*Review*

# Breeding Drought-Tolerant Maize (*Zea mays*) Using Molecular Breeding Tools: Recent Advancements and Future Prospective

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**Abstract:** As a most significant cereal crop, maize provides vital nutritional components to humans and livestock. Drought stress curtails maize growth and yield by impairing several morphological, physiological, and biochemical functions. The rising threats of drought stress significantly affect global food security and increase the ratio of hunger and starvation. The use of molecular breeding techniques has enabled maize researchers to deeply examine the genetic control of drought tolerance and the genetic differences between genotypes to drought stress. Despite the significant progress in molecular genetics, the drought tolerance mechanism is still not fully understood. With the advancements in molecular research, researchers have identified several molecular factors associated with maize tolerance to drought stress. Quantitative trait loci (QTL) mapping and genome-wide association study (GWAS) analysis have led to identifying QTL, and genes linked to drought tolerance in maize that can be further exploited for their possible breeding applications. Transcriptome and transcription factors (TFs) analysis has revealed the documentation of potential genes and protein groups that might be linked to drought tolerance and accelerate the drought breeding program. Genetic engineering has been used to develop transgenic maize cultivars that are resistant to drought stress. Clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) is a new ray of hope to edit the gene of interest to enhance drought tolerance in maize and save both time and cost in cultivar development. In the current review article, we have tried to present an updated picture of the advancements of drought tolerance in maize and its future prospects. These organized pieces of information can assist future researchers in understanding the basis of drought tolerance to adopt a potential breeding tool for breeding drought-tolerant maize cultivars.

**Keywords:** maize; drought; tolerance; genes; QTL; genetic mechanism; cultivars

## 1. Introduction

Crop production faces unpredictable encounters due to global climate variations [1,2]. Abiotic stresses such as drought, salinity, and heat stress constantly threaten the yield of essential crops and increase the risk of danger to global food security [3–6]. Improving food security is critical, given that over 800 million people remain affected by insufficient food supplies [7]. Conflicts and wars have pushed this situation to a dead end [8,9]. Rising

food prices may benefit food growers [10] and support their long-term development [11], but extreme food prices pose challenges to gaining food security [12,13]. Improving crop adaptation to climatic changes is essential for food security [14].

Maize originated from Mexico and spread to a wide latitudinal range [15]. Maize is the third most significant food crop after rice and wheat, grown in large areas worldwide. Maize meets 50–60% of the calorie requirements of people [16]. Maize provides several dietary elements used for livestock and significantly contributes to food and nutritional security. Maize is a substantial source of economy for farmers [17]. Drought stress is one of the most devastating and challenging abiotic stresses [18] due to the prolonged period of water shortage in the root zone of crops. The demand for maize production is growing as a source of food, oil, and biofuel for the rapidly increasing world human population. Drought stress caused about 15% of maize yield losses globally [19]. Areas of maize production will become drier and warmer, spreading many diseases under hot climatic conditions and significantly impacting maize yield [20]. This situation calls for improving drought tolerance in maize to offset estimated yield losses and maintain maize yield in dry areas [21]. Drought stress severely curtails maize seed germination (Figure 1), seedling development, photosynthesis, and root growth, reducing the yield in a large area [22]. Drought stress affects the soluble protein in maize cultivars, as studied by Mohammadkhani and Heidari [23]. Drought stress decreases seed yield and the harvest index of maize, as studied by Khalili et al. [24]. Drought stress reduced relative leaf water potential, leaf size, photosynthesis, stomatal conductance, and substomatal $CO_2$ concentration in maize [25,26].
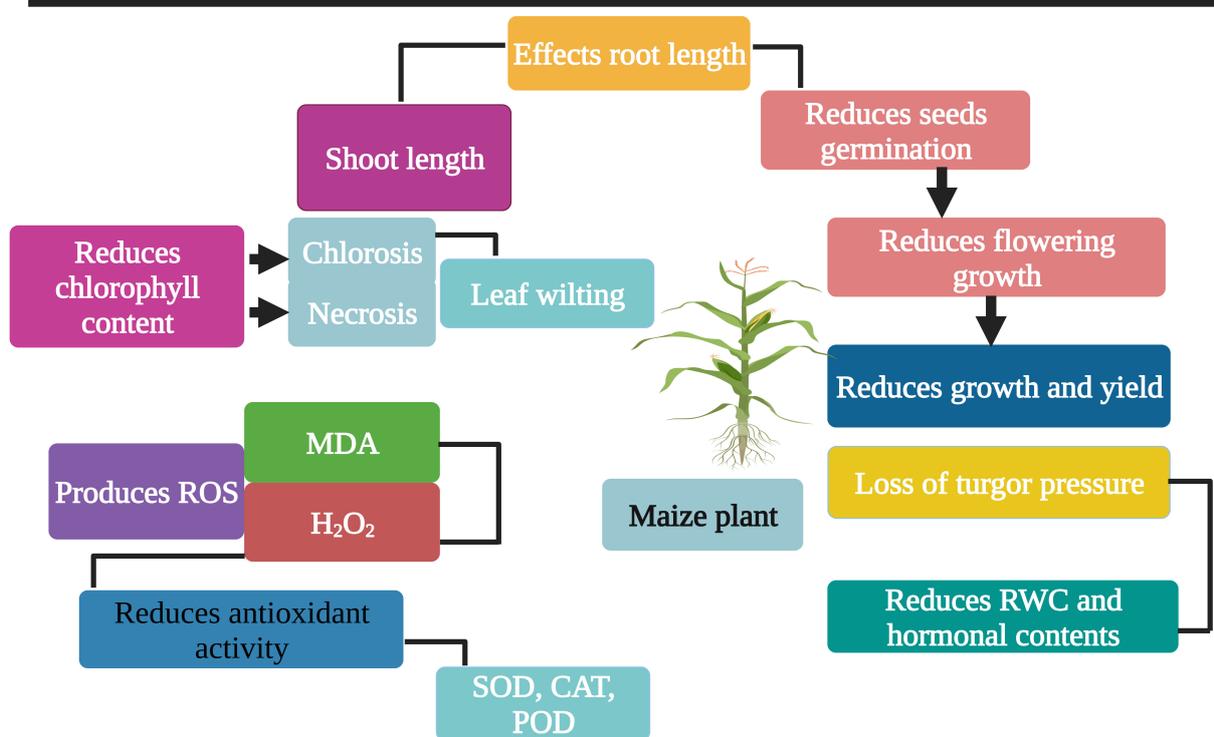


**Figure 1.** Drought stress inhibits maize growth, shoot and root length, antioxidant activity, RWC, and seed germination. Besides this, drought stress initiates loss of turgor pressure and a decrease in hormonal contents. This figure was created with Biorender.com, 10 April 2023.

Maize needs more water for its growth; therefore, improving its water usage efficiency (WUE) must improve its production under changing environmental conditions [27]. Understanding responses to drought stress is a prerequisite to understanding the molecular

mechanism, and, therefore, several potential genes have been identified at the transcriptional level [28,29]. Considering the importance of maize, increasing its yield under adverse environments has been the main objective of maize breeders [30]. Improving drought tolerance in maize is significant in ensuring food security [31]. Classical breeding methods have shown limited progress in improving maize tolerance to drought stress; hence, identifying how the plant responds to drought stress will provide new insights into how genetic modification affects crop yield under drought conditions [32]. Many molecular breeding tools have been employed to enhance the drought tolerance of maize, and hundreds of drought-tolerant maize cultivars have been developed.

QTL mapping is the most powerful way to identify novel genomic regions for drought tolerance. Earlier studies have identified many novel QTL governing the drought tolerance of maize. Hao et al. [33] conducted a meta-analysis of QTL for drought tolerance and studied 239 QTL under well-watered conditions and 160 under stressed conditions. These QTL were detected using 12 experiments and 22 populations [33]. GWAS has identified several genes, QTL, and single nucleotide polymorphisms ($SNP_S$) linked to drought tolerance in maize, and it is considered an effective molecular tool for developing tolerant maize cultivars. The use of GWAS has increased the speed of plant breeding to increase crop tolerance to abiotic stresses [27]. Transcriptome and TFs analyses provide a deep insight into the molecular mechanism of drought tolerance in maize by identifying gene/TF families under drought stress conditions. Hence, it is crucial to locate the drought-tolerant regulatory genes for the genetic improvement of maize [34]. An earlier study by Min et al. [35] detected several genes regarding drought tolerance in maize, which can be potential candidates for drought-tolerant breeding [35]. TF analysis identified several TF families that govern maize's drought tolerance [36]. Hence, these techniques have enormous potential to accelerate drought breeding [35]. Genetic engineering and CRISPR/Cas9 are potential molecular tools that play a crucial role in the genetic improvement of crops for desired traits [37–40]. Transgenic maize plants developed by Wei et al. [37] showed significant tolerance to drought stress [37]. This review summarizes the applications of potential molecular tools for developing drought-tolerant maize genotypes. There are sufficient studies on this aspect, but a detailed overview is missing. Hence, this comprehensive review will help future breeders to adopt more realistic molecular breeding methods to accelerate drought-tolerant breeding in maize. This information will expedite further research progress in the future.

