Genetic Engineering and Breeding of Drought-Resistant Crops

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Abstract

Drought is one of the most important environmental stresses affecting the productivity of most field crops. Elucidation of the complex mechanisms underlying drought resistance in crops will accelerate the development of new varieties with enhanced drought resistance. Here, we provide a brief review on the progress in genetic, genomic, and molecular studies of drought resistance in major crops. Drought resistance is regulated by numerous small-effect loci and hundreds of genes that control various morphological and physiological responses to drought. This review focuses on recent studies of genes that have been well characterized as affecting drought resistance and genes that have been successfully engineered in staple crops. We propose that one significant challenge will be to unravel the complex mechanisms of drought resistance in crops through more intensive and integrative studies in order to find key functional components or machineries that can be used as tools for engineering and breeding drought-resistant crops.

Contents

1. INTRODUCTION

Owing to climate change and the freshwater-supply shortage caused mainly by the increasing world population, drought has been the most important environmental stress affecting agriculture worldwide, particularly in terms of the productivity of field crops. Drought stress can occur at any growth stage and can affect this productivity to variable degrees depending on the onset time, duration, and intensity of drought treatment. Drought stress at the reproductive stage can directly result in an average yield loss of more than 50% (14, 114). Breeders have made exciting progress in improving and developing drought-tolerant crops, but these still cannot meet the demands of food security in the face of an increasing world population, global warming, and a water shortage. In China, developing rice with high water-use efficiency (WUE) and drought resistance (DR) is one of the main objectives of the Green Super Rice Project (142), which was put forward to cope with the challenges of rice production.

DR is a complex trait, and drought stress is often accompanied by heat or other stresses. Plants use multiple strategies to respond to drought stress and have evolved to adapt to drought via morphological and physiological changes through diverse signaling cascades and osmotic adjustment. Plants may avoid drought stress by having a short life cycle with accelerated flowering, or may cope with drought stress by reducing water loss (by closing stomata and increasing the thickness of the leaf cuticle) or by improving water uptake (by developing a deep and thick root system). Plants have also evolved tolerance mechanisms to survive severe drought stress through accumulation of osmoprotectants, antioxidants, and reactive oxygen species (ROS) scavengers. The processes of the plant response to drought stress include stress signal perception, signal transduction and amplification, and adaptation at the morphological, physiological, and molecular levels. In these processes, many proteins—including transcription factors (TFs), protein kinases, and diverse stress-related proteins—function to enhance DR via outputs such as growth delay, transpiration reduction, osmotic adjustment, and ROS scavenging. Hundreds of genes in these pathways that control the key processes of the plant response to drought stress have been identified by genetic, genomic (at the transcriptomic, proteomic, metabolomic, and epigenetic levels), and transgenic approaches. Some of them have been confirmed to have potential for improving the DR of crops in field trials. However, the biochemical and molecular basis for drought perception, signal transduction, and

WUE: water-use efficiency

DR: drought resistance

ROS: reactive oxygen species

stress adaptation remains largely unclear, which continues to be a major challenge for the genetic improvement of DR.

Many excellent reviews on DR have focused on particular molecules, signaling pathways, or crop engineering or provided an overall retrospective for model plants. This review starts with an overview of the genetic and genomic basis of DR in major crops, then focuses on recent studies of genes that have been well characterized in physiological, molecular, or biochemical processes affecting DR and recent studies of genes that have been tested in the genetic engineering of staple crops. The review also provides comprehensive information to the research community for future efforts in combining genetic and engineering approaches with conventional breeding to develop drought-resistant crops.

DA: drought avoidance

DT: drought tolerance

QTL: quantitative trait locus

2. THE GENETICS OF DROUGHT RESISTANCE

2.1. Genetic Control of Leaf Traits Related to Drought Resistance

Several DR mechanisms are associated with leaf traits, and these traits have often been used as criteria to evaluate the DR of crops. Morphological traits such as leaf rolling, stomatal density, and stomatal aperture control, along with physiological traits such as cuticular wax, abscisic acid (ABA) content, relative water content, water potential, and canopy temperature, are frequently used as criteria for drought avoidance (DA). Osmotic adjustment, cell membrane stability, proline and sugar content, and so on are generally considered criteria for drought tolerance (DT). DA and DT are two major mechanisms for DR (139), although DT has frequently been interpreted as equivalent to DR in some of the literature. Because of genetic variations in the leaf traits related to DR, researchers have made great efforts to elucidate the genetic basis of these leaf-related DR traits by quantitative trait locus (QTL) mapping approaches.

Stomatal density and stomatal aperture are two of the main factors that determine stomatal conductance and thus photosynthetic ability. Genotypic variations in these factors have been reported, but the genetic mechanisms behind these leaf traits are poorly understood. Ishimaru et al. (41) identified 4 QTLs that control adaxial and abaxial stomatal density in rice from a population of backcross inbred lines derived from a cross of the Nipponbare (*Oryza sativa* ssp. *japonica*) and Kasalah (*Oryza sativa* ssp. *indica*) varieties. Among these, the QTL for adaxial density overlapped with the QTL for abaxial density on chromosome 3, indicating that the same locus may pleiotropically control stomatal density on both surfaces. Using 101 recombinant inbred lines (RILs) derived from a cross of the *japonica* rice IR69093-41-3-2-2 and *indica* rice IR72 varieties, the authors detected 4 QTLs (on chromosomes 2, 6, and 10) controlling stomatal size and 10 QTLs (on chromosomes 1, 2, 3, 4, and 6) controlling stomatal density across growth stages and on leaf surfaces, respectively. The contributions of the QTLs ranged from 9.3% to 15.2% for density and from 9.7% to 14.3% for stomatal size (55). However, no common QTLs were detected for these two traits in this study.

Leaf rolling and leaf drying are traits that are relatively easy to score. Two QTLs for leaf rolling time (the number of days from the drainage of standing water in the field to the appearance of leaf rolling) were detected on chromosomes 8 and 3 in RILs derived from a cross between the lowland rice variety Co39 and the upland rice variety Moroberekan (61). Price et al. (88) detected only one QTL on chromosome 7 using the F_2 population from a cross of upland rice varieties, and no QTLs near these regions were detected from the populations derived from lowland varieties (41). Yue et al. (138, 139) detected 10 QTLs for leaf rolling time and 10 QTLs for leaf drying score in two separate years under different drought stress conditions using a RIL population derived from a cross of lowland rice ZS97 and upland rice IRAT109. Two of the QTLs, *qDlr9* and *qLds3b*, had

a significant effect on leaf rolling time and leaf drying score, respectively, in both years. Zhang et al. (145) identified 12 QTLs related to DT, and their contributions to phenotypic variation ranged from 3% to 14%. Among these, the QTL *qSDT12-1* on chromosome 12 was mapped close to the region containing the leaf rolling QTL (RM235–MRG5454) identified by Yue et al. (138), and *qSDT5-1* on chromosome 5 was also in the same location as the leaf rolling QTL (RM509–RM430) mapped by Yue et al. (138).

