



Review

# Exploitation of Drought Tolerance-Related Genes for Crop Improvement

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**Abstract:** Drought has become a major threat to food security, because it affects crop growth and development. Drought tolerance is an important quantitative trait, which is regulated by hundreds of genes in crop plants. In recent decades, scientists have made considerable progress to uncover the genetic and molecular mechanisms of drought tolerance, especially in model plants. This review summarizes the evaluation criteria for drought tolerance, methods for gene mining, characterization of genes related to drought tolerance, and explores the approaches to enhance crop drought tolerance. Collectively, this review illustrates the application prospect of these genes in improving the drought tolerance breeding of crop plants.

**Keywords:** drought tolerance; phenotypic criteria; gene mining; genetic improvement; molecular breeding; crop plants



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## 1. Introduction

With population growth and climate change, food security has become a major global challenge. It is predicted that the rising demand will require a two-fold increase of food production by 2050 [1]. Crop production is being impacted by increasing abiotic stresses. Drought is one of the most severe abiotic stresses on crop production, and its impact depends on its timing, duration, and intensity, caused by insufficient rainfall and/or altered precipitation patterns. According to the Intergovernmental Panel on Climate Change reports, the annual area of dry lands during 1961–2013 has increased at a rate of average of more than 1% per year. In 2015, around 500 million people experienced desertification [2]. Future droughts are predicted to be more frequent, severe, and longer-lasting [3]. Therefore, it is urgent to accelerate the genetic improvement of crop drought tolerance by using desirable genes through the application of new biotechnological tools.

In the history of crop breeding, conventional techniques such as cross, backcross, artificial mutagenesis, pedigree selection, and recurrent selection have played an important role in the genetic improvement of drought tolerance. Conventional breeding mainly relies on phenotypic selection in the field; however, it is greatly affected by the environmental conditions and requires many years of identification and evaluation, resulting in highly labor-intensive, time-consuming, and low efficiency. To accelerate drought tolerance breeding, it is vital to understand the physiological and genetic basis of crop responses to drought. However, drought tolerance is a complex quantitative trait controlled by multiple genetic loci and susceptible to environmental influences. Therefore, in the present review, the evaluation criteria of crop drought tolerance were reviewed, followed by mining approaches of drought tolerance genes and methods to improve the drought tolerance of crops.

## 2. Crop Drought Tolerance Evaluation Criteria

The drought tolerance evaluation criteria are dependent on the timing, duration, and intensity of drought stress, climatic conditions, measurement time, location, and instruments. It is the first step of crop drought tolerance breeding to select appropriate criteria to evaluate the drought tolerance of crops in a specific period and within a specific environment. The following selection criteria have been used to distinguish drought tolerance genotypes.

### 2.1. Agronomic Criteria

#### 2.1.1. Morphological Traits

The visible phenotypes of plants can be used as an indicator of crop drought tolerance. For example, the rate and speed of seed germination, length of coleoptile, speed of leaf emergence, plant height, panicle neck diameter, wax content, survival rate under extreme drought, days to heading, seed setting rate, normalized difference vegetation index (NDVI), plant biomass, and their variations under different water regimes have been associated with drought tolerance [4].

Besides the above morphological traits, leaf morphology, including leaf length, width, thickness and color, leaf distribution, and stomatal characteristics, is highly responsive to a water deficit. Leaf rolling is also a typical physiological phenomenon under drought stress. Crop plants with moderately rolled leaves are thought to reduce water loss by transpiration [5]. Drought-tolerant plants tend to have some common phenotypes, such as smaller and thicker leaves, smaller and denser stomata, more epidermal trichomes, thicker cuticle epidermis and palisade tissue, and well-developed vascular bundle sheath.

Root architecture traits, such as the root number, diameter, angle, depth, total length, distribution, and biomass, are also highly associated with drought tolerance. Despite the importance of root traits in crop drought tolerance, they are rarely used directly as the selection criteria in breeding, because the phenotypic detection of root-related traits is a time-consuming and laborious process. Nonetheless, the root depth shows a strong association with a cooler canopy temperature, which can be used as a proxy in selection [6].

#### 2.1.2. Yield-Related Traits

Yield-related traits such as the yield index (YI), yield stability index (YSI), harvest index (HI), water use (WU), and yield based water use efficiency (WUE) were used to evaluate the performances of genotypes under drought stress and limited irrigation conditions [7].

### 2.2. Physiological and Biochemical Criteria

Plants have evolved a series of mechanisms to withstand drought stress not only at the morphological level but, also, at the physiological and biochemical levels. The physiological and biochemical characteristics of plants in response to water stress include the capacities of photosynthesis, osmotic adjustment, antioxidant defense, the hormone level, and enzyme activity [8].

#### 2.2.1. Photosynthesis-Related Traits

Drought severely decreases CO<sub>2</sub> assimilation and affects photosynthesis by reducing the stomatal aperture and the concentration and activities of photosynthetic enzymes. Chlorophyll fluorescence is a relatively high throughput parameter to indicate photoinhibition due to a water deficit [9]. Fluorescence parameters were used to detect the changes of CO<sub>2</sub> assimilation, linear electron flux, and photosystem II photochemistry under drought stress. Fluorescence imaging can be used to screen a large number of plants with photosynthetic perturbation under drought conditions. Crops with higher chlorophyll contents have a more efficient utilization of light energy and stronger drought tolerance under drought stress [10].