## 2. Screening of Drought Tolerance in Maize

Improving drought tolerance is one of the main objectives of maize breeders [41] and drought tolerance is a complex process both at the physiological and molecular levels [41–43]. Drought tolerance is a multigenic trait with low heritability and is categorized by genotype–environment interaction (GEI) [44]. Screening for drought tolerance (Figure 2) requires the existence of considerable genetic variability in available germplasms. Many researchers have assessed the drought tolerance of maize by studying photosynthesis, hormone regulation, gene expression, and osmotic regulation [45,46]. Crop resilience refers to the adaptive ability to drought stress at a certain level. If the degree of stress does not exceed the resilience range of the crop, then the crop can recover after drought stress [47]. Plant genetic resources (PGRs) are valuable materials for present and future research studies. They have been categorized as an indispensable source of genetic variation for breeding new crop varieties [48]. Extensive efforts have been made to organize, store, and analyze the data collected during collection missions [49]. The global plan for preserving and utilizing PGRs was laid down by the Food and Agricultural Organization (FAO) in 2010. However, in the available germplasm stored in hundreds of gene banks, little information is known about the extent of genetic variability in traits of juvenile plants such as germination rate and seedling morphology traits in response to biotic and abiotic stresses. One of the largest gene banks in the world is the Federal Ex Situ Gene Bank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), which

is in Gatersleben [50–52]. Screening the large germplasm under drought stress conditions is essential to identify the tolerant genotypes. Badr et al. [53] evaluated the 40 accessions of maize under drought stress conditions for 27 responsive traits of germination percentage (GP) and seedling growth. The high heritability of shoot and root seedling traits provided a strong reason for further genetic analysis. Shoot and root traits showed a significant positive correlation under drought conditions [53]. The degree of drought tolerance in maize lines can be identified at the seedling stage by the measurement of dry shoot weight and root length of plants. These traits can be potentially used for drought-tolerant screening among maize lines [54]. Another study by Ali et al. [55] revealed that hybrid Sh-139×EV-340 showed significant variation for root and shoot development under water stress conditions at the seedling stage. This hybrid can be further evaluated to identify unknown loci for root and shoot development [55].
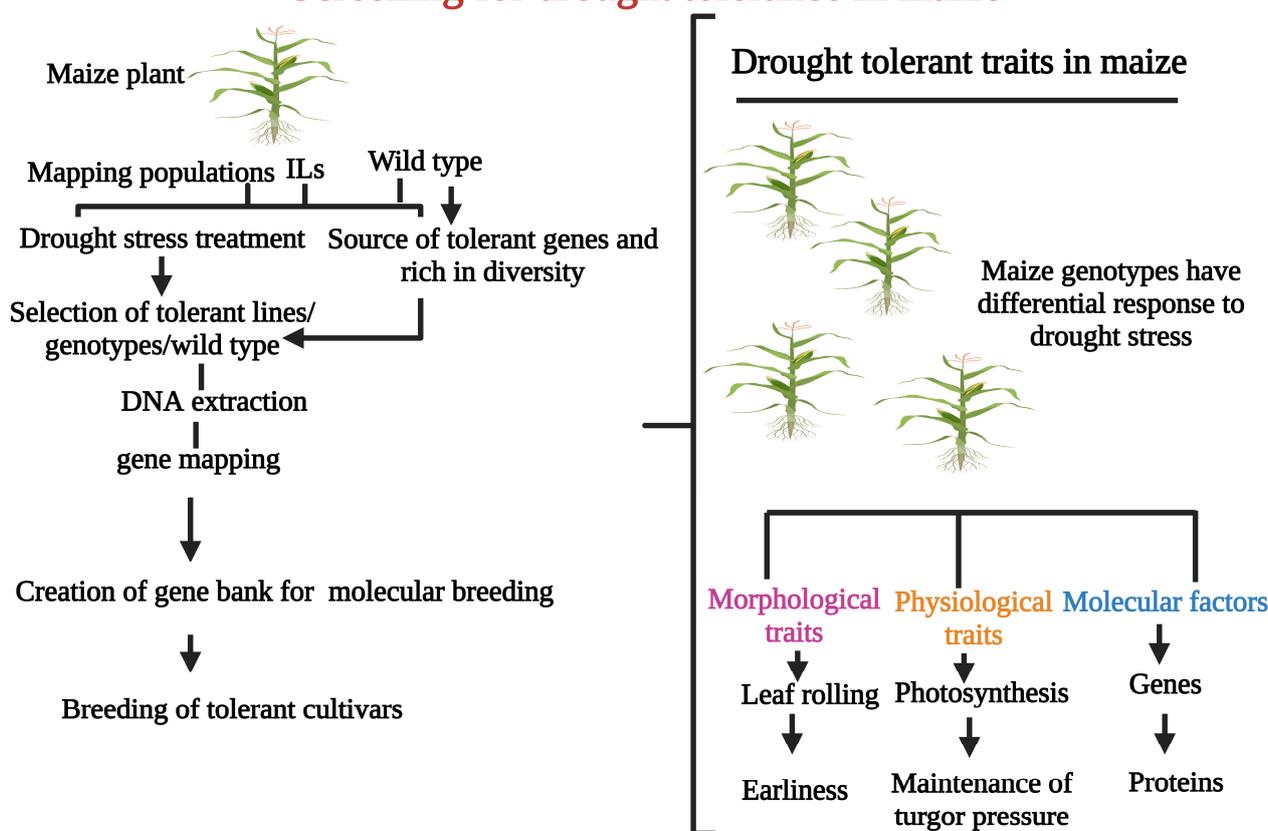


**Figure 2.** Screening drought-tolerant maize genotypes or wild type is a reliable way of identifying the drought-tolerant traits and genes regulating drought tolerance. Screening at the molecular level would help to adopt a suitable way of breeding drought-tolerant cultivars. This figure was created with Biorender.com, 10 April 2023.

The new indices such as PCI (the production capacity index) and YPSI (the yield production score index) are essential selection methods for identifying and categorizing maize hybrids based on their degree of drought tolerance. These methods were used by Bonea [56], and he selected HS 1156-14 with high resilience and productivity under drought stress [56]. In another experiment, seedlings of 55 inbred and five hybrids were shifted to the hydroponic solution. Seedlings were exposed to drought stress for five days. The inbred lines, CM140, CML422, and hybrid DMRH1306 were identified as drought tolerant. Results confirmed that this phenotypic screening method is very effective, short, and simple, and can be used to screen large germplasm of maize for drought tolerance [57]. Another method of screening is based on photosynthetic pigments and antioxidant enzymes. Different maize

genotypes and hybrids were evaluated under drought stress conditions for 50 days. The Yousafwala hybrid and FH-1137 exhibited a smaller decrease in photosynthetic and antioxidant parameters, indicating their tolerant nature [58]. Hence, the selection of genotypes and hybrids based on these traits is recommended for maize growth under water-deficit conditions [58]. Raj et al. [59] assessed the drought tolerance of maize using PEG-6000, which initiated the drought stress conditions. Single-sequence repeats (SSR) were used for drought tolerance screening at the molecular level. The hybrids AUK-30 and AUMH-8855 were tolerant to drought stress, which can be genetically analyzed for further studies [59].

It has been confirmed from research studies that screening for drought tolerance at the early growth stage can lead to a better understanding of the drought-tolerant nature of maize genotypes or lines. The maize inbred lines FLD 12, FLD 23, and FLD 24 were identified as drought tolerant in an earlier study conducted by Adhikari et al. [60]. This assessment was carried out based on morphological and physiological traits [60]. In another study, 24 super-sweet maize inbred lines were classified based on drought tolerance and yield potential. The inbred lines MPA90010/51-1 and MSH90011/82-1 were drought tolerant, as indicated by mean productivity (MP) values and the stress tolerance index (ST). These inbred lines could be used as source material for developing drought-tolerant maize genotypes [61]. The four inbred lines, Nub60 (1.56), Nub32, Nub66, and GZ603 (1.44) were the highest drought-tolerant inbred lines, as studied by Balbaa et al. [62]. Previous studies have extensively studied maize genotypes with phenotypic and genotypic variation for morphological, physiological, and biochemical traits. Emmanuel et al. [63] screened 12 Indian maize landraces for drought stress based on relative water content (RWC). RJ-2020, BPCH-6, and EC-3161 had high RWC and were selected as drought-tolerant landraces for further study [63]. Maize genotypes grown under drought stress conditions showed different degrees of drought tolerance. Masood et al. [64] revealed that genotypes 545, AES204, and WM13RA were selected based on superior performance under drought stress conditions. These genotypes can be used as parental material for developing drought-tolerant maize hybrids [64]. In an earlier study, maize accessions 14927, 15155, 19191, and 15233 were identified as drought tolerant based on tolerance indices such as root length, shoot length, and wall thermostability linked to drought stress [65]. These results showed that landraces and inbred lines are potential sources of genetic variability for drought tolerance in maize. There is an urgent need to evaluate the wild maize relatives to screen for drought-tolerant traits that can be used to improve commercial cultivars grown in water-deficit soils. Field screening is relatively tough and costly; hence, screening maize genotypes in lab conditions would save time and accelerate plant breeding.