MAS: marker-assisted selection

$\delta^{13}C$: carbon isotope discrimination

2.2. Genetic Control of Physiological Traits Related to Drought Resistance

Osmotic adjustment and cell membrane stability are commonly considered to be major physiological indices that are putatively associated with DT in crops. The genetic basis of these traits has also been analyzed by QTL mapping. Lilley et al. (61) reported that one QTL, *RG1*, on chromosome 8 that was identified by the analysis of variance (ANOVA) method was a major osmotic adjustment contributor. Zhang et al. (141) identified five QTLs (on chromosomes 1, 2, 3, 8, and 9) controlling osmotic adjustment using a doubled-haploid population from a CT9993/IR62266 cross, and all of the favorable alleles were contributed by the IR62266 parent. By using the BC_3F_3 families derived from a cross of IR62266-42-6-2 and IR60080-46A, Robin et al. (94) detected 14 QTLs (on chromosomes 1, 2, 3, 4, 5, 7, 8, and 10) for osmotic adjustment, and the phenotypic variation explained by individual QTLs ranged from 14% to 25%. In durum wheat, the QTLs for osmotic adjustment and leaf rolling were also detected on chromosomes 2B, 4A, 5A, and 7B (83). Nine putative QTLs (on chromosomes 1, 3, 7, 8, 9, 11, and 12) for cell membrane stability in a rice doubled-haploid population were identified, and the contributions ranged from 13.4% to 42.1% (111). Among these, one QTL for cell membrane stability was mapped to the same region as that for osmotic adjustment on chromosome 8 identified in the same population by Zhang et al. (141). Interestingly, this QTL has synteny with QTLs for osmotic adjustment in wheat (61) and barley (108), suggesting that it may be conserved for DT; this region could be an excellent target for the generation of drought-resistant genotypes by marker-assisted selection (MAS).

High WUE is an important breeding target for major crops. Direct measurement of WUE relies on leaf gas exchange data and long-term measurement of plant water consumption and biomass production, and it is logistically difficult in large-scale plant screening. Differences in carbon isotope discrimination ($\delta^{13}C$) have been used as an indirect indicator of WUE. Ishimaru et al. (42) found six QTLs (on chromosomes 2, 3, 4, 6, 8, and 11) for $\delta^{13}C$ that explained 0.628 (r^2) of the total phenotypic variation. Xu et al. (128) identified seven QTLs for $\delta^{13}C$ on chromosomes 1, 4, 7, 8, and 11, and the contributions of these QTLs to the total phenotypic variation ranged from 7.6% to 22.2%. However, there were no common QTLs reproduced in these two studies. Interestingly, the QTL *$\delta^{13}C1.1\_E4$* colocalized with a QTL for leaf length (128), indicating that leaf morphological and physiological traits may be significantly associated with $\delta^{13}C$. In soybean, five QTLs for the $\delta^{13}C$ trait on chromosomes G, H, J, and C1 and seven QTLs on chromosome L were identified (70, 71).

Canopy temperature has also been commonly used in evaluating DA. Yue et al. (138) detected six QTLs for this trait, and Babu et al. (6) detected one QTL on rice chromosome 2. There were no overlapping QTLs in these studies, which may imply that canopy temperature is strongly affected by the environment. In a wheat doubled-haploid population (RAC875/Kukri), two QTLs located on chromosome 3B had a considerable effect on canopy temperature and accounted for up to 22% of the variation (10). Another QTL on chromosome 3B-b identified in the wheat Seri/Babax RIL population explained 14% of canopy temperature variation under drought stress conditions (85). In general, no locus has been confirmed for either WUE or canopy temperature in crops.

Plants accumulate ABA under drought stress, which is also considered to be related to DA. Quarrie et al. (90) identified 10 QTLs for ABA content in rice and found that one minor QTL, only approximately 4% of the phenotypic variation, was coincident with the QTL that also encodes for leaf size. In maize, one QTL for leaf ABA was identified on chromosome 2 by two independent research groups (54, 112), and this QTL (*root-ABA1*) also affects root traits (31). Despite the physiological importance of ABA in drought responses, the genetic basis of drought-induced ABA accumulation remains elusive, especially in relation to morphological traits such as leaf size and root growth.

RSA: root system architecture

2.3. Genetic Control of Root Traits Related to Drought Resistance

Plant roots play a vital role in plant growth, development, and fitness and are also responsible for water and nutrient uptake. Root development is directly affected by environmental factors, and possession of a deep and thick root system that allows access to water deep in the soil has been considered important for DR in crops. The root system is complex and comprises traits such as root volume, root length, root penetration, root thickness, and the root/shoot ratio, which are controlled by many genes and have generally been considered to be contributors to DA (89). The plant root system architecture (RSA) is plastic and dynamic, allowing plants to respond to environmental changes, which then promotes root growth and development to avoid water deficit in the early stages of drought stress. Although numerous QTLs have been reported for root system–related traits, the genetic basis of the plastic RSA in crops grown in natural field conditions, particularly in response to drought stress, is poorly understood.

Using a doubled-haploid population derived from an IR64/Azucena cross, Yadav et al. (130) identified 39 QTLs related to six root traits. In the same population, Hemamalini et al. (37) identified 28 QTLs for five root traits, and Zheng et al. (148) detected 12 QTLs for four root traits. In a RIL-F_6 population derived from a Bala/Azucena cross, 17, 49, and 51 QTLs related to root traits were detected under three different drought stress conditions, respectively (67, 86, 87), but very few QTLs were repeatedly detected or mapped near similar genomic regions. Yue et al. (139) identified a total of 74 QTLs related to seven root traits from the ZS97/IRAT109 RIL population in a two-year experiment under well-watered and drought stress conditions. Among these, 38 QTLs were detected under drought stress conditions, but only 6 were reproduced in both years and only 9 were commonly detected in both water regime conditions. Qu et al. (89) identified 86 QTLs for six root traits at five growth stages, but only 12 of them appeared in more than two growth stages. These results indicate that the QTLs for root traits are highly sensitive to environmental conditions and developmental stages and could not be easily reproduced.

Among the numerous QTLs for different root traits, a significant portion are located in the same chromosome regions or in those in close proximity, and such colocalized QTLs were detected even in different populations. For example, in the RG104–RG348 marker region on chromosome 3, Yadav et al. (130) detected a QTL for total root weight, Hemamalini et al. (37) detected a QTL for both root dry weight and root volume, and Zheng et al. (148) detected a QTL for both root penetration ability and root thickness. A QTL (*QTL9-2*) with a major influence on root traits reported by Price et al. (87) corresponds to a locus affecting diverse root traits reported by MacMillan et al. (67). In the RM472–RM104 region on chromosome 1, a QTL for root volume identified by Yue et al. (139) was found to overlap with the QTL for root thickness and root weight detected by Zheng et al. (147). In the RM470–RM303 region on chromosome 4, QTLs detected by Yue et al. (139) affecting different traits—including deep-root rate, maximum root length, and root volume under drought stress—overlapped with the QTLs for root thickness, root penetration, and penetrated root dry weight in a different population, as reported by Zhang et al. (144).

All of these results indicate that many QTLs for root traits related to drought stress are pleiotropic. Despite this, some QTLs for the same root trait are also commonly detected. Price et al. (87) and Yadav et al. (130) detected a QTL for maximum root length at the RG650 marker on chromosome 7. Common QTLs for root thickness at the G1085 marker on chromosome 9 were also detected in different populations in several studies (37, 148). These colocalized QTLs and common QTLs are promising in MAS for root traits in crop DR breeding programs.

MABC: marker-assisted backcrossing

Despite the obviously important role of RSA in DR, direct root selection for optimal RSA characteristics in the field is difficult because RSA is composed of numerous component root traits, each with a complex genetic basis. Therefore, throughput and phenotyping accuracy remain bottlenecks for genetic analyses of RSA. To address this, developmental models of RSA have emerged during the past decade. New approaches for RSA phenotyping have been developed through direct imaging. Two-dimensional imaging enables the quantification of RSA using images of the root system captured by digital cameras or scanners, and the image acquisition is straightforward for non-soil-grown plants. However, this method can be used only when phenotyping relatively simple RSA traits. Several approaches have been developed using three-dimensional RSA imaging of plants grown in both soil and gel-based growth systems. X-ray computed tomography is a promising technique and has been applied in numerous species, including barley, maize, wheat, and *Arabidopsis* (35, 63, 65, 110); magnetic resonance imaging is another three-dimensional imaging technology that has been used to measure roots. However, both of these techniques are expensive and require long periods for scanning, and thus they have not been widely adopted for high-throughput RSA phenotyping. Multiple-energy computed tomography methods have been developed to resolve the above problems (32, 33). The application of these improved three-dimensional imaging methods should lead to identification of authentic and important loci underlying the genetic basis of RSA traits in the future.