### 2.2.2. Osmotic Adjustment-Related Traits

Since plants accumulate a variety of substances to maintain cell growth and leaf turgor, the concentration of these substances can reflect the crop drought tolerance to a certain extent. These substances can be classified into two catalogs: (1) inorganic ions, such as  $K^+$  and  $Cl^-$ ; (2) organic substances, such as trehalose, fructan, mannitol, proline, glycine, and betaine; and low molecular weight proteins such as late embryogenesis abundant (LEA) proteins, aquaporins (AQP), osmotin, and molecular chaperones.

### 2.2.3. Antioxidant Defense-Related Traits

Oxidative stress is commonly accompanied by drought stress. Reactive oxygen species (ROS) stress may disturb the membrane protein and enzyme configuration [11]. The protective enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxiredoxin (POD), are involved in reducing the ROS. Therefore, crop drought tolerance largely depends on the concentration of ROS and the activities of these protective enzymes.

### 2.2.4. Phytohormones-Related Traits

Among the endogenous phytohormones, abscisic acid (ABA) is regarded as the most closely related plant hormone to drought stress response. Drought stress induces the biosynthesis and accumulation of intracellular ABA, mainly in the root cap and wilted leaves, which activates the corresponding transcription factors and then promotes the expression of downstream drought-related genes [12].

## 2.3. Integrated Drought Tolerance Criteria

Predicting crop drought tolerance based on the expression of morphological traits and/or physiological and biochemical characters is an essential step in breeding drought-tolerant crops. However, it is not easy to accurately evaluate the crop drought tolerance due to its complexity. Research has focused on investigating the efficiency of several criteria and integrating these criteria in identifying crop genotypes, combining the drought tolerance and high yield potential under stressed and nonstressed environments. Sahar et al. compared 24 indices and found strong positive correlations between the grain yield and nine indices, such as the mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), mean relative performance (MRP), relative efficiency index (REI), modified stress tolerance indices 1 and 2 (MSTik), harmonic mean of yield (HM), and relative decrease in yield (RDY), which can be used for selecting drought tolerant and high-yield genotypes [13]. Khalili et al. proposed an integrated selection criterion index (SI) similar to MP, GMP, STI, stress susceptibility (SSI), and YSI as an effective selection criterion to distinguish the tolerant and desirable genotypes across multiple environments [14].

## 2.4. High-Throughput Phenotyping Platform

The emergence of a high-throughput phenotyping (HTP) platform makes it possible to capture trait information from a large number of plant samples under different water regimes [15]. The HTP platform integrated multiple imaging techniques, including red–green–blue (RGB), thermal infrared (TIR), chlorophyll fluorescence (ChlF), and multi-spectral and hyperspectral imaging and other sensor equipment [16]. Besides ground-based imaging, aerial imaging by unmanned aerial vehicles is also used to monitor the responses of crops to drought [17]. HTP technology provides a strong support for dissecting the genetic basis of drought tolerance in crops.

## 3. Methods for Mining Genes Related to Drought Tolerance

There are lots of omics approaches for mining genes related to drought tolerance in crop plants, such as genomics, transcriptomics, proteomics, metabolomics, ionomics, and epigenomics.

### 3.1. Genomics Analysis

With the development of high-throughput sequencing technology, the whole genome sequences of many crops have been unveiled (plaBiPD. Available online: <http://www.plabipd.de/index.ep> (accessed on 12 September 2021)). The availability of genomics platforms has made it easier to identify genes related to drought tolerance in crops. Nowadays, several genomics tools have been applied to elucidate drought tolerance-related genes, such as quantitative trait loci (QTL) mapping, map-based cloning, ectopic expression or suppression, genome-wide association studies (GWAS), and candidate gene association studies (CGAS).

#### 3.1.1. QTL Mapping

A number of QTL/genes for drought tolerant traits have been mapped, such as maize leaf temperature [18], maize leaf rolling index [19], rapeseed growth-related phenotypes [20], rice leaf rolling index [21], rice root angle [22], wheat stay-green trait under diverse water regimes [23], wheat stomatal conductance [24], density and size [25], and wheat yield-related phenotypes [26].

#### 3.1.2. Map-Based Cloning of Mutant

A major advantage of the map-based cloning approach is based on mutant phenotypes, and a series of genes involved in the mutant phenotypes can be discovered without prior assumptions or knowledge of these genes. With the improvement of crop reference genomic information, the availability of molecular markers, and the establishment of crop transformation systems, map-based cloning has become a conventional technique for gene discovery. Some genes related to drought tolerance were cloned, such as *PHOTOSENSITIVE LEAF ROLLING 1* [5], *LEAF WILTING 3* [27], and the ABA receptor gene [28].