## 3. QTL Mapping Analysis for Drought Tolerance in Maize

QTL mapping has been effectively used for the genetic dissection of drought-tolerant genomic regions in maize [66]. Earlier and recent studies have identified the QTL linked with drought tolerance in maize, and some have been cloned successfully. Sheoran et al. [67] summarized the 542 QTL reported in 33 published papers for abiotic stress tolerance to conduct a meta-analysis. They have detected 32 meta-QTL possessing 1907 genes for abiotic stresses (waterlogging and drought). These QTL could facilitate drought-tolerant breeding in maize [67]. A double haploid (DH) population of 217 lines was subjected to drought stress to identify the genomic regions associated with drought tolerance in maize. A total of 9 QTL were detected for water stress conditions. QTL, *qWS-GY3-1*, and *qWS-ESP3-1* (Table 1) contributed to drought tolerance, and these QTL were in edQTL regions (environment-dependent QTL) [68]. Another study reported 54 QTL affecting different photosynthetic traits in maize under well-watered and drought stress conditions. A total of 43 QTL identified under drought stress indicated that tolerance to photoinhibition is a key factor influencing drought tolerance in maize. These QTL could support MAS-based breeding of drought tolerance in maize [69]. QTL sequence analysis is one of the reliable ways to identify the potential QTL/genes underlying drought tolerance in maize.

Zhang et al. [70] used leaf relative water content (LRWC) as drought tolerance indices for maize drought tolerance. QTL sequence analysis identified four QTL, *qLRWC2*, *qLRWC10a*, *qLRWC10b*, and *qLRWC10c* for LRWC under water-deficit conditions. These QTL may help carry out additional research studies in the future [70]. In two QTL, *qDTA3-3 and qDTA10*, days to anthesis (DTA) were identified under drought stress conditions, which may be promising candidates for drought-tolerant breeding in maize [71].

Identifying QTL linked with yield-related traits can play a key role in developing high-yielding maize cultivars that sustain yield under water-deficit conditions. A total of 9 QTL were identified under water stress conditions in maize which linked to weight, kernel weight, and 100-kernel weight. The QTL *qEL4s* and *qKW4s* could be used for fine mapping and acceleration of MAS breeding as they were found in the same region in water stress conditions [72]. In the same way, Almeida et al. [73] identified 83 and 63 QTL for grain yield (GY) and anthesis-silking interval (ASI) through the individual environmental analysis of these traits. QTL, *qWS-GY-10*, and *qWS-GY-7* were identified for GY under water stress conditions [73]. Another study identified 45 QTL (5 for yield and 40 for eight yield components) for yield and yield components. These QTL were distributed on all maize chromosomes except chromosome 8 [74]. A total of 203 QTL were identified for the traits ear per plant (EPP), stay green (SG), plant-to-ear height ratio (PEH), and anthesis-silking interval (ASI) linked with drought tolerance in maize. The QTL linked with SG and EPP were distributed in chromosomes 1 and 2. These QTL should be further analyzed for their use in MAS selection [75]. The haplotype QTL *qPH-HP383-10* was detected on chromosome 10 and linked with drought tolerance, as reported by Lu et al. [76]. Another study identified many QTL for yield and yield-related traits under drought stress conditions. The QTL *qES3* for ear setting (ES) was located in chromosome 3, which can contribute to drought tolerance in maize [77]. This comprehensive analysis of drought-tolerant QTL showed that these QTL could be used for QTL pyramiding and MAS breeding to develop drought tolerance in maize genotypes. However, additional studies are required to improve the efficiency of QTL mapping and the development of novel mapping populations, which can increase the chances of identifying novel regions for drought tolerance.

**Table 1.** List of drought-tolerant QTL in maize.

| Parents/Population | QTL | Chromosome | References |
| --- | --- | --- | --- |
| 121 RIL | *qDTA3-3* and *qDTA10* | 3, 10 | [71] |
| 217 DH | *qWS-GY3-1* and *qWS-ESP3-1* | 3 | [68] |
| 419 F$_2$ individuals | *qLRWC2* and *qLRWC10a* | 2, 10 | [70] |
| 13 F$_{2:3}$ families | *qEL4s* and *qKW4s* | 4 | [72] |
| 234 RIL, 247 F$_{2:3}$, 300 F$_{2:3}$ | *mQTL_SG_1a* and *mQTL_EPP_2* | 1, 2 | [75] |
| 234 RIL, 247 F$_{2:3}$, 300 F$_{2:3}$ families | *qWS-GY-10* and *qWS-GY-7* | 10, 7 | [73] |
| 116 F$_3$ families | *qGY2* and *qGY10* | 2, 10 | [74] |
| 305 maize lines | *qPH-HP383-10* | 10 | [76] |
| The population of F$_{2:3}$ families | *qGY1* and *qGY9* | 1, 9 | [78] |
| 180 F2:3 families | *qES3* | 3 | [77] |

## 4. Overview of Association Mapping Studies for Drought Tolerance

Genome-wide association study (GWAS) is one of the most powerful and influential ways to dissect the genetic makeup of drought tolerance in crops. GWAS gives a reliable estimate of traits–marker association by screening many germplasms. A detailed overview of GWAS-based QTL/genes identification is critical to search for the most potential candidate genomic regions which can be exploited in drought-tolerant breeding. Wang et al. [79] reported the natural variation for drought tolerance in the maize seedling stage using GWAS. They have reported that natural variation in *ZmVPP1* contributed significantly to the trait. *ZmVPP1* overexpression in maize transgenic lines increased drought tolerance by increasing photosynthesis and root development [79]. GWAS identified 365 SNPs in 354 genes, and 52 of these genes exhibited significant differential expression in inbred

line B73 under well-watered and water-stressed conditions. These alleles could improve drought tolerance in maize [27]. In another study, Wang et al. [80] evaluated 201 maize ILs and identified 206 significant SNPs in 115 candidate genes for drought tolerance and linked traits, including GY, plant height, etc. Besides this, GWAS also identified nine QTL, and these genomic regions might contribute to drought tolerance in maize [80]. SNPs are a common form of genetic variability in the maize genome. A pair linkage disequilibrium and association mapping analysis with phenotypic traits performed under well-watered and water stress conditions revealed a decline within 100–500 kb along with the physical distance of each chromosome. Results showed a strong association of 29 SNPs with two phenotypic traits in one or more environments. SNPs can be converted to functional markers and used for MAS programs [81].

Xue et al. [82] dissected the genetic complexity of drought tolerance using 350 maize ILs genotyped by using 1365 SNPs. GWAS has identified 42 linked SNPs in 33 genes for 126 traits × environment × treatment combination. Gene *GRMZM2G12577* was linked with hundred kernel weight and relative ear position [82]. The morphological or yield traits and secondary metabolites can also contribute to drought tolerance in maize. In an earlier study, 123 significant SNPs linked with 63 loci were identified for phenotypic and metabolic traits under two water conditions. Of 63, 23 loci exhibited a significant effect between QTL and water status, indicating that these loci linked with metabolites were associated with drought tolerance [83]. The flowering stage is the most critical because any abiotic stress can cause a decline in the function of seed development. Hence, identifying loci linked with drought tolerance at the flowering stage should be a key priority for maize breeders. Recently, Khan et al. [84] evaluated 279 maize ILs under drought stress and well-watered conditions for three consecutive years. Plant height (PH), anthesis-to-silk interval (ASI), and ear biomass at silking stage (EBM) were studied for drought tolerance. GWAS identified 71, 159, and 21 SNPs (Table 2) linked with three traits, ASI, EBM, and PH. Only a few genes were linked with drought-tolerant traits. For example, *ARABIDILLO 1* was linked with ASI and SF16 protein for PH [84]. Drought mainly affects the root traits of crops; hence, an improvement of root-based drought tolerance is critical to sustaining maize growth under drought stress. GWAS-based analysis of seminal root length (SRL) has identified 62 loci under two water treatments. This strongly supports the further analysis and use of GWAS for a better understanding of drought tolerance in maize [85]. Grain yield (GY) and flowering time are critical traits affected by drought stress. GWAS and genomic prediction (GP) analyses of 300 maize ILs identified 688 candidate genes under drought stress and well-watered (WW) conditions. These genes were enriched into 15 ontology terms, and 46 genes showed significant differential expression under WW and water stress conditions. The genetic information and loci of flowering time and grain yield (GY) identified in this study can be used to rapidly develop stress-tolerant maize germplasm [86].