2.4. Validation of Important Quantitative Trait Loci for Crop Performance Under Drought Stress Conditions

Hundreds of QTLs have been identified for DR-related traits, but very few have been verified in appropriate breeding programs for improving crop DR in the field. Among the many important crops, rice has received the most attention for improving DR by MAS. For example, Shen et al. (100) used marker-assisted backcrossing (MABC) to transfer four QTLs for deeper roots (on chromosomes 1, 2, 7, and 9) from doubled-haploid lines derived from Azucena/IR64 into IR64, and some BC3F3 near-isogenic lines (NILs) showed significantly improved root depth and maximum root length under water-limited conditions compared with IR64. Steele et al. (105) also used MABC to introduce four QTLs (for root thickness and root length) from Azucena into Kalinga III. Twenty-two NILs with the target QTLs were evaluated in five field experiments, and one NIL, containing the target segment on chromosome 9 (RM242–RM201) from Azucena, showed markedly increased root length under well-watered and drought stress conditions. By evaluating the grain yield of the MABC products from this study under drought stress conditions for three years, Steele et al. (106) developed a highly drought-tolerant rice variety in India named Birsa Vikas Dhan 111 (PY84) that has high grain yield and good grain quality.

Bernier et al. (12) identified a QTL on chromosome 12 (*qtl12.1*) with a significant effect on grain yield from the F_3 lines of two upland rice varieties, Vandana and Way Rarem, grown under drought stress conditions in the field for two years. The effectiveness of this QTL in improving DT was reproduced when tested in different locations and in different upland drought conditions (13). Interestingly, Mishra et al. (72) tested the effect of *qtl12.1* in the rice variety Sabitri, which is a high-yielding but drought-susceptible recipient, and found that *qtl12.1* has a strong effect on

grain yield under lowland drought conditions. These studies suggest that *qtl12.1* is a major QTL controlling grain yield under drought stress and that it can be used in molecular breeding for improved DR.

Recently, Uga et al. (113) cloned and characterized the QTL *Deeper Rooting 1* (*DRO1*), which controls root growth angle. Higher expression of *DRO1* increases the root growth angle so that the roots grow in a more downward direction. When *DRO1* was introduced into a shallow-rooting rice cultivar by backcrossing, the NIL lines exhibited enhanced DA by increasing deep rooting and maintained high-yield performance under drought conditions. *DRO1* is the first root QTL that has been cloned in crops, and this work further confirmed that control of RSA can contribute significantly to DA.

Some QTLs conferring DR in other crops were also validated by MABC. In maize, a major QTL (*root-ABA1*) for root traits and leaf ABA has been identified (31, 54), and the effect of this QTL was also tested by MABC. The backcross-derived NILs growing in the field showed higher leaf ABA content, confirming the strong and steady effect of *root-ABA1* on ABA induction under different watering conditions (53, 54). In sorghum, four QTLs (*stg1–4*) for the stay-green trait were detected using a population from BT × 642 and RT × 7000, in which BT × 642 is the resource for the stay-green trait. Physiological evaluation showed that RT × 7000 NILs harboring the *stg2* region from BT × 642 displayed more green leaf area than other NILs under terminal drought conditions (36). Multiple NILs with QTLs for yield- and drought-related physiological traits in cotton exhibited the expected phenotype, with lower osmotic potential and higher $\delta^{13}C$ compared with the recipient parent, examined in two field trials with two irrigation regimes (57).

All of the above-mentioned reports clearly show that considerable improvement of DR in crops can be achieved by MAS, which allows for the pyramiding of genes at two or more loci to improve DR. However, the accuracy and precision in QTL identification, the complexity of genetic and environmental interactions, the large number of genes controlling grain yield, and the erroneous use of mapping populations have affected the progress of MAS for DR improvement. With the accessibility of genome sequence information for each crop, integrated genetic and physical maps with a high density of single-nucleotide polymorphisms and other markers for specific DR traits will lead to a substantial role for MAS in molecular breeding for DR.

3. FUNCTIONAL GENOMICS OF DROUGHT RESISTANCE AT VARIOUS LEVELS

Because DR has a complex genetic basis and diverse mechanisms, it is not unexpected that hundreds to thousands of genes or proteins are up- or downregulated under drought stress conditions, and these changes occur mostly on a subcellular scale and often for a short time in specific organs. Functional genomics approaches have recently been adopted to study the molecular basis of DR at the transcriptomic, proteomic, metabolomic, and epigenetic levels.

The early transcriptomic studies were based on large numbers of expressed sequence tags from either normalized or nonnormalized cDNA libraries of drought-stressed tissues from plant genotypes with distinct resistance to drought stress. Bioinformatic analysis of the publicly available expressed sequence tags for various crops provides another option to identify drought-responsive genes. However, the functions of these genes identified by bioinformatic analyses need to be confirmed by laboratory research. With the rapid development of microarray and DNA chip technologies, genome-wide transcript profiling has been widely applied to identify drought-responsive genes in crops. By using Affymetrix GeneChip technology, Wang et al. (115) found that 5,284 genes were differentially expressed in drought-stressed rice seedlings. Lenka et al. (56) compared the transcriptomes of the drought-tolerant rice variety N22 and the drought-susceptible rice variety

IR64 and found that DT is contributed mainly by the enhanced expression of several enzyme-encoding genes. In the same IR64 cultivar, Ray et al. (91) identified 1,563 upregulated genes and 1,746 downregulated genes under water-deficit stress conditions. Degenkolbe et al. (21) investigated the gene expression profiles of four rice varieties (the drought-tolerant varieties IR57311 and LG93-4 and the drought-sensitive varieties Nipponbare and Taipei 309) under drought stress conditions using a 20K oligonucleotide microarray constructed with support from the National Science Foundation. They found that only 245 and 413 genes were commonly suppressed and induced, respectively, in all four cultivars, and more genes were regulated in the sensitive varieties than in the tolerant varieties.

With the advent of next-generation sequencing technology, RNA sequencing (RNA-seq) has been widely applied in expression profiling because of its advantages in finding novel transcripts, splicing models, allele-specific expression, and splicing junctions (68). By using this technique, Dugas et al. (24) identified 28,335 unique transcripts, compared against the transcript databases of rice, maize, and *Arabidopsis*, from sorghum root and shoot treated with polyethylene glycol or ABA. Of the genes producing these transcripts, more than 50 were novel drought-responsive genes. In maize, RNA-seq analysis of ovary and leaf meristems exposed to well-watered or drought conditions revealed that many cell division– and cell cycle–related genes in the drought-stressed ovary were downregulated, whereas many genes in ABA-related pathways were upregulated (48). Although these profiling data were derived from different techniques and are not sufficient to completely explain the diverse DR mechanisms of crops, useful clues can be found by intensively exploring these large data sets along with other functional genomics data sets.

A few studies have reported proteomic or metabolomic changes in crops under drought stress. For instance, Ali & Komatsu (2) investigated the initial responses of rice seedlings to drought stress and found that 10 and 2 proteins were induced and suppressed, respectively, in which an actin depolymerizing factor could be considered a target protein induced by drought, because the expression of the actin depolymerizing factor was higher in a drought-tolerant rice variety before stress and increased further in leaf blades, leaf sheath, and roots under drought stress conditions. Shu et al. (103) identified 60 proteins in rice seedlings that were responsive to drought stress, with the upregulated proteins related mainly to protein folding and assembly. The authors also identified 37 metabolites differentially expressed under drought stress conditions by employing a gas chromatography–mass spectrometry method using plants exposed to the same treatment conditions. Combination analysis of the proteomic and metabolic data revealed that the increased expression of enzymes involved in anabolic pathways may result in increased levels of six amino acids. Another study showed that protective and stress-related proteins (mainly chaperones and dehydrins) were commonly upregulated in both drought-sensitive and drought-tolerant maize varieties under drought stress conditions, but the number and induction levels of the upregulated proteins were generally lower in the sensitive genotypes (9).