#### 3.1.3. Ectopic Expression or Suppression

The ectopic expression or suppression of target genes in different tissues or species is also an effective approach to identifying drought-tolerant genes. Through screening a wheat cDNA yeast library, wheat stress-responding genes were identified [29]. In addition, the overexpression, downregulation, or tissue-specific expression of artificially modified promoters is also an effective way to study gene functions [30]. For example, the drought response functions of *dehydration responsive element-binding protein/C-repeat-binding factor (DREB/CBF)* family genes, *GmDREB1* [31], *ZmDREB4.1* [32], *OsDREB2* [33], and *TaDREB3* [34] were identified by this approach.

#### 3.1.4. GWAS and CGAS

With the development of high-density SNP detection techniques such as a DNA chip, high-throughput sequencing and genome-wide association studies on drought tolerance traits in many plant species have been widely carried out, such as soybean canopy wilting [35], maize survival rate [36], and wheat root depth [37], and a series of genes or loci were discovered. A candidate gene association study is based on the sequence polymorphism of genes, and through the analysis of the correlation between candidate genes and phenotypes, the favorable alleles are finally identified [38].

### 3.2. Transcriptomics Analysis

A transcriptomics analysis has been used to decipher drought tolerance-related genes such as *OsbZIP6* [39], the *LATERAL ROOT DENSITY* gene discovered from root transcriptome data [40]. Combining the transcriptomics data with the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis, the genes involved in some specific pathways could be predicted [41]. The expression of QTL (eQTL) and expression of GWAS (eGWAS) were also used to dissect the genetic basis of the drought-tolerant phenotype [42].

### 3.3. Proteomics Analysis

Drought stress has profound impacts on the protein abundance (such as protective proteins, chaperones, and ROS scavenging enzymes); post-translational modifications; protein interactions; and the ultimate functions of the proteins [43]. The comparison of protein profiling before and after drought stress or in genotypes with contrasting responses to drought stress will enhance our understanding of drought tolerance at the protein level [44].

#### 3.3.1. Mass Spectrometry

A series of techniques based on mass spectrometry (MS) are used for the proteomic analysis, such as gas chromatography MS (GC-MS), liquid chromatography MS (LC-MS), electrospray ionization (ESI), matrix-assisted laser desorption ionization with a time-of-flight analyzer (MALDI-TOF), and isobaric tags for relative and absolute quantification (iTRAQ) [45]. Using these techniques, not only the protein type and abundance can be detected but, also, protein modifications such as phosphorylation and ubiquitination can be analyzed.

#### 3.3.2. Two-Dimensional Gel Electrophoresis

A large number of proteomic data, including rice, tobacco, and Arabidopsis, were obtained using the techniques of two-dimensional gel electrophoresis and two-dimensional difference gel electrophoresis and are available at WORLD-2D-PAGE (Available online: <https://world-2dpage.expasy.org/list/> (accessed on 12 September 2021)) [46].

#### 3.3.3. Protein Interaction Technique

Yeast two-hybrid (Y2H), yeast three-hybrid (Y3H), bimolecular fluorescence complementation (BiFC), luciferase complementation imaging assay (LCI), coimmunoprecipitation (CoIP), pull-down, and other protein interaction detection techniques can be used to detect drought tolerance-related proteins. For example, the ABA receptor was identified through the yeast two-hybrid screening of ABA-insensitive interaction proteins [47].

### 3.4. Metabolomics and Ionomics Analysis

Metabolomics is the study of small molecule profile based on the MS and nuclear magnetic resonance (NMR), including metabolites of small molecule substrates, intermediates, and products, which can be a prediction tool for plants' performances under stress [48]. A better understanding of the metabolic response mechanisms under drought stress using metabolic QTL and metabolic GWAS could be beneficial to crop improvements [49].

Ionomics is a high-throughput elemental profiling method for studying the complete set of ions in living organisms. Therefore, it is widely used in forward [50] and reverse [51] genetics for detecting natural variants [52] and mutant screening to understand the mechanisms of abiotic stress tolerance in crop plants.

### 3.5. Epigenomics Analysis

Epigenetics studies epigenetic modifications at the genomic level, such as DNA methylation [53], histone modification [54], chromatin remodeling [55], and noncoding RNA [56]. Under environment stress, plants can transiently or lastingly change epigenetic modifications to adjust a gene expression to adapt to the environment [57]. For example, under drought stress, maize *natural antisense transcript* (NAT) genes are induced, and their gene regions are enriched with high levels of histone modifications, such as H3K36me3, H3K9ac, and H3K4me3 [58]. Under continuous stress, the identification of epigenetic markers that are heritable and transmitted to their progeny is of primary importance [59]. The role of DNA methylation variations in rice adaptations to drought stress was discovered by applying drought conditions to two rice varieties for 11 successive generations [60]. Plant microRNAs (miRNAs) also play a key role in the epigenetic regulation of drought

tolerance [61]. Therefore, epigenomics study will also enhance our understanding of plant drought tolerance.

In conclusion, the comprehensive utilization of multi-omics technologies will facilitate mining drought tolerance-related genes for improving crop productivity under abiotic stress.