Genomic selection (GS) has gained importance because of the hindrances in traditional breeding methods. Two hundred forty maize subtropical lines were phenotyped for drought tolerance using 29,619 SNPs. A total of 77 SNPs were significantly associated with 10 TFs responsible for drought tolerance. TFs were involved in the drought tolerance of morphological and physiological traits [87]. The comprehensive analysis of 240 maize lines was performed using high-density markers for the genetic mechanism of drought tolerance. Out of 61 SNPs, 48% were critically linked with drought-tolerant genes. These SNPs were associated with stomatal closure, flowering, root development, detoxification, and reduced water potential. Interaction of SNPs *via* functional traits can improve drought tolerance in maize. SNPs linked with ABA signaling improved drought tolerance by regulating flowering, auxin, root development, etc. [88]. Earlier researchers, Osuman et al. [89], conducted GWAS analysis for yield and yield-related traits under terminal drought conditions. One hundred sixty-two maize tropical lines were evaluated under drought stress and genetically analyzed for potential SNPs/genes. In total 66, 27, and 24 SNPs were associated with traits evaluated under combined heat and drought (CHD), terminal drought (TD), and their combined effects. Four SNPs had pleiotropic effects on days to anthesis (AD), and

silking (SD) under CHD. A total of 12 genes were implicated in regulating plant response to multiple abiotic stresses [89]. A collection of 210 maize ILs was analyzed for genomic regions governing drought tolerance. GWAS identified 696 traits- associated SNPs under water-stressed (WS) and 413 SNPs under well-watered (WW) conditions. These results showed the importance of genomic selection (GS) for drought breeding [90].

Maize landraces have been a potential source of genetic diversity for drought tolerance. Identifying genes in landraces and their transfer can eventually improve drought tolerance in maize; however, it demands the large-scale GWAS-based genetic analysis of landraces. A GWAS technique was employed on 1326 maize landraces developed by the CIMMYT Genetic Resource Program. GWAS was employed to identify the marker-trait and grain yield (GY) associations for two irrigation treatments. Two genes were linked to drought stress response and showed that landraces had a significant genetic diversity and could be used to improve drought tolerance in maize [91]. As mentioned in earlier analyses, GWAS showed that it is a powerful technique to unfold the genetic background of drought tolerance and can be widely exploited for other drought breeding programs. More studies may be conducted to analyze the biochemical-based drought tolerance in maize using GWAS analysis. Candidate genes identified in GWAS studies must be transformed using the latest molecular breeding techniques.

**Table 2.** GWAS-based identification of genomic regions linked to drought tolerance in maize.

| Population/Parents | QTL/Genes/SNPs | Function | Reference |
|---|---|---|---|
| 279 maize ILs | 71, 159, and 21 SNPs | *ARABIDILLO 1* for ASI and SF16 protein for PH | [84] |
| 420 RIL | 28 and 16 SNPs | Genes improved seedling growth and plant development | [92] |
| 162 tropical maize lines | 66, 27, and 24 SNPs | Four SNPs linked with three traits under CHD | [89] |
| 1326 maize landraces | 5695 SNPs | Two genes linked with drought tolerance | [91] |
| 209 diverse accessions | 62 loci | SRL loci as a potential candidate for drought tolerance | [85] |
| 300 ILs | 688 candidate genes | 46 genes showed significant differential expression | [86] |
| 210 maize ILs | 696 under WS and 413 under WW | The SNPs reflected significant genetic variability | [90] |
| 240 maize lines | 29,619 SNPs | 77 SNPs were significantly related with 10 TFs | [87] |
| 367 inbred lines | *ZmVPP1* genes | Enhanced photosynthesis and root development | [79] |
| 318 maize ILs | 123 significant SNPs | 23 metabolic loci linked with drought tolerance | [83] |
| 5000 inbred lines | 365 SNPs located in 354 genes | 52 genes showed differential expression | [27] |
| 201 ILs | 206 significant SNPs in 115 candidate genes | These genes might play a role in drought tolerance | [80] |
| 240 maize lines | 61 SNPs | SNPs related to ABA signaling improved drought tolerance | [88] |
| 350 ILs | 1365 SNPs | 42 SNPs linked with 33 genes and gene *GRMZM2G12577* associated with hundred kernel weight | [82] |
| 80 ILs | 1356 SNPs | 29 SNPs linked to phenotypic traits | [81] |

GWAS, genome-wide association studies; SNPs, single nucleotide polymorphisms; QTL, quantitative traits loci; TFs, transcription factors; ILs, inbred lines; SRL, seminal root length; ASI, anthesis-to-silk interval; ABA, abscisic acid.

## 5. TFs Analysis and Their Role in Drought Tolerance

TFs analysis is one of the most powerful techniques to recognize the genomic regions involved in tolerance to abiotic stresses. TFs regulate gene expression for several abiotic stresses [93]. Various TFs families such as WRKY, NAC, and bZIP have a key role in plant response to abiotic stress [94–96]. A detailed and updated picture of TF iden-

tification and application in drought tolerance in maize is critical. WRKY is one of the largest families of TFs, which regulates several critical responses under drought stress. Zhao et al. [97] screened a new WRKY IIa TF, a substrate protein of *ZmMPK6*. Overexpression of *ZmMPK6* enhanced drought tolerance in maize. *ZmWRKY104* phosphorylated by *ZmMPK6* was key in the ABA-induced antioxidant defense response (Table 3) [97]. Earlier, Wang et al. [98] identified a WRKY TF, *ZmWRKY40*, which is in the nuclei of mesophyll protoplasts. *ZmWRKY40* was induced by drought stress and ABA. This study has provided a candidate gene and genetic mechanism of *ZmWRKY40* to improve drought tolerance in maize [98]. The function of another maize WRKY TF, *ZmWRKY79* was elucidated by drought stress and ABA-biosynthesis. *ZmWRKY79* boosted the ROS scavenging, reduced the $H_2O_2$ content, and increased the antioxidant defense system. *ZmWRKY79* also targeted the gene *ZmAAO3* in maize protoplast *via* acting on the W-boxes of corresponding gene promoters [99].

The basic helix-loop-helix (bHLH) TFs family contributes to maize's root and low phosphate development. *ZmPTF1* of the bHLH family regulated ABA synthesis, signaling pathways, and drought tolerance. The *ZmPTF1*-overexpressed maize lines had improved root systems and enhanced ABA content. Hence, *ZmPTF1* was found to be a useful gene for transgenic breeding in maize [100]. The bHLP TF can be a potential breeding target for drought tolerance in maize. *CgbHLH001* is a bHLP TF from *Chenopodium glaucum* that increased drought tolerance in maize. *CgbHLH001*-overexpressed maize lines showed improved drought tolerance by accumulating soluble sugars and increased activities of antioxidants such as superoxide dismutase (SOD), and catalase (CAT) [101]. The BRI1-EMS suppressor 1 (BES1)/brassinazole-resistant 1(BZR1) TFs regulate drought tolerance in maize *via* different mechanisms. Two genes, *ZmBES1/BZR1-3* and *ZmBES1/BZR1-9*, were cloned from a maize inbred line, B73, and they were functionally characterized by investigating their pattern of expression and transcriptional activation activity. These genes were linked to responses to oxidative stress and amino acid metabolic processes. Further analysis is required for the detailed mechanism of drought tolerance in maize and *Arabidopsis* [102]. The Apetala2/ethylene response factor (AP2/ERF) family of TFs plays a major role in abiotic stress tolerance in maize. Unfortunately, few members of this family have been characterized in maize and demand more studies. *ZmEREBP60*, a member of TFs, was functionally characterized in maize, and its expression was induced by drought stress in roots and leaves. *ZmEREBP60* enhanced drought tolerance in maize by reducing the content of $H_2O_2$. Hence, *ZmEREBP60* is a positive regulator of drought tolerance in maize and can be used for drought breeding [103]. NAC TFs play a key role in response to abiotic stresses. However, information about stress-induced NAC genes in maize is insufficient. Wang et al. [104] identified 87 NAC TFs in maize under drought stress conditions that showed differential expression [104]. Mao et al. [105] cloned a NAC TF, *ZmNAC55*, and studied its role in drought tolerance, and expression of this TF was induced by drought stress. *ZmNAC55* enhanced drought tolerance in wild *Arabidopsis* and was a positive regulator of drought tolerance [105]. The NAC TF, *ZmNAC49*, improved drought tolerance in maize by reducing stomatal density. *ZmNAC49* overexpression decreases the stomatal density, rate of transpiration, and stomatal conductance. The expressions of genes *ZmTMM* and *ZmSDD1* were also induced by *ZmNAC49* [36]. Many studies have reported the identification and cloning of genes that showed the differential mechanism for drought tolerance. HD-ZIP is one of the largest families of TFs, characterized by its role in drought tolerance in maize. Qiu et al. [106] identified the 42 ZmHDZ genes using maize transcriptome data. Results showed that the expression of several ZmHDZ genes was induced by drought stress, and maize lines with overexpression of *ZmHDZ9* showed enhanced activity of SOD and peroxidase (POD) and enhanced soluble protein content. Hence, this study reported the evolutionary HD-ZIP TFs homologs in maize [106]. Another HD-ZIP TF, ATHB-6, reduced the content of malondialdehyde in lines overexpressing *ATHB-6* compared to the wild type. Hence, further studies will help to deeply understand the functions of HD-ZIP TFs in maize [107]. MYB TFs family is mainly found in eukaryotes