Upon exposure to drought stress, plants produce diverse compounds (metabolites) to adapt to the stress. Alvarez et al. (3) detected and quantified the concentration changes of 31 compounds, including ABA, cytokinin, cytokinin-6-benzylaminopurine (BAP), and some phenylpropanoids in maize xylem sap exposed to drought stress conditions. Another study used gas chromatography–mass spectrometry to compare the metabolomic differences of 21 rice cultivars with different DR under drought stress conditions, and the data showed that eight metabolites were positively correlated with DR performance (20). Such comparative proteomic and metabolomic analyses may help reveal the complex mechanisms of DR and provide good candidates for molecular breeding to improve DT.

Evidence suggests that the drought stress response may also be regulated at the epigenetic level, which may be an important mechanism for regulating DR. Wang et al. (117) compared

the genome-wide DNA methylation changes in the drought-resistant rice variety DK151 and the drought-susceptible rice variety IR64. They found that 12.1% of the total site-specific methylation was induced by drought, and the differential methylation/demethylation between DK151 and IR64 was associated with DR performance. Zong et al. (150) investigated the genome-wide distribution and changes in histone H3 lysine 4 trimethylation (H3K4me3) in rice under drought stress conditions and found 3,927 and 910 genes that exhibited increased and decreased H3K4me3 modification, respectively. The modification level was increased mainly in genes with low expression levels and decreased in those with high expression levels. In another study, Shaik & Ramakrishna (98) investigated the scope and extent of epigenetic modification and miRNA-mediated regulation under drought stress conditions through bioinformatic analyses of 5,468 drought-responsive genes. The results showed that one-third of the drought-responsive genes are targeted by two-thirds of all known predicted miRNAs, and approximately 75% of the drought-responsive genes possibly involved in chromatin remodeling were downregulated under drought stress conditions.

Overall, the functional genomics studies to date in crops exposed to drought stress conditions are fragmental and insufficient to reveal the molecular basis of DR. However, such large data sets derived from genomics-based approaches provide valuable resources for discovering novel information, especially for identifying important molecular components for DR and elucidating their functions.

4. GENETIC ENGINEERING OF DROUGHT-RESISTANCE GENES IN CROPS

To adapt to drought stress, plants have evolved multiple interconnected chains of signaling processes to regulate different sets of drought-responsive genes for producing various classes of proteins, including TFs, enzymes, molecular chaperones, and other functional proteins. These proteins function accordingly to enhance plant resistance under drought conditions. Hundreds or even thousands of genes (as well as proteins and other regulatory elements) that control the processes of plant responses have been identified by the diverse functional genomics approaches described above. In parallel to this, many of these genes have been genetically manipulated or engineered to test their effects on improving DR in crops by overexpression and suppression transgenic technologies (**Table 1**).

The ectopic expression or suppression of regulatory genes could potentially activate multiple mechanisms of stress tolerance. Genes encoding various TF family members, such as dehydration-responsive element-binding factor (DREB), basic leucine zipper (bZIP), zinc-finger proteins, and NAM-ATAF-CUC2 (NAC), have been identified as involved in regulating DT and are promising for improving DR in crops (4, 16, 26, 132). ABA-responsive element-binding proteins/factors (AREBs/ABFs) are bZIP TFs known to function in ABA-dependent regulatory systems in response to drought stress. Overexpression of AREB1/ABF2, ABF3, or AREB2/ABF4 in *Arabidopsis* enhanced ABA sensitivity and DT (27, 49). When *ABF3* was overexpressed in rice under the control of the maize ubiquitin promoter (Ubi1P), rice seedlings showed delayed leaf rolling and wilting after drought stress treatment (79). Other rice bZIP TFs, such as OsbZIP23 (driven by Ubi1P), OsbZIP46 in the constitutively active form (driven by Ubi1P), and OsbZIP72 (driven by the CaMV35P promoter), have been tested in rice, and overexpression of the genes encoding these TFs resulted in improved DT (64, 107, 123). Moreover, *S1AREB1*-overexpressing tomato plants showed increased tolerance to water stress (80), and overexpression of the *GmbZIP* gene in soybean resulted in not only enhanced DT but also enhanced tolerance to salt and cold stresses (28).

NAC is a plant-specific TF family with a highly conserved DNA-binding domain, and many genes belonging to this family are responsive to drought stress (26, 34). *Stress-responsive NAC*

Table 1 Major genes that have been tested for drought resistance in major crops

Functional category		Gene	Protein function	Origin	Transformation receptor	Materials, expression	Testing conditions and stages	Parameters	Reference
Signaling factors									
Protein kinases	MAPKs	*OsMAPK5*	MAPK	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Survivability	126
		NPK1	MAPKKK	*N. tabacum*	*O. sativa*	OE, rice *Actin1P/LEA3-1P* (stress inducible)	FD, RS	Seed setting rate	124
		DSM1	MAPKKK	*O. sativa*	*O. sativa*	OE, maize *Ubi1P*	HO, VS	Plant growth	76
	CIPK	*OsCIPK12*	CBL-interacting protein kinase	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Survivability	122
	CDPK	*OsCDPK7*	Calcium-dependent protein kinase	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Plant growth, F_v/F_m	96
	Other kinase	*OsSIK1*	Receptor-like kinase	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Survivability	81
		SOS2	Serine/threonine kinase	*A. thaliana*	*O. sativa*	OE, rice *LEA3-1P*	FD, RS	Seed setting rate	124
Transcription factors	AP2/ERF	*OsDREB1A, -1B*	DREB1/CBF	*O. sativa*	*O. sativa*	OE, *CaMV35SP*/maize *Ubi1P*	DH/GH, VS	Survivability	43
		DREB1A, -1B, -1C	DREB1/CBF	*A. thaliana*	*O. sativa*	OE, maize *Ubi1P*	DH/GH, VS	Survivability	43
		DREB1A	DREB1/CBF	*A. thaliana*	*O. sativa*	OE, maize *Ubi1P*	GH, VS	Survivability	79
		DREB1A	DREB1/CBF	*A. thaliana*	*O. sativa*	OE, rice *LEA3-1P*	FD, RS	Yield, seed setting rate	124
		HvCBF4	DREB1/CBF	*H. vulgare*	*O. sativa*	OE, maize *Ubi1P*	GH, VS	Survivability	78
		OsDREB1F	DREB1/CBF	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	DH, VS	Survivability	116
		OsDREB1G, -2B	DREB1/CBF, DREB2	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Survivability	15
		OsDREB2A	DREB2	*O. sativa*	*O. sativa*	OE, *ARPC* (stress inducible)	HO/GH, VS	Survivability	17
		TaDREB2, -3	DREB	*T. aestivum*	*T. aestivum*/ *H. vulgare*	OE, double *CaMV35SP*/maize *Rab17P*	GH, VS	Multiple	73
		HARDY	AP2/ERF-like	*A. thaliana*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Survivability, WUE, photosynthesis	50