#### 4. Genes Related to Drought Tolerance

A number of genes involved in drought tolerance have been discovered, and they fall into two main categories. One is signal transduction factors, including protein kinases and transcription factors. Another is functional factors, including proteins involved in metabolism, osmotic regulation, protein turnover, protein modification, and ROS scavenging and transportation. The detailed information is listed in Table 1.

##### 4.1. Signal Transduction Factors

###### 4.1.1. ABA Receptors

Pyrabactin resistance 1/PYR1-like/regulatory components of the ABA receptor (PYR1/PYL/RCAR), as ABA receptors that were discovered through two methods: map-based cloning and Y2H at the same time, can perceive drought stress and transduce ABA signals [28,47]. In rice, the overexpression of *PYL9* [62] conferred drought tolerance. The overexpression of *TaPYL4* increased the water use efficiency and drought tolerance in wheat [63].

###### 4.1.2. Protein Kinases and Protein Phosphatases

The phosphorylation and dephosphorylation of proteins in plants play an important role in response to drought stress. A series of kinases were discovered to be participating in this process, such as calcium-dependent protein kinases (CDPKs), calcineurin B-like interacting protein kinase (CIPK), mitogen-activated protein kinases (MAPKs), receptor-like kinases (RLKs), and sucrose nonfermenting-related kinase 2s (SnRK2s). For example, (1) in CDPKs, *GmCDPK3* was shown to positively regulate drought tolerance in soybeans [64]; (2) in CIPKs, CIPK genes have been known to be involved in abiotic stress tolerance in rice [65]; (3) in MAPKs, *MPKK10.2* promoted the drought tolerance in rice [66]; (4) in RLKs, the RLK family member *GbRLK* from *Gossypium barbadense* enhanced the drought tolerance [67]; and (5) in SnRK2s, the SnRK2 family member *TaSnRK2.9* conferred drought tolerance to transgenic tobacco [68]. Moreover, PP2C-type protein phosphatases are also highly associated with drought tolerance in both pathways: ABA-dependent, such as *ZmPP2C-A* [69], and ABA-independent signal transduction, such as *OsPP2C09* [70].

###### 4.1.3. Transcription Factors and Cofactors

Diverse transcription factors such as AP2/ERF, bZIP, MYB, NAC, WRKY, and zinc finger protein have been known to be involved in the response to drought stress. The AP2/ERF transcription factor *NtERF172* enhanced the drought tolerance through regulating *NtCAT* [71]. The ABA-responsive element-binding proteins/factors (AREBs/ABFs), which belong to the bZIP transcription factor, were involved in ABA-dependent drought response in wheat [12] and soybeans [72]. *OsMYB102* negatively regulated ABA biosynthesis and the expression of downstream genes responding to abiotic stress [73]. Drought tolerance was enhanced in plants overexpressing the NAC family members *ZmNAC48* [74] and *TaNAC69* [75]. A zinc finger protein *DST* regulated the drought tolerance of rice by regulating the stomatal aperture [76].

A few nuclear proteins have been discovered as transcriptional cofactors in the drought response. *MED25* subunit, which interacts with several stress response transcription factors as a RNA polymerase II coactivator and was involved in stress tolerance [77]. A rice homolog of the human ski-interacting protein *OsSKIPa* positively regulated the stress tolerance [78]. An overexpression of *OsRIP18*, rice ribosome-inactivating protein gene 18, increased the drought tolerance [79].

#### 4.1.4. Epigenetic-Related Genes

In rice, drought can induce ~12.1% site-specific DNA methylation differences at the genome level [80]. Increasing evidence indicates that epigenetic regulators, especially histone deacetylases, HDA9 [81] and HDA15 [82], and chromatin remodeling ATPase BRAHMA [83] were involved in the drought stress responsiveness. A number of drought-induced microRNAs have been identified in plants [84].

### 4.2. Functional Factors

#### 4.2.1. Metabolism-Related Genes

Plenty of metabolic-related proteins have been identified to participate in drought responses. The enzymes involved in ABA metabolism are one example. ABA plays an important role in drought tolerance by triggering stomatal closure to reduce the water loss. Zeaxanthin epoxidase (ZEP), 9-cis-epoxycarotenoid dioxygenase (NCED), and ABA-aldehyde oxidase (AAO) are involved in ABA biosynthesis. Plants overexpressing these genes enhance the drought tolerance [85,86]. In addition to these, LOS5/ABA3, a molybdenum cofactor sulfurase of AAO, increased the ABA accumulation and improved the drought tolerance in cotton [87] and soybeans [88]. The DSM2 gene encoded the enzyme  $\beta$ -carotene hydroxylase, which converts  $\beta$ -carotene into zeaxanthin (a precursor of ABA) and can improve the drought tolerance of rice [89]. There are also genes (cytokinin biosynthetic gene *IPT*) involved in the metabolism of other hormones that affect the drought tolerance in cotton [90], rice [91], and tobacco [92].