and contributes to crop drought tolerance. The research study showed that the mechanism of ZmMYB-CC in maize is unknown. *ZmMYB-CC10* enhanced drought tolerance in maize by reducing oxidative damage and decreasing the content of $H_2O_2$. The activation of the *ZmAPX4* function by *ZmMYB-CC10* (Table 3) alleviated drought stress in maize [108]. Nuclear factors Y (NF-Ys) are significant TFs; however, their function in the underlying tolerance mechanism in crops is largely unknown. Two nuclear factors Y (NF-Ys) members were functionally characterized for their role in drought tolerance. *ZmNF-YB16-* and *ZmNF-YA1* improved drought tolerance in maize by similar biological functions under drought stress; however, they had different roles in other biological processes. The TF, *ZmNF-YA1*, promoted root development in maize [34]. The ethylene response factor (ERF) also plays a key role in improving drought tolerance in maize. An ERF TF, *ZmERF21*, is mainly expressed in roots and leaves, and its expression was induced by drought stress. The overexpression of *ZmERF21* increased chlorophyll content and antioxidant activities under drought stress. *ZmERF21* may regulate the genes linked to hormones and calcium signaling [109]. The detailed functional analysis of MYB and NF-Ys needs further studies because their genetic mechanism for drought tolerance is largely unknown. Identifying more TFs of MYB and NF-Ys would be useful.

**Table 3.** TFs for the improvement of drought tolerance in maize.

| Families | TFs | Role | Reference |
|---|---|---|---|
| WRKY | *ZmWRKY104* | Involved in ABA-induced antioxidant defense system | [97] |
| WRKY | *ZmWRKY40* | Enhanced drought tolerance | [98] |
| WRKY | *ZmWRKY79* | Boosted the ROS scavenging | [99] |
| bHLH | *ZmPTF1* | Enhanced ABA content and improved root system | [100] |
| bHLP | *CgbHLH001* | Increases soluble sugar contents | [101] |
| BES1/BZR1s | *ZmBES1/BZR1-3,* *ZmBES1/BZR1-9* | Linked with oxidative stress response and amino acid metabolic process | [102] |
| AP2/ERF | *ZmEREBP60* | Alleviated the drought-induced $H_2O_2$ content | [103] |
| NAC | *ZmNAC55* | Positive regulator of drought tolerance | [105] |
| NAC | *ZmNAC49* | Decreased stomatal conductance | [36] |
| HD-ZIP | *ZmHDZ9* | Improved antioxidant defense system | [106] |
| HD-ZIP | *ATHB-6* | Decreased the content of malondialdehyde | [107] |
| MYB | *ZmMYB-CC10* | Reduced oxidative damage and enhanced activity of *ZmAPX4* | [108] |
| (NF-Ys) | *ZmNF-YB16-, ZmNF-YA1* | *ZmNF-YA1* improved root development | [34] |
| ERF | *ZmERF21* | Increased antioxidant activities | [109] |
| DREB/CBF | *TsCBF1* | Increased relative water content (RWC) and higher grain yield (GY) | [110] |

## 6. Transcriptome Analysis for Drought Tolerance

Transcriptome analysis gives an understanding of the molecular mechanisms of drought tolerance in maize and has been regarded as the best research tool. Hence, analysis of the gene expression profile of maize genotypes under drought stress conditions will enhance our understanding of genetic control of drought tolerance [111]. Dozens of studies have been published on this topic, but an updated picture of recent studies is critical for future researchers Zheng et al. [111]. analyzed the gene expression profile of two maize inbred lines with different levels of drought tolerance. They detected 24, 220, and 4551 differentially expressed genes (DEGs) in inbred line 287M (drought tolerant) after 24, 48, and 72 h of drought stress. Likewise, 16, 29, and 2641 genes were identified in 753F (drought sensitive) after 24, 48, and 72 h of drought stress. The difference between the two inbred lines was linked with the scavenging of ROS, signal interaction network, etc. In an earlier study, 957 DEGs in maize genotypes subjected to melatonin and non-melatonin treatment were identified. MT-based regulated genes were linked with calcium signaling transduction and the biosynthesis of jasmonic acid (JA). MT interacted with other hormones to enhance drought tolerance. This drought tolerance mechanism would be highly useful for drought breeding [112].

One of the best ways is to conduct the comparative transcriptome analysis of drought-tolerant and sensitive genotypes. A transcriptome study has identified 4552 DEGs in C7–2t (drought-tolerant mutant) and C7–2 wild-type maize. Results showed that drought tolerance of inbred line C7–2t (drought-tolerant mutant) was due to its water-holding capacity and improved photosynthesis rate [113]. Two constating maize ILs, CML69 (drought-tolerant) and LX9801 (susceptible), were treated with drought stress at the seedling stage for five days. RNA sequence analysis has identified 10,084 DEGs. Drought-tolerant genes were divided into two sets; 4687 were genotype-specific, and 2219 were common drought-responsive genes. Tolerant genes are linked with the scavenging of ROS, drought avoidance, osmotic regulation, etc. [114]. Along with introgression lines (ILs), different hybrids were evaluated to study the transcriptome analysis under drought stress conditions in maize. Two hybrids, ND476 (drought tolerant) and ZX978 (drought sensitive), were evaluated under drought stress and control conditions. Transcriptome analysis identified 3451 and 4088 DEGs in ND476 and ZX978. Genes in ND476 were associated with starch and sucrose metabolism, while genes in ZX978 were linked to the ribosome and pentose [115]. Another hybrid, Zhengdan538, showed an enhanced response to drought stress in maize. A total of 2994–4692 DEGs were identified under WW and drought stress conditions, which were recognized by comparison with their parents. The potential genes were involved in energy biosynthesis and photosynthesis. This expression level dominance (ELD) was key in hybrid tolerance to water-deficit conditions [116]. Recently, Gillani et al. [117] evaluated two contrasting maize ILs, 478 (drought tolerant) and H21 (susceptible), to conduct the transcriptome analysis for drought tolerance under water stress conditions. They identified 68%, 48%, and 32% of drought-responsive genes (DRGs) in 478, and genes involved in drought tolerance were linked with starch and sucrose metabolism, plant hormonal signal transduction, etc. [117].

627 RNA sequence analyses were performed for 224 maize accessions under three water regimes. Results showed that 73,573 eQTL were identified for 30,000 genes. Ninety-seven genes were prioritized as related to drought tolerance because of their different expressions. These prioritized drought-tolerant genes may be a direct target for alleles mining [118]. These results showed the significance of RNA sequence analysis in identifying the DEGs in maize under drought stress conditions. Dong et al. [119] identified 666, 2417, and 7375 DGEs at the flare, tasseling, and grain-filling stages. Gene, *DnaJ*, and a putative WAK family receptor-like protein kinase were linked with molecular chaperon activities and cell signal transduction. *DnaJ* gene expression can be the best tolerance index for drought tolerance screening [119].

Identifying genes at the tasseling stage may help mitigate the harmful effects of drought stress in maize and avoid yield loss. Ten maize ILs were evaluated under drought stress to perform the RNA sequence analysis of early-developing tassels under control and water stress conditions. A total of 19,001 DEGs were identified in 10 ILs [120]. The application of growth hormones and osmolytes such as glycine betaine (GB) also enhanced drought tolerance in maize, as shown by transcriptome analyses. Two drought-tolerant and susceptible inbred lines, Chang 7-2 and TS141, were subjected to drought stress and GB treatment, and 562 up-regulated and 824 downregulated DEGs were found in ILs. The exogenous application of GB induced the upregulation of 1061 and 424 DEGs in ILs. The expression of 9 DEGs was constant with their transcriptome expression profile [1]. Pollen development is one of the most sensitive stages of maize reproduction. Hence, transcriptome analysis at the pollen-setting stage helps identify the potential genes involved in maize reproduction. A maize hybrid was evaluated under drought stress. It was observed by transcriptome analysis that the expression profile of 6424 genes and 1302 (Table 4) transcripts were changed in pollen grains after seven days of drought stress. Different genes were involved in pollen development and responses to drought stress. The identification of tolerant genes during pollen development and change in the expression profile of transcripts can help to prevent the loss caused by water shortage during the pollen development stage [121]. Transcriptome analysis to identify the potential genes in

wild relatives of maize would accelerate drought-tolerant breeding. These ideas should be implemented in future research studies.