	bZIP	*OsbZIP23*	bZIP	*O. sativa*	*O. sativa*	OE, maize *Ubi1P*	GH, VS/RS	Relative yield	123
		OsbZIP46 (constitutive active form)	bZIP	*O. sativa*	*O. sativa*	OE, maize *Ubi1P*	HO/GH, VS/RS	Survivability, relative yield	107
		OsbZIP72	bZIP	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	DH, VS	Survivability	64
		ABF3	bZIP	*A. thaliana*	*O. sativa*	OE, maize *Ubi1P*	GH, VS	Survivability, F_v/F_m	79
		SlAREB1	bZIP	*S. lycoper-sicum*	*S. lycopersicum*	OE, *CaMV35SP*	DH/VS	Multiple	80
	NAC	*SNAC1*	NAC	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH/FD, VS/RS	Survivability, seed setting rate	39
		OsNAC9	NAC	*O. sativa*	*O. sativa*	OE, rice *RCc3P* (root-specific expression)	GH/FD, VS/RS	Multiple	92
		OsNAC10	NAC	*O. sativa*	*O. sativa*	OE, rice *RCc3P*	GH/FD, VS/RS	Multiple	45
		OsNAC5	NAC	*O. sativa*	*O. sativa*	OE, rice *RCc3P*	GH/FD, VS/RS	Multiple	46
		OsNAC6	NAC	*O. sativa*	*O. sativa*	OE, maize *Ubi1P*	DH, VS	Survivability	74
		SNAC1	NAC	*O. sativa*	*T. aestivum*	OE, maize *Ubi1P*	GH, VS	RLWC, chlorophyll content	95
		TaNAC69	NAC	*T. aestivum*	*T. aestivum*	OE, barley *HvDhn8s/HvDhn4s* (stress inducible)	GH, VS	Multiple	129
	Zinc finger	*DST*	C2H2 zinc finger			EMS mutant	HO/GH, VS	Survivability	40
		ZFP252	C2H2 zinc finger	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Survivability	127
		Zat10	C2H2-EAR zinc finger	*A. thaliana*	*O. sativa*	OE, rice *Actin1P/LEA3-1P*	FD, RS	Yield, seed setting rate	124
Other transcription factors		*OsMYB2*	MYB	*O. sativa*	*O. sativa*	OE, maize *Ubi1P*	HO/GH, VS	Survivability	133
		TaPIMP1	R2R3 MYB	*T. aestivum*	*T. aestivum*	OE, maize *Ubi1P*	GH, VS	Water loss, proline content	146
		StMYB1R-1	R1-type MYB-like	*S. tuberosum*	*S. tuberosum*	OE, *CaMV35SP*	GH, VS	Multiple	101
		OsWRKY11	WRKY	*O. sativa*	*O. sativa*	OE, rice *HSP101P*	GH, VS	Survivability	121
		OsWRKY30	WRKY	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	HO, VS	Survivability	99

(*Continued*)

Table 1 (*Continued*)

Functional category	Gene	Protein function	Origin	Transformation receptor	Materials, expression	Testing conditions and stages	Parameters	Reference
Protein degradation	*OsDIS1*	E3 ubiquitin ligase			RI	GH, VS	Survivability	77
	OsDSG1	E3 ubiquitin ligase			KM	GH, VS	Fresh weight	82
	OsSDIR1	E3 ubiquitin ligase	*O. sativa*	*O. sativa*	OE, maize *Ubi1P*	GH, VS	Survivability	29
	OsRDCP1	E3 ubiquitin ligase	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Survivability	8
Protein modification	*SQS1*	Farnesyltransferase/squalene synthase			RI	GH, VS/RS	Survivability, relative yield	69
Other nuclear proteins	*OsSKIPa*	Ski-interacting protein	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS/RS	Survivability, yield	38
	OSRIP18	Ribosome-inactivating protein	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	HO/GH, VS/RS	Survivability	47
Functional proteins								
Metabolism of abscisic acid	*DSM2*	Carotene hydroxylase	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS/RS	Survivability, seed setting rate	22
	LOS5	Molybdenum cofactor sulfurase	*A. thaliana*	*O. sativa*	OE, rice *Actin1P/LEA3-1P*	FD, RS	Yield, seed setting rate	124
	LOS5	Molybdenum cofactor sulfurase	*A. thaliana*	*G. max*	OE, superpromoter (enhancer and promoter)	GH/FD, VS	Multiple	60
	LOS5	Molybdenum cofactor sulfurase	*A. thaliana*	*G. hirsutum*	OE, superpromoter	GH, VS	Multiple	140
Metabolism of other hormones	*IPT*	Isopentenyltransferase	*A. tumefaciens*	*O. sativa*	OE, bean *SAPKP* (maturation and stress inducible)	GH, RS	Yield, biomass	83
	IPT	Isopentenyltransferase	*A. tumefaciens*	*G. hirsutum*	OE, bean *SAPKP* (maturation and stress inducible)	GH, VS	Multiple	51

Osmotic adjustment	Trehalose	*OsTPS1*	Trehalose-6-phosphate synthase	*O. sativa*	*O. sativa*	OE, rice *Actin1P*	DH/HO, VS	Survivability	58
		TPSP (*otsA* + *otsB*)	Trehalose-6-phosphate synthase/ phosphatase	*E. coli*	*O. sativa*	OE, *ARPC*/rice *rbcSP* (mesophyll-specific expression)	GH, VS	Plant growth, F_v/F_m	30
		TPSP (*otsA* + *otsB*)	Trehalose-6-phosphate synthase/ phosphatase	*E. coli*	*O. sativa*	OE, maize *Ubi1P*	GH, VS	Plant growth, F_v/F_m	44
	Proline	*P5CS*	Δ^1-Pyrroline-5-carboxylate synthetase	*P. aconiti-folius*	*O. sativa*	OE, *ARPC*	GH, VS	Fresh weight	149
	Mannitol	*mtlD*	Mannitol-1-phosphate dehydrogenase	*E. coli*	*T. aestivum*	OE, maize *Ubi1P*	GH, VS	Multiple	1
Dehydrin/LEA		*OsLEA3-1*	LEA protein	*O. sativa*	*O. sativa*	OE, *CaMV35SP*/rice *LEA3-1P*	GH/FD, RS	Yield, seed setting rate	125
		OsLEA3-2	LEA protein	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	GH, VS	Survivability, grains per spike	23
		HVA1	LEA protein	*H. vulgare*	*O. sativa*	OE, rice *Actin1P*	GH, VS	Plant growth, survivability, relative water content	7
		HVA1	LEA protein	*H. vulgare*	*T. aestivum*	OE, maize *Ubi1P*	FD, VS	WUE, biomass	104
Other transporters		*AtNHX1*	Na^+/H^+ antiporter	*A. thaliana*	*O. sativa*	OE, rice *Actin1P*	FD, RS	Seed setting rate	124
		OsPIN3t	Auxin efflux carrier	*O. sativa*	*O. sativa*	OE, *CaMV35SP*	HO/GH, VS	Plant growth, survivability	143
		betA, *TsVP*	Choline dehydrogenase (*betA*), V-H^+-PPase (*TsVP*)	*E. coli* (*betA*), *T. halophila* (*TsVP*)	*Z. mays*	OE, maize *Ubi1P*	GH/VS	Multiple	120
Reactive oxygen species scavenging		*OsSRO1c*	Similar to RCD1	*O. sativa*	*O. sativa*	OE/KM, maize *Ubi1P*	HO/GH/FD, VS	Multiple	136
Amino acid metabolism		*OsOAT*	Ornithine δ-aminotrans-ferase	*O. sativa*	*O. sativa*	OE, maize *Ubi1P*	GH/FD, VS/RS	Survivability, relative seed setting rate	135

Species: *A. hypogaea*, *Arachis hypogaea*; *A. thaliana*, *Arabidopsis thaliana*; *A. tumefaciens*, *Agrobacterium tumefaciens*; *E. coli*, *Escherichia coli*; *G. hirsutum*, *Gossypium hirsutum*; *G. max*, *Glycine max*; *H. vulgare*, *Hordeum vulgare*; *N. tabacum*, *Nicotiana tabacum*; *O. sativa*, *Oryza sativa*; *P. aconitifolius*, *Phaseolus aconitifolius*; *S. lycopersicum*, *Solanum lycopersicum*; *S. tuberosum*, *Solanum tuberosum*; *T. aestivum*, *Triticum aestivum*; *T. halophila*, *Thellungiella halophila*; *Z. mays*, *Zea mays*. Testing conditions: DH, dehydration; HO, high osmotic medium supplied with polyethylene glycol or mannitol; GH, greenhouse or growth chamber (with plants grown in pots containing soil); FD, field. Testing stages: VS, vegetative or seedling stage; RS, reproductive or flowering stage. Other abbreviations: KM, knockout mutant; OE, overexpression; RI, RNA interference repression; RLWC, relative leaf water content; WUE, water-use efficiency. "Multiple" denotes that more than three parameters analyzed.

