system. *Scientific Reports,* **13**(1), 12086.

Kui, L., Majeed, A., Wang, X., Yang, Z., Chen, J., He, L., ... & Li, F. (2023). A chromosome-level genome assembly for *Erianthus fulvus* provides insights into its biofuel potential and facilitates breeding for improvement of sugarcane. *Plant Communications*, 4(4).

Kumar, A., Saripalli, G., Jan, I., Kumar, K., Sharma, P. K., Balyan, H. S., & Gupta, P. K. (2020). Meta-QTL analysis and identification of candidate genes for drought tolerance in bread wheat (*Triticum aestivum* L.). *Physiology and Molecular Biology of Plants*, **26**, 1713-1725.

Kumar, D. & Kirti, P. B. (2023). The genus Arachis: an excellent resource for studies on differential gene expression for stress tolerance. *Frontiers in Plant Science,* **14**, 1275854.

Kumari, M., Dubey, A. K., Kumar, R., & Kumar, A. (2024). Marker-assisted selection in plant breeding for stress tolerance. *In Improving Stress Resilience in Plants* 371-387.

Li, L.F., Zhang, Z.B., Wang, Z.H., Li, N., Sha, Y., Wang, X.F., ... & Liu, B. (2022). Genome sequences of five Sitopsis species of Aegilops and the origin of polyploid wheat B subgenome. *Molecular plant*, **15**(3), 488-503.

Liu, J., Wei, L., Wu, Y., Wang, Z., Wang, H., Xiao, J., ... & Sun, L. (2024). Characterization of sucrose nonfermenting-1-related protein kinase 2 (SnRK2) gene family in Haynaldia villosa demonstrated SnRK2. 9-V enhances drought and salt stress tolerance of common wheat. *BMC genomics*, **25**(1), 209.

Mangrauthia, S. K., Molla, K. A., Sundaram, R. M., Chinnusamy, V., & Bansal, K. C. (2024). Genomics and Genome Editing for Crop Improvement. In Transformation of Agri-Food Systems (pp. 297-322). Singapore: Springer Nature Singapore.

Maurel, C. & Nacry, P. (2020). Root architecture and hydraulics converge for acclimation to changing water availability. *Nature plants*, **6**(7), 744-749.

Mishra, N., Tripathi, M. K., Tripathi, N., Tiwari, S., Gupta, N., & Sharma, A. (2021). Validation of drought tolerance gene-linked microsatellite markers and their efficiency for diversity assessment in a set of soybean genotypes. *Curr. J. Appl. Sci. Technol*, **40**, 48-57.

Mondal, S., Atta, K., Mukherjee, S., Chowdhury, S. R., Pal, A., Maitra, S., & Hossain, A. (2023). Advancement of transgenic wheat (*Triticum aestivum* L.) to survive against abiotic stresses in the era of the changing climate. In Abiotic Stresses in Wheat (pp. 357-374). Academic Press.

Mou, Y., Sun, Q., Yuan, C., Zhao, X., Wang, J., Yan, C., ... & Shan, S. (2022). Identification of the LOX gene family in peanut and functional characterization of AhLOX29 in drought tolerance. *Frontiers in plant science*, **13**, 832785.

Nardino, M., Perin, E. C., Aranha, B. C., Carpes, S. T., Fontoura, B. H., de Sousa, D. J. P., & Freitas, D. S. D. (2022). Understanding drought response mechanisms in wheat and multi-trait selection. *PloS one*, **17**(4), e0266368.

Nouraei, S., Mia, M. S., Liu, H., Turner, N. C., & Yan, G. (2022). Transcriptome analyses of near isogenic lines reveal putative drought tolerance controlling genes in wheat. *Frontiers in Plant Science*, **13**, 857829.