**Table 4.** A List of genes expressed under drought stress conditions in maize.

| Genotypes/ILs/Hybrids | DEGs Genes | Role | References |
|---|---|---|---|
| 287M, 753F | 24, 220, 4551 and 16, 29, 2641 | ROS scavenging and hormonal metabolism | [111] |
| Maize seedling | 957 | MT interaction with other hormones enhanced drought tolerance | [112] |
| C7–2t, C7–2 | 4552 | C7–2t had a stable photosynthesis rate | [113] |
| CML69, LX9801 | 4687 genotype-specific and 2219 common drought-tolerant genes | Drought avoidance and osmotic regulation | [114] |
| 224 maize accessions | 73,573 eQTL for 30,000 genes | 97 genes associated with drought tolerance due to expression variant | [118] |
| Maize genotypes | 666, 2417, 7375 | *DnaJ* and a putative WAK family receptor-like protein kinase improved drought tolerance | [119] |
| 10 ILs | 19,001 | Metabolic regulation at the RNA level | [120] |
| Hybrids ND476 and ZX978 | 3451 and 4088 | Sucrose, starch, pentose, and ribosome | [115] |
| 478, H21 | 68%, 48% and 32% DEGs in 478 | Sucrose and starch metabolism | [117] |
| Chang 7-2, TS141 | 562, 824 | GB-based upregulation and downregulation of DEGs can enhance drought tolerance | [1] |
| Hybrid, ZhongDan909 | 6424 genes and 1302 transcripts | Genes involved in pollen development | [121] |
| Zhengdan538 | 2994–4692 | Energy biosynthesis and photosynthesis | [116] |
| Hybrid ND47 | 3451 | Increased photosynthesis rate and amino acid metabolism | [122] |
| *Zea mays* cv. B73 | 619 genes and 126 transcripts | 20 drought-tolerant genes involved in flowering time | [123] |
| H082183 (drought tolerant), Lv28 (drought susceptible) | 688 and 3 363 in H082183 and 1428 and 512 in Lv28 | Plant hormone transduction and starch metabolism | [124] |

## 7. Transgenic Breeding and CRISPR/Cas9

Genetically modified (GM) crops were first introduced in the United States (US) in the mid-1990s, and they were extensively adopted by growers around the world [125]. A total of 189.8 million hectares of land were used to plant GM crops alone in 2017 [126]. To save time and money, it has become essential to employ GM tools to combat the rising threats of drought stress in maize. A transgenic drought-tolerant maize line, *SbSNAC1-382*, with overexpression of the gene, *SbSNAC1*, showed enhanced tolerance to drought stress, as studied by Zeng et al. [127]. Earlier, two transgenes, *BetA*, and *TsVP*, were expressed in maize plants produced by cross-pollination. Pyramided transgenic maize plants showed higher contents of GB and H$^+$-PPase activity compared to parental lines. Pyramid plants had vigorous growth and higher yields than their wild type and parents. This study proved that several transgenes could improve drought tolerance in transgenic maize plants [37]. To obtain effective transgenic plants, a suitable promoter that permits transgene expression at desired levels is very significant [128]. Therefore, scientists must isolate various promoters and identify their characteristics. The isolation and functional characterization of abscisic acid (ABA) promoters are of great importance for the genetic improvement of drought tolerance in maize. A *ZmSOPro* gene was isolated from the maize genome and analyzed for its role in drought tolerance. The minimal *ZmSOPro* was induced by drought stress in

transgenic maize plants. This gene and promoter, the 119-bp promoter, could be an ideal candidate for the genetic engineering of drought tolerance in maize [129].

Earlier, *TsVP* was transformed from a *Thellungiella halophila* into maize. Transgenic maize plants exhibited a higher percentage of seed germination and less membrane damage than wild-type plants. V-H⁺-PPase action of transgenic maize enhanced drought tolerance in maize plants [130]. Further characterization and analysis of transgenes can improve the speed of drought-tolerant breeding in maize. The identification and transformation of genes from other crops can also increase maize's tolerance to drought stress. ERECTA (ER) is a leucine-rich repeat-receptor-like kinase gene (LRR-RLK) encoding a protein identified in *Arabidopsis*. Two ERECTA (ER) genes, *SbER1–1* and *SbER2–1*, were isolated from drought-tolerant sorghum (*Sorghum bicolor*). The *SbER2–1* gene increased drought tolerance in maize by increasing the net photosynthetic rate. Transgenic maize plants have a higher lignin content and upgraded metabolism under drought stress. *SbER2–1* can be used to breed genetically modified cultivars [131].

In the same way, *HVA1* from barley was transferred to develop transgenic maize plants to cope with drought stress conditions. The T3 plants with transgene overexpression showed higher root and leaf biomass and RWC, indicating GE's promising role in drought tolerance [132]. Plant hormones play an important role in abiotic stress response. *AtG2ox1*, a member of the GA2ox family, is used to develop maize plants with gibberellins (GA) deficiency. *AtG2ox1* enhanced the chlorophyll content and growth of transgenic plants compared to wild-type plants. *AtG2ox1* can be used as a candidate gene for breeding drought-tolerant maize cultivars [133]. It has been concluded that studies on genetically modified drought-tolerant maize are limited. This issue must be taken seriously to map the potential genes from local genotypes or wild type and other crop genomes to breed the transgenic maize plants.

CRISPR/Cas9, a novel gene editing tool [134], is valuable in accelerating molecular breeding for abiotic stress tolerance in crops (Figure 3). The genome editing tools, transcription activator-like effector nucleases (TALENs), meganucleases, zinc-finger nucleases (ZFN), and CRISPR/Cas9 have ensured the targeted gene editing in plants [135–137]. CRISPR/Cas9 is highly efficient and easiest to implement. Cas9 edits the targeted DNA sequence in the genome. CRISPR/Cas9 gene editing is accomplished by introducing a DNA double break (DSB) in the targeted genes through Cas9, followed by DNA repair using non-homologous end joining (NHEJ) and homology direct repair (HDR). This tool has been used in maize [138]. *ARGOS8* is a negative regulator of ethylene responses. To study the targeted use of *ARGOS8* native expression variation in drought-tolerant breeding, a set of 400 inbred maize was examined for *ARGOS8* mRNA expression. The CRISPR-Cas9 tool was used to generate new variants of the *ARGOS8* gene. Compared to the wild type, a field study showed that *ARGOS8* variants enhanced grain yield by five bushels per acre under flowering stress conditions and had no loss in yield under well-watered conditions. These outcomes showed the greater ability of CRISPR/Cas9 to generate novel drought-tolerant mutants [38]. Recently, Pan et al. [139] generated two allelic mutants of *ZmSRL5* using CRISPR/Cas9. The wax-related genes were not changed in the *srl5* mutant. Hence, *ZmSRL5* is obligatory for the structure formation of cuticular wax and can enhance drought tolerance [139]. In another study, the *zmcpk37* mutant was generated using CRISPR/Ca9. The transgenic lines of *ZmCPK35* and *Zmcpk37* were obtained using *Agrobacterium*-mediated transformation in maize IL. Maize hybrid and IL with overexpression of *ZmCPK35* and *Zmcpk37* showed higher yields under drought stress [140]. Despite all of the progress, the use of CRISPR/Cas9 in maize is limited regarding breeding for drought tolerance. Targeted genes can be edited to generate desired mutants in maize to expand the use and efficiency of CRISPR/Cas9. New editing systems (base editing and prime editing) would increase the precision of genome editing in maize.
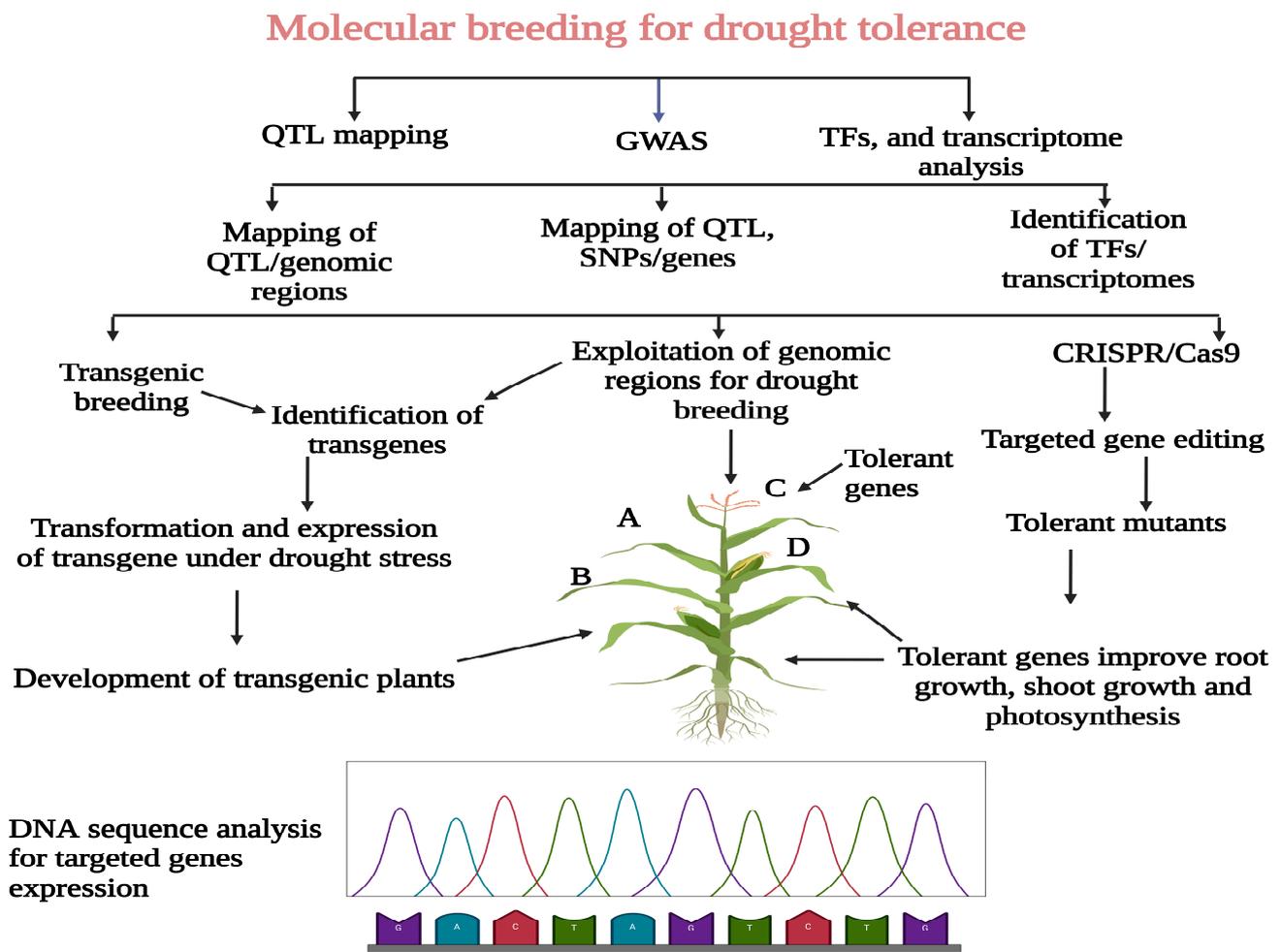
**Figure 3.** The application of molecular tools such as CRISPR/Cas9 and genetic engineering have greatly accelerated the speed of drought breeding in maize. Expanding these tools would be highly useful in breeding tolerant genotypes that can combat the threats of drought stress in the future. This figure was created with Biorender.com, 10 April 2023.