1 (*SNAC1*) is a member of the rice NAC family and is specifically induced in guard cells by drought stress conditions. Overexpression of *SNAC1* in rice resulted in significantly enhanced DR under severe drought conditions in the field at the reproductive stage (22–34% higher seed setting) without any phenotypic changes or yield penalty under normal growth conditions (39). The *SNAC1*-overexpressing rice showed a significant reduction in stomatal aperture, and the enhanced DT appeared to be due partially to the increased WUE and increased ABA sensitivity in guard cells (39). When *SNAC1* was overexpressed in wheat, transgenic wheat also showed improved DR (95), providing additional promising evidence for the value of this gene.

In addition to *SNAC1*, several other NAC gene family members in rice, such as *OsNAC6*, *OsNAC10*, *OsNAC5*, and *OsNAC9*, have roles in DR (45, 46, 74, 92). *OsNAC6*-overexpressing transgenic rice plants under the control of the maize ubiquitin ZmUbi promoter had 42–57% higher rates of recovery after being removed from hydroponic medium for 12 h, but the transgenic plants exhibited a growth retardation phenotype (74). OsNAC5 can interact with OsNAC6 and SNAC1, and transgenic rice overexpressing *OsNAC5* driven by the root-specific promoter RCc3 showed higher grain yields than wild-type rice under drought stress conditions in the field, but the constitutive overexpression of *OsNAC5* had no obvious effect on DR (46). Interestingly, with the same strategy, RCc3::OsNAC9 and RCc3::OsNAC10 transgenic rice also showed significantly enhanced DT at the reproductive stage in field trials (45, 92). NAC genes from other crops were also tested for their effect on DT. When the *TaNAC69* gene from bread wheat was overexpressed in bread barley leaves and roots through use of a barley drought-inducible HvDhn4s promoter, the transgenic plants produced more shoot biomass under water-deficit conditions (129).

Some DREBs/CBFs function in ABA-independent drought-responsive pathways, and members of this family from many plant species have been characterized as improving DT. The function of the DREBs (which include two subclasses, DREB1 and DREB2) in stress tolerance was found first in *Arabidopsis* (62). The DREB1/CBF regulatory function has been intensively explored to determine whether it can improve DT in crops. *Arabidopsis DREB1A*, *-1B*, and *-1C* and rice *OsDREB1A*, *-1B*, *-1E*, *-1F*, and *-1G* driven either by the CaMV35S, ZmUbi, or OsActin1p promoters or by the drought-inducible HVA22P promoter have been transformed in rice or other crops, and the transgenic plants showed increased DT in greenhouse and field trials (15, 43, 79, 116, 124). OsHVA22P::AtDREB1A plants showed significantly higher relative spikelet fertility and yield than wild-type control plants after severe drought stress at the panicle development stage (124). Unlike the plants with growth defects, which resulted from the overexpression of *OsDREB1A* and *OsDREB1B* (43), transgenic rice overexpressing *OsDREB1F* and *OsDREB1G* showed no obvious growth retardation (15, 116).

DREB2 TFs have also been tested for their functions in DT. For example, introducing *OsDREB2A* under the control of the stress-inducible promoter ABRC and *OsDREB2B* driven by the CaMV35S promoter into rice resulted in transgenic plants that exhibited improved DT (17, 116). Similarly, the drought-induced expression of wheat *TaDREB2* and *TaDREB3* resulted in improved survival rates of transgenic wheat and barley under severe drought stress conditions, whereas no obvious growth defects were observed under normal growth conditions. These results are in contrast to the growth retardation and delayed flowering observed in transgenic plants constitutively overexpressing *TaDREB2* and *TaDREB3* (73). In general, these reports suggest that DREB TFs have promising potential for crop engineering for DT when suitable drought-inducible promoters are used to avoid the unwanted side effects.

Quite a few zinc-finger proteins confer DR in crops. Transgenic rice overexpressing *OsZFP252* showed enhanced DR, with 74–79% higher survival rates and increased accumulation of proline and soluble sugars (127). DST, another rice zinc-finger protein, acts as a negative regulator of

stomatal closure by modulating H_2O_2 homeostasis, and the *dst* mutant exhibited enhanced DT and salt tolerance through increased stomatal closure and reduced stomatal density (40). When *Arabidopsis ZAT10* induced by DREB1A/CBF3 was overexpressed in rice, the transgenic plants showed 17–36% increased yield production compared with the wild type under severe drought stress conditions in the field (124).

Numerous TFs from other families and nuclear proteins also function in DT. Overexpression of *OsWRKY11* in rice by the heat-inducible promoter HSP101 significantly improved drought and heat tolerance (121), and *OsWRKY30* also regulates DT in rice (99). Transgenic rice overexpressing *OsMYB2* showed improved DT and increased accumulation of soluble sugars but decreased levels of H_2O_2 and malondialdehyde (133). Two additional studies reported that *TaPIMP1*-overexpressing transgenic wheat and *StMYB1R-1*-overexpressing potato plants also showed improved tolerance to dehydration stress (101, 146). Nuclear factor Y (NF-Y) is a CCAAT-binding TF composed of A, B, and C subunits; in *Arabidopsis*, *AtNF-YB1* and *AtNF-YA5* were able to improve DR when constitutively overexpressed (59, 75). Overexpression of maize *ZmNY-YB2*, an ortholog of *AtNF-YB1*, in maize plants resulted in improved DT, with an increased yield of ~50% under relatively severe drought stress conditions in the field (75).

A few nuclear proteins, such as OsSKIPa (38) and OsRIP18 (47), which function as transcriptional coregulators, have also been identified in stress tolerance. OsSKIPa is a rice homolog of the human Ski-interacting protein (SKIP), and *OsSKIPa*-overexpressing transgenic rice exhibited significantly enhanced DT at the reproductive stage, which was partially contributed by the increased ROS-scavenging ability and activation of many stress-related genes (38).

Posttranscriptional protein modifications such as protein phosphorylation/dephosphorylation, protein degradation/modification, and sensing of secondary messengers such as Ca^{2+} play important roles in stress signaling and regulation. Some TFs and drought-responsive proteins need to be phosphorylated/dephosphorylated or modified by posttranslational regulation to become active, and genes encoding mitogen-activated protein kinase (MAPK) cascades, calcineurin B–like protein interacting protein kinase (CIPK), calcium-dependent protein kinase (CDPK or CPK), and receptor-like kinases have roles in drought stress signaling and regulation pathways (**Table 1**). OsMAPK5 was the first characterized protein kinase in rice for regulating drought and other abiotic stresses, but it negatively regulates biotic stress (126). When the kinase domain of *NPK1* [a tobacco MAPK kinase kinase (MAPKKK)] was constitutively overexpressed in maize, the transgenic lines showed enhanced DT by maintaining a high photosynthetic rate. The drought-stressed transgenic maize plants produced kernels comparable to those produced under well-watered conditions (102). Similarly, when *NPK1* was overexpressed in rice by a drought-inducible promoter, the transgenic rice showed significantly increased rates of spikelet fertility (23% higher than those of the controls) and grain production (28% higher than those of the controls) under severe drought stress conditions in the field (124). Another MAPKKK, DSM1, also confers osmotic stress tolerance in rice, mainly through ROS-scavenging mechanisms (76). Two types of Ca^{2+}-sensing protein kinases, CIPK and CDPK, are also involved in stress signaling. *OsCDPK7*- and *OsCIPK12*-overexpressing rice showed increased tolerance to drought (96, 122). The observed DT in rice conferred by OsCIPK12 correlated with a significant increase in the proline and soluble sugar content after exposure to drought stress conditions (122). Among the other cases, overexpression of the receptor-like protein kinase gene *OsSIK1* resulted in improved drought and salt tolerance, with increased activities of peroxidase and superoxide and lower accumulation of H_2O_2 (81). Overexpression of the *Arabidopsis* serine/threonine kinase gene *SOS2* under the control of a rice drought-inducible promoter enhanced DR under field conditions (124).