The components involved in mRNA metabolism, including alternative splicing, RNA export, and pre-mRNA processing, play important roles in drought response. For example, the overexpression of *MeSCL30*, a cassava alternative splicing-related gene, enhanced the drought tolerance through maintaining ROS homeostasis and inducing drought-responsive gene expression [93]. Highly ABA-Induced 1 Interacting protein HIN1 with a pre-mRNA splicing function enhanced the splicing efficiency during drought acclimation [94]. Cold shock protein (CSP), functioning as an RNA chaperone, also participated in drought tolerance in *Arabidopsis* and maize [95].

Some genes involved in other substance metabolisms also contributed to the drought tolerance in crop plants. For example, arginine decarboxylase (ADC) modulated the polyamine biosynthetic pathway conferred drought tolerance of transgenic rice [96]. The overexpression of aspartic protease gene *APA1* also conferred a drought tolerance [97]. A C-5 sterol desaturase gene *FvC5SD* overexpression enhanced the drought tolerance in soybeans by reactive oxygen species scavenging [98]. A Caffeoyl-CoA O-methyltransferase gene conferred drought tolerance by promoting lignin synthesis [99]. The expression of flavodiiron proteins/reductases genes *Flv2*, *Flv3*, and *Flv4* in *Arabidopsis* and tobacco plants enhanced the drought tolerance [100].

#### 4.2.2. Osmotic Adjustment-Related Genes

Genes related to the synthesis and accumulation of osmotic adjustment substances can improve the drought tolerance of plants. For example, (1) dehydrin, an overexpression of the dehydrin gene *SiDHN*, promoted the cold and drought tolerance of transgenic tomato plants [101]; (2) glycine betaine, an overexpression of the betaine aldehyde dehydrogenase gene *BADH* from spinach, enhanced the drought and salinity tolerance of potato plants [102], and the choline oxidase gene *codA* converts choline into glycinebetaine, which was also associated with plant drought tolerance [103]; (3) LEA proteins, in which *LEA* genes were proven to play important roles in rice drought tolerance [104]; (4) mannitol, where an overexpression of the mannitol dehydrogenase gene *mtlD* imparted drought tolerance in finger millet [105]; (5) proline, where P5CS is a rate-limiting enzyme in proline biosynthesis, and its transformation increased proline production and enhanced the tolerance to water and salt stresses in rice [106]; an overexpression of the ornithine  $\delta$ -aminotransferase gene  $\delta$ -OAT increased the proline concentration and improved the drought tolerance [107]; and (6) trehalose, where trehalose-6-phosphate synthase (TPS) and

trehalose-6-phosphate phosphatase (TPP) are two main enzymes in trehalose biosynthesis, and the overexpression of genes *TPS/TPP* increased the trehalose accumulation and drought tolerance in plants [108].

#### 4.2.3. Protein Turnover-Related Genes

Protein degradation is also a way for crop plants to adapt to drought stress. For example, the E3 ubiquitin ligase gene is involved in protein degradation during drought stress, which includes several subfamilies: (1) RING finger gene family members *OsDSG1* [109], *OsRDCP1* [110], *OsSDIR1* [111], *StRFP2* [112], and *TaSDIR1* [113]; (2) U-box gene family member *PUB11* [114]; (3) F-box gene family members *SAGL1* and *ECERIFERUM3* [115]; and (4) CUL4-DDB1 gene family member *HOS15* [116].

#### 4.2.4. Protein Modification-Related Genes

The genes involved in protein glycosylation, sumoylation, and farnesylation are also involved in response to drought stress. For example, *LEW3* [27] was involved in protein glycosylation and the abiotic stress response. SUMO protease *ASP1* [117] and SUMO E3 ligase *SIZ1* [118] had functions in ABA signaling. Protein farnesyltransferase was proven as a key negative ABA signal regulator in the guard cells of plants [119].

#### 4.2.5. ROS-Related Genes

Plant ROS scavenging has been demonstrated as an effective way to improve drought tolerance. Some ROS homeostasis-related genes have been proven to have functions in drought tolerance. For example, *OsSRO1c* conferred a rice drought tolerance through regulating hydrogen peroxide [120]. A pea superoxide dismutase *SOD* gene enhanced the drought tolerance in rice [121]. *EcAPX1*, ascorbate peroxidase from *Eleusine coracana*, played an important role in response to drought stress [122].

#### 4.2.6. Transporter Related Genes

Maize vacuolar-type H<sup>+</sup> pyrophosphatase gene *ZmVPP1* contributed to seedling drought tolerance [36]. Pyramiding *TsVP* (V-H<sup>+</sup>-PPase from *Thellungiella halophila*) and *BetA* (choline dehydrogenase from *Escherichia coli*) through co-transformation enhanced the drought tolerance in maize [123]. *AQP* improved the drought tolerance, such as *OsRWC3* [124] and *MaPIP1;1* [125]. An auxin efflux carrier such as *OsPIN3t* was also involved in drought tolerance [126].

#### 4.2.7. Wax Related Genes

Lipid transfer proteins are involved in the biosynthesis of protective hydrophobic layers such as cutin and suberin [127]. Other wax-related genes, such as *DWA1* [128] and *Glossy1* homologous genes [129] in rice and *Glossy13* in maize [130], were involved in wax accumulation and drought tolerance.