## 8. Conclusions and Outlook

Maize is one of the most significant cereal crops that provides nutrition to human beings and livestock. Maize plays a key role in the gross domestic product (GDP) of many countries. Drought stress has curtailed the maize growth and yield in a large area and created an imbalance in the food supply chain. Human popul0ation growth and increasing consumption tend to increase the emissions of climate-changing greenhouse gases. Global warming has negative consequences on crop growth and yields in large areas. The threat of drought stress will be increased soon because of the continuous depletion of water resources and global warming. Maize breeders have used several conventional breeding tools to breed drought-tolerant maize genotypes and have achieved significant results. However, these breeding tools are time-consuming and costly, hindering the success of drought breeding. Maize tolerance to drought stress is polygenic, and, hence, the complex genetic mechanism of drought tolerance has hindered the large-scale use of conventional breeding methods. The maize plant uses several morphological, physiological, and biochemical processes to cope with drought stress; however, this is not a long-term solution to increase crop growth. In the past years, scientists have brought a significant revolution in molecular breeding to counter the threat of rising climatic changes. QTL mapping has identified the novel genomic regions controlling drought tolerance in maize and has shown marvelous success. It has also enhanced the use of MAS selection as well as the pyramiding of QTL,

which enhanced the drought tolerance of several maize genotypes. Despite its large-scale use, QTL mapping has several limitations, such as the use of large sample sizes and recombination during the development of RIL. These challenges for QTL mapping must be addressed in future studies to enhance the efficiency of this technique. The above-reported QTL should be cloned and transferred to susceptible genotypes to increase their tolerance to rising episodes of drought stress. GWAS has effectively mapped the novel QTL and SNPs by analyzing a large sample size. Several QTL and SNPs have been identified that are involved in maize drought tolerance. GWAS cannot identify all genetic determinants of complex traits and can only identify the modest action of heritability.

Transcriptomes and TF analysis techniques have been used to identify the novel genomic regions for drought tolerance in maize. However, not all of them have been cloned and targeted by molecular breeding. It is necessary to screen the identified genes and examine their regulatory networks for their role in drought tolerance in maize. Some families of TFs are not completely understood for their possible role in drought tolerance. Genetic engineering techniques have shown tremendous success in many crops and maize to increase the resistance to certain abiotic stresses. The transgenic genotypes of many crops have significant resistance to drought stress; however, there is still insufficient knowledge about transgenic breeding applications in maize. Therefore, developing transgenic maize genotypes is urgently needed to cope with drought stress. The large-scale screening of maize germplasm is critical in identifying the tolerant genotypes as well as tolerant traits associated with drought tolerance. Abiotic stresses have threatened maize genetic diversity; hence, it is important to safeguard it using several molecular breeding tools. Comprehensive genome sequencing can give a better overview of the gene networks involved in drought tolerance in wild types.

We should expand the use of CRISPR/Cas9, a novel gene editing tool, to generate drought-tolerant mutants in maize. The use of CRISPR/Cas9 will accelerate drought-tolerant breeding. Using CRISPR/Cas9 will increase the success rate of drought breeding and enhance the precision of genome editing. We suggest using novel gene editing systems such as prime editing (PE) and base editing (BE), which can edit large fragments of genetic material. Overall, the studies mentioned above showed that drought stress is still a burning issue and will continue to affect crops in large areas. Hence, improving existing molecular breeding tools and developing new methods is necessary to improve the genetic makeup of crops to deal with drought stress issues. This comprehensive and well-organized review paper will serve as a valuable source of information for future maize researchers to understand the basis of drought tolerance and adopt a more powerful method to breed drought-tolerant maize genotypes.

**Author Contributions:** A.R. conceptualized and prepared the manuscript; H.J., P.H., L.Z., Y.M. and H.X., participated in the literature search; B.A., S.H.Q., M.U.H. and M.R.H. reviewed and edited the manuscript; Y.J. supervised the study. All authors have read and agreed to the published version of the manuscript.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Bai, M.; Zeng, W.; Chen, F.; Ji, X.; Zhuang, Z.; Jin, B.; Wang, J.; Jia, L.; Peng, Y. Transcriptome expression profiles reveal response mechanisms to drought and drought-stress mitigation mechanisms by exogenous glycine betaine in maize. *Biotech. Lett.* **2022**, *44*, 367–386. [CrossRef]

2. Gao, Z.; Zhao, J.; Wang, C.; Wang, Y.; Shang, M.; Zhang, Z.; Chen, F.; Chu, Q. A six-year record of greenhouse gas emissions in different growth stages of summer maize influenced by irrigation and nitrogen management. *Field Crops Res.* **2023**, *290*, 108744. [CrossRef]

3. Notununu, I.; Moleleki, L.; Roopnarain, A.; Adeleke, R. Effects of plant growth-promoting rhizobacteria on the molecular responses of maize under drought and heat stresses: A review. *Pedosphere* **2022**, *32*, 90–106. [CrossRef]

4. Wang, Y.; Cao, Y.; Liang, X.; Zhuang, J.; Wang, X.; Qin, F.; Jiang, C. A dirigent family protein confers variation of Casparian strip thickness and salt tolerance in maize. *Nat. Comm.* **2022**, *13*, 2222. [CrossRef] [PubMed]

5. Pei, Y.-Y.; Lei, L.; Fan, X.-W.; Li, Y.-Z. Effects of high air temperature, drought, and both combinations on maize: A case study. *Plant Sci.* **2023**, *327*, 111543. [CrossRef]

6. Rahmat, B.P.N.; Octavianis, G.; Budiarto, R.; Jadid, N.; Widiastuti, A.; Matra, D.D.; Ezura, H.; Mubarok, S. SlIAA9 Mutation Maintains Photosynthetic Capabilities under Heat-Stress Conditions. *Plants* **2023**, *12*, 378. [CrossRef]

7. FAO; UNICEF; WFP; WHO. *Building Resilience for Peace and Food Security*; FAO: Rome, Italy, 2017.

8. Bellemare, M.F. Rising food prices, food price volatility, and social unrest. *Amer. J. Agricul. Econ.* **2015**, *97*, 1–21. [CrossRef]

9. Kalkuhl, M.; Von Braun, J.; Torero, M. *Food Price Volatility and Its Implications for Food Security and Policy*; Springer Nature: Berlin/Heidelberg, Germany, 2016.