Farnesylation is a posttranslational modification in which a farnesyl group is added to inactive target proteins so that they are targeted to membranes. Transgenic canola plants in which

farnesyltransferase FTB or FTA was suppressed by the drought-inducible RD29A promoter exhibited significantly higher yields than control plants under drought stress conditions in field trials (118, 119). Suppressing the rice farnesyltransferase SQS by RNA interference greatly enhanced DR through reduced stomatal conductance and higher relative water content at both the vegetative and reproductive stages (69). Protein degradation is another way to inactivate protein function. Diverse proteins such as E3 ligases, RING-domain-containing proteins, and F-box proteins are involved in the protein degradation pathway. Among the E3 ligases identified in rice, OsSDIR1 and OsRDCP1 positively regulate DT (8, 29), whereas OsDIS1 and OsDSG1 negatively regulate DT (77, 82).

Under drought stress conditions, ABA is synthesized and accumulates in guard cells, where it triggers stomatal closure to reduce water loss (97). The roles of many proteins in the ABA signaling pathway—such as TFs, protein kinases, and other regulatory proteins—have been reviewed (134), and some of them were described above in terms of their ability to improve DR in crops. Some genes in the ABA metabolism pathway have also been tested for their roles in DR in crops. LOS5/ABA3, a molybdenum cofactor sulfurase, is a key enzyme functioning in the final step of ABA biosynthesis in *Arabidopsis*. When *LOS5/ABA3* was overexpressed in rice, the transgenic plants showed improved spikelet fertility and grain yield under drought stress conditions in the field (124). LOS5 also had a positive effect on improving DR when overexpressed in soybean and cotton (60, 140). NCED (9-*cis*-epoxycarotenoid dioxygenase) is a drought-inducible and rate-limiting enzyme involved in ABA biosynthesis; positive effects on DR were observed in transgenic rice overexpressing *Arabidopsis* NCED2 (124) and tomato plants overexpressing the endogenous gene *LeNCED1* (109). Recently, the rice gene *DSM2*, encoding a β-carotene hydroxylase that converts β-carotene to zeaxanthin (an ABA precursor), has been identified as having a positive role in improving DR in rice (22).

In addition to genes associated with the ABA pathway, several genes functioning in other hormone metabolism pathways have been investigated for their effects on stress tolerance. Overexpression of a rice *IPT* gene, encoding a critical enzyme for cytokinin biosynthesis, resulted in increased DR and increased grain yield by regulating hormone synthesis and homeostasis (84). Similar results were obtained by overexpressing different *IPT* homolog genes in other crops, including cotton and tobacco (51, 93). These results indicate that IPTs from different species have the same functions, thus providing promising candidates for engineering DR in crops.

Genes encoding late embryogenesis abundant (LEA) proteins are often strongly induced at the later stages of drought stress, and some of them act as chaperones. Overexpression of barley *HVA1* (encoding a LEA protein) in rice and wheat led to improved growth performance and WUE under drought stress conditions, and the increased DR of *HVA1*-overexpressing transgenic wheat has been verified in several field trials (7, 104). Recently, two rice LEA genes, *OSLEA3-1* and *OsLEA3-2*, have been reported to play roles in drought stress regulation (23, 125). Transgenic rice overexpressing *OsLEA3-1* exhibited significantly less yield loss than control plants under severe drought stress conditions in field trials (125).

Osmoprotectants are small, nontoxic molecules that can stabilize proteins and cell membranes or scavenge ROS during exposure to stress conditions. Most crops cannot synthesize the osmoprotectants that accumulate in stress-tolerant species. Increasing the osmoprotectant synthesis/metabolic pathways for the production of osmolytes has also been attempted in order to increase the DR of crops. Transformation of a mannitol dehydrogenase gene (*mtlD*) into wheat resulted in enhanced DT under water-limiting conditions (1). However, no significant differences in osmotic adjustment between the *mtlD* transgenic wheat and control plants were observed, suggesting that the effect of mannitol resulted from protective mechanisms, mainly by stabilization of macromolecules, rather than by osmotic adjustment.

Proline is one of the common plant osmoprotectants, and during drought stress, its biosynthesis is promoted and its degradation is prevented. Overexpression of a gene encoding 1-pyrroline-5-carboxylate synthetase (P5CS), the rate-limiting enzyme in proline biosynthesis, improved the DT of transgenic rice (149). Recently, similar results in enhancing DT were reported in transgenic petunia plants overexpressing the *AtP5CS* and *OsP5CS* genes (131). Suppression of the gene encoding 1-pyrroline-5-carboxylate reductase (P5CR) in soybean resulted in increased sensitivity to water deficit and heat stresses (19), whereas overexpression of the *P5CR* gene resulted in increased relative water content in leaves with overaccumulation of proline under drought stress conditions (18). Ornithine δ-aminotransferase (δ-OAT) is a pyridoxal-5′-phosphate-dependent enzyme that has been proposed to be involved in proline and arginine metabolism. Transgenic rice overexpressing *OsOAT* showed increased proline levels and enhanced tolerance to drought and oxidative stresses (135).

Trehalose plays an important role in abiotic stress and functions as a reserve carbohydrate and stress protectant, stabilizing proteins against denaturation. In plants, trehalose biosynthesis is catalyzed by two key enzymes: trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP). To improve the DT of rice by increasing the accumulation of trehalose, a TPP/TPS fusion gene from the *Escherichia coli* trehalose biosynthetic genes (*otsA* and *otsB*) was introduced into rice, and the transgenic plants indeed exhibited increased amounts of trehalose and improved DT (30, 44). In addition, the transgenic rice exhibited less photooxidative damage under salt and low-temperature stresses. Similar results were obtained from the overexpression of *OsTPS1* (58) and *AtTPS1* (5). These results confirm the role of trehalose in carbon metabolism; however, these transgenic plants were seldom tested under field conditions.

5. DROUGHT-RESISTANCE BREEDING IN CROPS

New crop varieties with improved DR and/or WUE have been developed through conventional breeding approaches in the past decade, and some DR breeding programs have adopted MAS technology. However, no public research institutes have reported engineering-based DR crops, although some private companies have claimed to have a few cases of genetically modified crops undergoing field trials. Here, we briefly review the progress in conventional and MAS-integrated DR breeding in crops.

Conventional breeding for DR adopts mainly a large-scale backcross strategy to develop new varieties with improved DR and high yield potential. For example, in rice, the International Rice Research Institute made a total of 322 crosses between 3 elite recurrent parents (IR64, Teqing, and IR68552-55-3-2) and 163 donor varieties of diverse origins (137). The backcross progenies derived from these crosses that showed higher yields than the recurrent parents were selected in severe-drought lowland or upland nurseries (52). Similarly, to develop new rice varieties with improved DR and high yield, the Shanghai Agrobiological Gene Center performed large-scale crossing and backcrossing using elite paddy rice varieties as recurrent parents and upland drought-resistant rice as donors. In recent years, a series of water-saving and drought-resistant rice (WDR) varieties have been developed and released, such as Huhan3, Huhan2B, and Hanyou3 (66). Because of the polygenic nature, low heritability, and high levels of genotype–environment interaction of DR-related traits, the progress of conventional DR breeding in crops is still quite slow.