Pandey, B. B., Ratnakumar, P. B. U. K., Usha Kiran, B., Dudhe, M. Y., Lakshmi, G. S., Ramesh, K., & Guhey, A. (2021). Identifying traits associated with terminal drought tolerance in sesame (*Sesamum indicum* L.) genotypes. *Frontiers in Plant Science*, **12**, 739896.

Parade, S. H., Huffhines, L., Daniels, T. E., Stroud, L. R., Nugent, N. R., & Tyrka, A. R. (2021). A systematic review of childhood maltreatment and DNA methylation: candidate gene and epigenome-wide approaches. *Translational psychiatry*, **11**(1), 134.

Pradhan, M. (2023). Chapter-7 Adoption of MAS in Plant Breeding. Dr. Chandana Behera, 117. researchgate.net

Rauf, S., Ortiz, R., Malinowski, D. P., Clarindo, W. R., Kainat, W., Shehzad, M., ... & Hassan, S. W. (2021). Induced polyploidy: A tool for forage species improvement. *Agriculture*, **11**(3), 210.

Rayaprolu, L., Deshpande, S. P., & Gupta, R. (2022). Genotyping-by-Sequencing (GBS) method for accelerating marker-assisted selection (MAS) Program. *Genomics of Cereal Crops*, 245-257.

Raza, A., Mubarik, M. S., Sharif, R., Habib, M., Jabeen, W., Zhang, C., ... & Varshney, R. K. (2023). Developing drought-smart, ready-to-grow future crops. *The Plant Genome*, **16**(1), e20279.

Saini, P., Kaur, H., Tyagi, V., Yadav, A. N., Saini, P., Sharma, V., ... & Sheikh, I. (2023). Genetic enhancement of nutritional and end-use quality in bread wheat through alien introgressions from wild relatives. *Cereal Research Communications*, **51**(2), 295-314.

Sharma, D., Gahtyari, N. C., Sharma, P., Khulbe, R. K., Pal, R. S., & Kant, L. (2022). Doubled haploidy: an accelerated breeding tool for stress resilience breeding in cereals. In Next-Generation Plant Breeding Approaches for Stress Resilience in Cereal Crops (pp. 199-240). Singapore: *Springer Nature Singapore*. researchgate.net

Sharma, P., Kapoor, N., Dhiman, S., Kour, J., Singh, A. D., Sharma, A., & Bhardwaj, R. (2023). Role of sugars in regulating physiological and molecular aspects of plants under abiotic stress. In The Role of Growth Regulators and Phytohormones in Overcoming Environmental Stress, 355-374.

Sharma, P., Mishra, S., Pandey, B., & Singh, G. (2023). Genome-wide identification and expression analysis of the NHX gene family under salt stress in wheat (*Triticum aestivum* L). *Frontiers in Plant Science*, **14**, 1266699.

Shende, R. T. & Shinde, R. M. (2023). Marker Assisted Selection (MAS) for Crop Improvement. *Agricultural Biotechnology*, 137.

Shokat, S., Großkinsky, D. K., Singh, S., & Liu, F. (2023). The role of genetic diversity and pre-breeding traits to improve drought and heat tolerance of bread wheat at the reproductive stage. *Food and Energy Security*, **12**(6), e478.

Song, Y., Zeng, M., Wang, X., Shi, P., Fei, M., & Zhu, J. (2024). Hierarchical Engineering of Sorption‐Based Atmospheric Water Harvesters. *Advanced Materials*, **36**(12), 2209134.

Srivastava, R. K., Yadav, O. P., Kaliamoorthy, S., Gupta, S. K., Serba, D. D., Choudhary, S., ... & Varshney, R. K. (2022). Breeding drought-tolerant pearl millet using conventional and genomic approaches: Achievements and prospects. *Frontiers in plant science*, **13**, 781524.

Sun, X., Zhang, L., Xu, W., Zheng, J., Yan, M., Zhao, M., ... & Yin, Y. (2024). A Comprehensive Analysis of the Peanut SQUAMOSA Promoter Binding Protein-like Gene Family and How AhSPL5 Enhances Salt Tolerance in Transgenic Arabidopsis. *Plants*, **13**(8), 1057.