**Table 1.** Genes related to drought tolerance in crop plants.

Functional Category	Protein Function	Gene Name	Species	Method	Reference
<b>Signal Transduction Factor</b>					
ABA receptor	PYR/PYL/RCAR	<i>OsPYL9</i> , <i>TaPYL4</i>	<i>Oryza sativa</i> , <i>Triticum aestivum</i>	Ectopic expression, transcriptomics	[62,63]
	CDPK	<i>GmCDPK3</i>	<i>Glycine max</i>	Transcriptomics	[64]
Protein kinase and protein phosphatase	CIPK	<i>OsCIPK</i>	<i>Oryza sativa</i>	Transcriptomics	[65]
	MAPK	<i>OsMPKK10.2</i>	<i>Oryza sativa</i>	Ectopic expression	[66]
	RLK	<i>GbRLK</i>	<i>Gossypium barbadense</i>	Ectopic expression	[67]



Table 1. Cont.

Functional Category	Protein Function	Gene Name	Species	Method	Reference
Transcription factor and cofactor	SnRK	<i>TaSnRK2.9</i>	<i>Triticum aestivum</i>	Ectopic expression	[68]
	PP2C	<i>OsPP2C09</i> , <i>ZmPP2C-A</i>	<i>Oryza sativa</i> , <i>Zea mays</i>	CGAS, ectopic expression, transcriptomics	[69,70]
	AP2/ERF	<i>NtERF172</i>	<i>Nicotiana tabacum</i>	Yeast one-hybrid	[71]
	bZIP	<i>GmbZIP1</i> , <i>TaAREB3</i>	<i>Glycine max</i> , <i>Triticum aestivum</i>	Ectopic expression, transcriptomics	[12,72]
	HD-ZIP	<i>ZmOCL5</i>	<i>Zea mays</i>	QTL mapping	[18]
	MYB	<i>OsMYB102</i>	<i>Oryza sativa</i>	Ectopic expression	[73]
	NAC	<i>TaNAC69</i> , <i>ZmNAC48</i>	<i>Triticum aestivum</i> , <i>Zea mays</i>	CGAS, ectopic expression, transcriptomics	[74,75]
	Zinc finger	<i>OsDST</i>	<i>Oryza sativa</i>	Map-based cloning	[76]
	Transcription cofactor	<i>OsSKIPa</i> , <i>OsRIP18</i>	<i>Oryza sativa</i>	Ectopic expression	[78,79]
<b>Functional factor</b>					
Metabolism	Abscisic acid metabolism	<i>OsDSM2</i> , <i>PvNCED1</i>	<i>Oryza sativa</i> , <i>Phaseolus vulgaris</i>	Ectopic expression, map-based cloning	[86,89]
	mRNA turnover	<i>MeSCL30</i>	<i>Manihot esculenta</i>	Transcriptomics	[93]
	Other substance metabolism	<i>ADCs</i>	<i>Avena Sativa</i>	Ectopic expression	[131]
Osmotic regulation	Dehydrin	<i>SiDHN</i>	<i>Saussurea involucrata</i>	Ectopic expression	[101]
	Glycine betaine	<i>BADH</i>	<i>Spinacia oleracea</i>	Ectopic expression	[102]
	LEA	<i>OsLEA3-2</i>	<i>Oryza sativa</i>	Ectopic expression	[104]
	Mannitol	<i>mtlD</i>	<i>Eleusine coracana</i>	Ectopic expression	[105]
	Proline	<i>OsP5CS</i> , <i>OsOAT</i>	<i>Oryza sativa</i>	Ectopic expression	[106,107]
	Trehalose	<i>ZmT6P</i>	<i>Zea mays</i>	Ectopic expression	[108]
Protein turnover	E3 ubiquitin ligase	<i>StRFP2</i> , <i>TaSDIR1</i>	<i>Solanum tuberosum</i> , <i>Triticum aestivum</i>	CGAS, ectopic expression	[112,113]
Protein modification	Farnesylation	<i>ERA1</i>	<i>Brassica napus</i>	Ectopic expression	[119]
	Sumoylation	<i>OsSIZ1</i>	<i>Oryza sativa</i>	Ectopic expression	[118]
ROS scavenging	Ascorbate peroxidase	<i>EcAPX1</i>	<i>Eleusine coracana</i>	Ectopic expression	[122]
	Similar to RCD One	<i>OsSRO1c</i>	<i>Oryza sativa</i>	Ectopic expression	[120]
	Superoxide dismutase	<i>SOD</i>	<i>Pisum sativum</i>	Ectopic expression	[121]
Transporters	Aquaporin	<i>MaPIP1;1</i> , <i>OsRWC3</i>	<i>Musa acuminata</i> , <i>Oryza sativa</i>	Ectopic expression	[124,125]
	Auxin efflux carrier	<i>OsPIN3t</i>	<i>Oryza sativa</i>	Ectopic expression	[126]
	Choline Dehydrogenase, V-H <sup>+</sup> -PPase	<i>ZmbetA</i> , <i>ZmTsVP</i>	<i>Zea mays</i>	Ectopic expression	[123]

Table 1. Cont.