With the successful application of MAS in crop breeding for relatively simple traits (such as *R* genes for disease resistance), this technology has also been deployed in breeding practices to improve crop DR. The common strategy of MABC is to introgress major QTLs for drought-resistant donor genotypes into high-yielding but less drought-resistant or drought-sensitive recipient

MARS: marker-assisted recurrent selection

parents. This means that the superior cultivars developed contain only the major QTLs from the donor, and the whole genome of the recurrent parent remains. For example, the drought-tolerant variety PY84 was developed by introgressing root trait QTLs in the elite rice cultivars IR64 and Kalinga (100, 105).

MABC has not been very successful in improving DR, however, mainly because DR is controlled by multiple minor QTLs or by epigenetic QTLs. To overcome such limitations, a marker-assisted recurrent selection (MARS) approach has been applied, involving selecting individuals with the most significant effects in each selection cycle (11). MARS was successfully used to develop superior varieties of sweet corn, sunflower, and soybean (25). Genome-wide selection or genomic selection is another way to develop superior lines with overall excellent performance and DR, but so far this approach has not generated any drought-resistant crop products.

6. CONCLUSIONS AND PERSPECTIVES

Based on the progress discussed above and our knowledge, an integrated strategy for dissecting the mechanisms of DR and improving DR in crops is summarized in **Figure 1**. Under natural conditions, the timing, strength, and endurance of drought stress are highly dynamic and unpredictable, which further complicates DR evaluation. In addition, DR should be evaluated, if

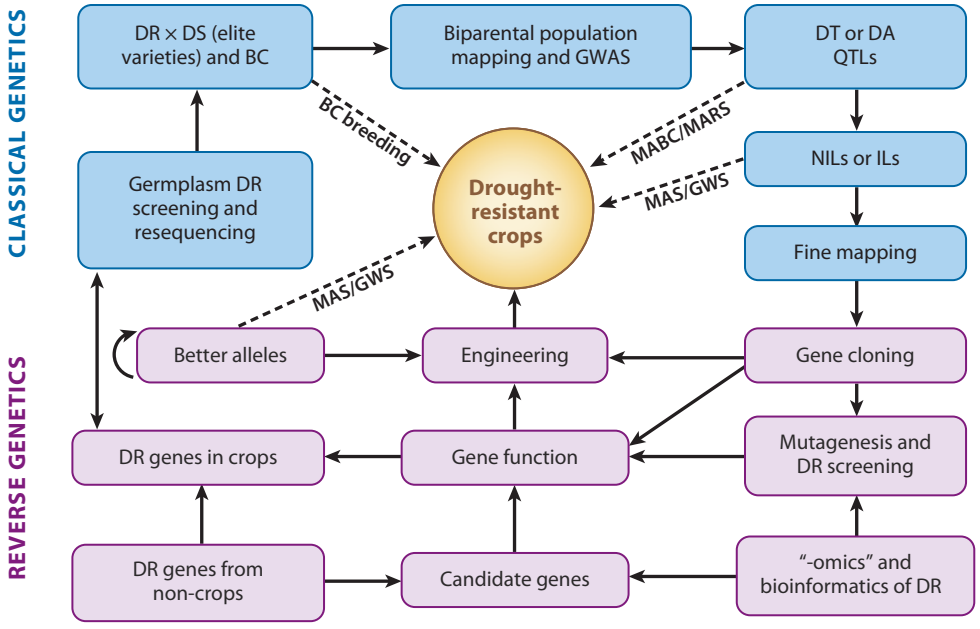

Figure 1

Approaches for engineering and breeding drought-resistant crops. Both classical (*blue*) and reverse (*purple*) approaches are integrated to study the genetic and molecular basis of drought resistance and to identify genes or loci for engineering or breeding drought-resistant crops. Abbreviations: BC, backcrossing; DA, drought avoidance; DR, drought resistance; DS, drought sensitivity; DT, drought tolerance; GWAS, genome-wide association study; GWS, genome-wide selection; IL, isogenic line; MABC, marker-assisted backcrossing; MARS, marker-assisted recurrent selection; MAS, marker-assisted selection; NIL, near-isogenic line; QTL, quantitative trait locus.

possible, in association with other major abiotic stresses, such as high temperature and salinity, because drought stress frequently occurs along with high-temperature stress, and crosstalk occurs between the responses to these stresses at various levels.

Numerous QTLs for drought-tolerant traits have been identified in major crops, and many attempts have been made to use these major QTLs to develop drought-tolerant crops. However, very few have proven successful, owing mainly to the influence of genetic background and the environment. Similarly, recent advances in functional genomics have made it easy to conduct large-scale and high-throughput genotyping (such as resequencing hundreds of germplasms affordably in a short period of time), which facilitates the identification of major QTLs or candidate genes for DR. However, accurate and high-throughput phenotyping of DR traits, especially in field conditions, is currently the major limiting factor for both genetic dissection and breeding of DR in crops. There is no doubt that successful cloning of QTLs for DR traits will enable us to better understand the genetic basis of the traits and more effectively manipulate the traits for DR breeding. In addition to the commonly used MABC, newly emerging breeding approaches such as MARS and genome-wide selection provide more powerful tools for pyramiding multiple QTLs or integrating multiple traits for DR (**Figure 1**).

Many genes have been identified that could improve crop DR through transgenic approaches. However, the effects of these genes on DR have been tested mainly in greenhouses with plants grown in small pots or only at the seedling stages. Only a few have been tested in fields (**Table 1**), including the *FTA/FTB* genes in canola (118, 119); *SNAC1*, *OsNAC9*, *OsNAC10*, and *OsLEA3-1* in rice (39, 124, 125); and *ZmNF-YB2* in maize (75). Owing to the complex nature of drought stress in the field, those genes proven to be effective in improving DR in the greenhouse must be further evaluated in the field before being used in breeding programs. Some genes also have negative side effects on normal growth or yield potential (84). Identification and application of tissue-specific and drought-inducible promoters may overcome this problem, but practically, useful promoters for such purposes are limited so far.

Although hundreds of genes and QTLs related to DR have been identified in both the model plant *Arabidopsis* and many crops, our knowledge of signaling networks and the complete picture of the genetic and molecular mechanisms of DR is still limited. Most genetic and molecular studies of DR have focused on the aboveground parts of plants; the underground parts have received much less attention because of the difficulties in phenotyping. Root architecture and plasticity, which play important roles in crop growth in drought-prone environments, and stomatal movement should be focus areas for studying DR. More integrative studies that link the genetics, genomics, physiology, systems biology, and agronomics of DR are challenging but will advance our knowledge of this complex trait. Another challenge in the near future is to identify the signaling components missing in the current models of drought-response pathways and the crosstalk between the response pathways of different stresses. The last but most important challenge is how to efficiently explore the large data sets derived from "-omics" (such as genomics, transcriptomics, proteomics, and metabolomics) and phenotyping studies of DR (**Figure 1**) in order to find or crystallize key functional units or machinery that can be used as tools for engineering and breeding DR in crops.

SUMMARY POINTS

1. Hundreds of QTLs for the diverse traits related to drought resistance have been mapped, but only a small portion of them can be repeatedly detected in different environments and populations, and very a few have been verified or cloned.

2. Increasingly huge data sets are being derived from genome-wide studies at the transcriptomic, proteomic, and metabolomic levels, but how to efficiently explore these data sets to extract the essential functional pathways or networks for genetic improvement of drought resistance remains a significant challenge.
3. Hundreds of genes that control the key processes of drought responses or adaptation have been identified. The genes that have been proven effective for improving drought resistance in crops were summarized by highlighting those being tested in the field.
4. New crop varieties with improved drought resistance have been developed by conventional breeding approaches. Very few QTLs or functional genes have been successfully integrated into conventional breeding programs.
5. Approaches for engineering and breeding drought-resistant crops based on intensive and integrative studies that link genetics, genomics, physiology, systems biology, and agronomics will advance our knowledge of drought resistance.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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