Sunilkumar, V. P., Krishna, H., Devate, N. B., Manjunath, K. K., Chauhan, D., Singh, S., ... & Singh, P. K. (2023). Marker-assisted selection for transfer of QTLs to a promising line for drought tolerance in wheat (*Triticum aestivum* L.). *Frontiers in Plant Science*, **14**, 1147200.

Takahashi, M., Saccò, M., Kestel, J. H., Nester, G., Campbell, M. A., Van Der Heyde, M., ... & Allentoft, M. E. (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. *Science of the Total Environment*, **873**, 162322.

Tian, G., Wang, S., Wu, J., Wang, Y., Wang, X., Liu, S., ... & Wang, M. (2023). Allelic variation of TaWD40-4B. 1 contributes to drought tolerance by modulating catalase activity in wheat. *Nature Communications*, **14**(1), 1200.

Verma, H. & Sarma, R. N. (2021). Identification of markers for root traits related to drought tolerance using traditional rice germplasm. Molecular Biotechnology.

Walkowiak, S., Gao, L., Monat, C., Haberer, G., Kassa, M. T., Brinton, J., ... & Pozniak, C. J. (2020). Multiple wheat genomes reveal global variation in modern breeding. *Nature*, **588**(7837), 277-283.

Wang, Y., Tang, H., Wang, X., Sun, Y., Joseph, P. V., & Paterson, A. H. (2024). Detection of colinear blocks and synteny and evolutionary analyses based on utilization of MCScanX. *Nature Protocols*, 1-24.

Wight, C. P., Blake, V. C., Jellen, E. N., Yao, E., Sen, T. Z., & Tinker, N. A. (2024). One hundred years of comparative genetic and physical mapping in cultivated oat (*Avena sativa*). *Crop and Pasture Science*, 75(2).

Xie, W., Bai, B., & Wang, Y. (2024). Chromosome-scale genome assembly of *Apocynum pictum*, a drought-tolerant medicinal plant from the Tarim Basin. bioRxiv. biorxiv.org

Xu, T., Meng, S., Zhu, X., Di, J., Zhu, Y., Yang, X., & Yan, W. (2023). Integrated GWAS and transcriptomic analysis reveal the candidate salt-responding genes regulating Na^+/K^+ balance in barley (*Hordeum vulgare* L.). *Frontiers in Plant Science*, **13**, 1004477.

You, S., Zhao, Z., Yu, X., Zhu, S., Wang, J., Lei, D., ... & Wan, J. (2023). A toxin-antidote system contributes to interspecific reproductive isolation in rice. *Nature Communications*, **14**(1), 7528.

Zhang, Y., Chai, M., Zhang, X., Yang, G., Yao, X., & Song, H. (2022). The fate of drought-related genes after polyploidization in *Arachis hypogaea* cv. Tifrunner. *Physiology and Molecular Biology of Plants*, **28**(6), 1249-1259.

Zhou, H., Xu, L., Li, F., & Li, Y. (2022). Transcriptional regulation by CRISPR/dCas9 in common wheat. *Gene*, **807**, 145919.

Zhou, Y., Liu, J., Guo, J., Wang, Y., Ji, H., Chu, X., ... & Ma, Y. (2022). GmTDN1 improves wheat yields by inducing dual tolerance to both drought and low‐N stress. *Plant Biotechnology Journal*, **20**(8), 1606-1621.

Zwyrtková, J., Blavet, N., Doležalová, A., Cápal, P., Said, M., Molnár, I., ... & Hřibová, E. (2022). Draft sequencing crested wheatgrass chromosomes identified evolutionary structural changes and genes and facilitated the development of SSR markers. *International Journal of Molecular Sciences*, **23**(6), 3191.

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