Functional Category	Protein Function	Gene Name	Species	Method	Reference
	Vacuolar-type H <sup>+</sup> pyrophosphatase	<i>ZmVPP1</i>	<i>Zea mays</i>	GWAS	[36]
	ABC transporter	<i>ZmGLOSSY13</i>	<i>Zea mays</i>	Map-based cloning	[130]
Cuticle wax	Megaenzyme	<i>OsDWA1</i>	<i>Oryza sativa</i>	Map-based cloning	[128]
	Sterol desaturase	<i>OsGLOSSY1</i>	<i>Oryza sativa</i>	Ectopic expression	[129]

## 5. Genetic Improvement of Drought Tolerance in Crops

In the history of crop breeding, breeders have unconsciously selected target genes by evaluating phenotypic traits. However, conventional breeding, which relies on multilo-cation phenotypic selection for many years, is inefficient. The application of molecular breeding technology will facilitate the direct selection of genotypes, speed up the breeding process, and improve the efficiency of developing drought-tolerant varieties.

### 5.1. Marker-Assisted Selection

Marker-assisted selection (MAS) is a selection strategy based on QTL or gene markers employed by breeders to accelerate plant breeding programs. According to the purpose of selection, MAS can be divided into marker-assisted pedigree selection (MAPS), genomic selection (GS) or genome-wide selection (GWS), marker-assisted recurrent selection (MARS), and marker-assisted backcrossing (MABC). Gupta et al. [132] reviewed the wheat genetics of important quantitative traits, including a tolerance to abiotic stress, and summarized the potential value of QTLs for the improvement of drought tolerance using MAS. Despite the availability of a large number of major QTL for drought tolerance, little progress has been made for MAS. For example, in rice, the pyramiding of six large-effect QTL for drought adaptation has improved the drought tolerance of Asian cultivars [133]. In wheat, using MAS, a major QTL (Qyld.csdh.7AL) was introgressed into wheat cultivars to develop a high-yield genotype under rainfed conditions [134]; the introgression of QTL on 7AS and 2BS led to the improvement of the grain yield under the drought treatment [135]. Functional molecular markers of candidate genes for drought tolerance have also been developed for use in breeding [136].

### 5.2. Genomic Selection

MAS has been implemented for crop breeding, but its efficiency is limited due to the small number of molecular markers that can be used. Drought and most agronomic traits are quantitative traits controlled by multiple minor effect genes. Using genome-wide markers to predict the breeding values of individuals, genomic selection as a promising breeding method presents a new alternative to traditional MAS [137]. GS has been shown to improve the efficiency and speed up breeding in maize [138], rice [139], and wheat [140], and high prediction accuracies have been obtained for the yield and a number of other traits. However, due to the genetic complexity of its characteristics, the genomic selection for improving drought tolerance should be investigated.

### 5.3. Genetic Improvement Using Transgene and Genome Editing Techniques

Along with the identification of the candidate genes, we can also use transgenic technology to improve the crop drought tolerance, such as OE, RNAi, VIGS, ZFN, TALEN, and CRISPR [141]. At present, genetically modified crops are widely used in the world. The genes used in transgenic crops mainly include herbicide resistance gene (*Bar*), insect resistance gene (*Bt*), and disease resistance genes, while drought tolerance genes are

rarely used. Transgenic technology and gene editing technology will promote the genetic improvement of crop drought resistance. For example, CRISPR technologies can not only introduce a small insertion or deletion mutations at the target loci but can also provide precision editing, such as base editing, prime editing, and gene targeting [142].

#### 5.4. Genetic Improvement Combined with the Chemical Approach

As an important hormone, ABA plays a vital role in the drought tolerance of crops, while, due to the chemical instability, the rapid catabolism of ABA limits its application in the field. Scientists have conducted vast amounts of research to search for more stable ABA analogs, such as pyrabactin, AM1 (ABA mimic 1)/quinabactin, cyanabactin, opabactin, and AMFs [143]. Furthermore, combining chemical and genetic approaches, AMFs are applied to *PYL2* overexpression transgenic plants, increasing their drought tolerance [144]. Another avenue to improve crop drought tolerance is engineering an ABA receptor. An ABA receptor PYR1 variant, which is sensitive to the agrochemical mandipropamid, improved the drought tolerance in transgenic plants [145].

#### 5.5. Molecular Design Breeding

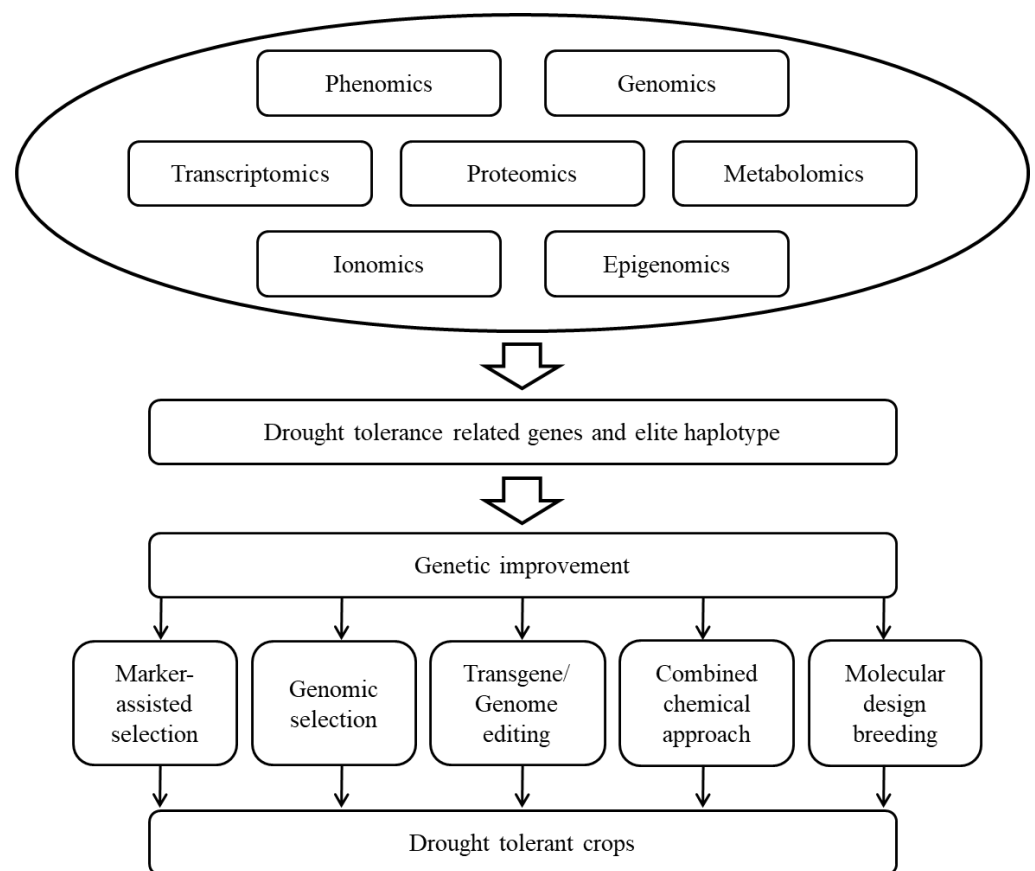
Molecular design breeding, a highly integrated system based on biotechnology and bioinformatics [146], may be an effective approach to enhance drought tolerance [147]. It can design and manipulate genotypes to meet various breeding objectives in different ecological regions under diverse water conditions [148]. Based on the principle of molecular design breeding, the ideal genotypes can be identified during crossing, selection and transgene and genome editing.

## 6. Conclusions and Perspectives

Most transgenic plants with drought tolerance phenotypes were based on the overexpression of genes using constitutive promoters, such as actin, CaMV35S, and ubiquitin. However, the overexpression of genes is often accompanied by a yield penalty due to stress-induced energy consumption. Therefore, mining drought tolerance-related genes that are not subjected to yield penalties or fine-tuning these genes through suitable stress-inducible promoters to minimize the yield penalties should be considered [149].

Most of the genes reviewed in this paper have been shown to enhance drought tolerance in growth chambers or greenhouses, and the utility of most of the genes has not been verified in the field. This is especially problematic for water stress, since root growth in pots in controlled environments cannot be compared to their response in deep soil water profiles, which has a profound impact on their adaptation and productivity. Therefore, the use of these drought-tolerant genes in crop breeding still needs the joint efforts of scientists and breeders to achieve a proof of concept.

Crop plants are subjected to drought stress at various stages of growth and development. Plants can adapt to drought stress through a series of morphological, physiological, and biochemical changes, but these genetically regulated responses are extremely complex. Therefore, it is necessary to conduct phenotypic and genotypic identification and evaluation through multidisciplinary approaches and to comprehensively use conventional, physiological, and molecular breeding techniques to improve the drought tolerance of crops (Figure 1).



**Figure 1.** Approaches of breeding for drought tolerance in crops.

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## Abbreviations

AAO	ABA-aldehyde oxidase
ABA	Abscisic acid
AQP	Aquaporins
AREB/ABF	ABA-responsive element-binding proteins/factors
CDPK	Calcium dependent protein kinase
CGAS	Candidate gene association study
CIPK	Calcineurin B-like interacting protein kinase
CSP	Cold shock protein
DREB/CBF	Dehydration responsive element binding protein/C-repeat binding factor
GWAS	Genome wide association studies
HTP	High throughput phenotyping
LEA	Late embryogenesis abundant
MAPK	Mitogen-activated protein kinase

MAS	Marker-assisted selection
PYR1/PYL/RCAR	Pyrabactin resistance 1/PYR1-like/Regulatory components of the ABA receptor
QTL	Quantitative trait loci
RLK	Receptor like kinase
ROS	Reactive oxygen species
SnRK2	Sucrose nonfermenting related kinase 2
SOD	Superoxide dismutase
TPS	Trehalose-6-phosphate synthase

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