10. Hertel, T.W. Food security under climate change. *Nat. Clim. Chang.* **2016**, *6*, 10–13. [CrossRef]

11. Swinnen, J.; Squicciarini, P. Mixed messages on prices and food security. *Science* **2012**, *335*, 405–406. [CrossRef]

12. Ray, D.K.; Gerber, J.S.; MacDonald, G.K.; West, P.C. Climate variation explains a third of global crop yield variability. *Nat. Comm.* **2015**, *6*, 5989. [CrossRef]

13. Webber, H.; Ewert, F.; Olesen, J.E.; Müller, C.; Fronzek, S.; Ruane, A.C.; Bourgault, M.; Martre, P.; Ababaei, B.; Bindi, M. Diverging importance of drought stress for maize and winter wheat in Europe. *Nat. Comm.* **2018**, *9*, 4249. [CrossRef] [PubMed]

14. Wakatsuki, H.; Ju, H.; Nelson, G.C.; Farrell, A.D.; Deryng, D.; Meza, F.; Hasegawa, T. Research trends and gaps in climate change impacts and adaptation potentials in major crops. *Curr. Opin. Environ. Sust.* **2023**, *60*, 101249. [CrossRef]

15. Zhao, Y.; Zhao, B.; Xie, Y.; Jia, H.; Li, Y.; Xu, M.; Wu, G.; Ma, X.; Li, Q.; Hou, M. The evening complex promotes maize flowering and adaptation to temperate regions. *The Plant Cell* **2023**, *35*, 369–389. [CrossRef] [PubMed]

16. Strable, J.; Scanlon, M.J. Maize (*Zea mays*): A model organism for basic and applied research in plant biology. *Cold Spr. Harb. Prot.* **2009**, *2009*, pdb.emo132. [CrossRef] [PubMed]

17. Palacios-Rojas, N.; McCulley, L.; Kaeppler, M.; Titcomb, T.J.; Gunaratna, N.S.; Lopez-Ridaura, S.; Tanumihardjo, S.A. Mining maize diversity and improving its nutritional aspects within agro-food systems. *Compreh. Rev. Food Sci. Food Safety* **2020**, *19*, 1809–1834. [CrossRef]

18. Aliniaeifard, S.; Rezayian, M.; Mousavi, S.H. Drought Stress: Involvement of Plant Hormones in Perception, Signaling, and Response. In *Plant Hormones and Climate Change*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 227–250.

19. Adewale, S.; Akinwale, R.; Fakorede, M.; Badu-Apraku, B. Genetic analysis of drought-adaptive traits at seedling stage in early-maturing maize inbred lines and field performance under stress conditions. *Euphytica* **2018**, *214*, 145. [CrossRef]

20. Edmeades, G. *Progress in Achieving and Delivering Drought Tolerance in Maize-an Update*; ISAAA: Ithaca, NY, USA, 2013; Volume 130.

21. Tesfaye, K.; Kruseman, G.; Cairns, J.E.; Zaman-Allah, M.; Wegary, D.; Zaidi, P.; Boote, K.J.; Erenstein, O. Potential benefits of drought and heat tolerance for adapting maize to climate change in tropical environments. *Clim. Risk Mana.* **2018**, *19*, 106–119. [CrossRef]

22. Aslam, M.; Zamir, M.; Afzal, I.; Yaseen, M.; Mubeen, M.; Shoaib, A. Drought stress, its effect on maize production and development of drought tolerance through potassium application. *Agron. Res. Mol.* **2012**, *46*, 1–16.

23. Mohammadkhani, N.; Heidari, R. Effects of drought stress on soluble proteins in two maize varieties. *Turk. J. Biol.* **2008**, *32*, 23–30.

24. Khalili, M.; Naghavi, M.R.; Aboughadareh, A.P.; Rad, H.N. Effects of drought stress on yield and yield components in maize cultivars (*Zea mays* L.). *Int. J. Agro. Plant Prod.* **2013**, *4*, 809–812.

25. Zhang, X.; Lei, L.; Lai, J.; Zhao, H.; Song, W. Effects of drought stress and water recovery on physiological responses and gene expression in maize seedlings. *BMC Plant Biol.* **2018**, *18*, 68. [CrossRef]

26. Javed, N.; Ashraf, M.; Akram, N.A.; Al-Qurainy, F. Alleviation of adverse effects of drought stress on growth and some potential physiological attributes in maize (*Zea mays* L.) by seed electromagnetic treatment. *Photochem. Photob.* **2011**, *87*, 1354–1362. [CrossRef] [PubMed]

27. Li, C.; Sun, B.; Li, Y.; Liu, C.; Wu, X.; Zhang, D.; Shi, Y.; Song, Y.; Buckler, E.S.; Zhang, Z. Numerous genetic loci identified for drought tolerance in the maize nested association mapping populations. *BMC Gen.* **2016**, *17*, 894. [CrossRef]

28. Kakumanu, A.; Ambavaram, M.M.; Klumas, C.; Krishnan, A.; Batlang, U.; Myers, E.; Grene, R.; Pereira, A. Effects of drought on gene expression in maize reproductive and leaf meristem tissue revealed by RNA-Seq. *Plant Physiol.* **2012**, *160*, 846–867. [CrossRef] [PubMed]

29. Thirunavukkarasu, N.; Sharma, R.; Singh, N.; Shiriga, K.; Mohan, S.; Mittal, S.; Mittal, S.; Mallikarjuna, M.G.; Rao, A.R.; Dash, P.K. Genomewide expression and functional interactions of genes under drought stress in maize. *Int. J. Gen.* **2017**, *2017*, 2568706. [CrossRef] [PubMed]

30. Mahajan, S.; Tuteja, N. Cold, salinity and drought stresses: An overview. *Arch. Biochem. Biophy.* **2005**, *444*, 139–158. [CrossRef] [PubMed]

31. Yang, H.; Wang, C.; Chen, F.; Yue, L.; Cao, X.; Li, J.; Zhao, X.; Wu, F.; Wang, Z.; Xing, B. Foliar carbon dot amendment modulates carbohydrate metabolism, rhizospheric properties and drought tolerance in maize seedling. *Sci. Total Environ.* **2022**, *809*, 151105. [CrossRef] [PubMed]

32. Tuberosa, R.; Salvi, S. Genomics-based approaches to improve drought tolerance of crops. *Trend. Plant Sci.* **2006**, *11*, 405–412. [CrossRef]

33. Hao, Z.; Li, X.; Liu, X.; Xie, C.; Li, M.; Zhang, D.; Zhang, S. Meta-analysis of constitutive and adaptive QTL for drought tolerance in maize. *Euphytica* **2010**, *174*, 165–177. [CrossRef]

34. Yang, Y.; Wang, B.; Wang, J.; He, C.; Zhang, D.; Li, P.; Zhang, J.; Li, Z. Transcription factors *ZmNF-YA1* and *ZmNF-YB16* regulate plant growth and drought tolerance in maize. *Plant Physiol.* **2022**, *190*, 1506–1525. [CrossRef]

35. Min, H.; Chen, C.; Wei, S.; Shang, X.; Sun, M.; Xia, R.; Liu, X.; Hao, D.; Chen, H.; Xie, Q. Identification of drought tolerant mechanisms in maize seedlings based on transcriptome analysis of recombination inbred lines. *Front. Plant Sci.* **2016**, *7*, 1080. [CrossRef]

36. Xiang, Y.; Sun, X.; Bian, X.; Wei, T.; Han, T.; Yan, J.; Zhang, A. The transcription factor *ZmNAC49* reduces stomatal density and improves drought tolerance in maize. *J. Exp. Bot.* **2021**, *72*, 1399–1410. [CrossRef] [PubMed]

37. Wei, A.; He, C.; Li, B.; Li, N.; Zhang, J. The pyramid of transgenes TsVP and BetA effectively enhances the drought tolerance of maize plants. *Plant Biotech. J.* **2011**, *9*, 216–229. [CrossRef] [PubMed]

38. Shi, J.; Gao, H.; Wang, H.; Lafitte, H.R.; Archibald, R.L.; Yang, M.; Hakimi, S.M.; Mo, H.; Habben, J.E. *ARGOS 8* variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotech. J.* **2017**, *15*, 207–216. [CrossRef] [PubMed]

39. Li, X.; Xu, S.; Fuhrmann-Aoyagi, M.B.; Yuan, S.; Iwama, T.; Kobayashi, M.; Miura, K. CRISPR/Cas9 Technique for Temperature, Drought, and Salinity Stress Responses. *Curr. Issues Mol. Biol.* **2022**, *44*, 2664–2682. [CrossRef] [PubMed]

40. Jiang, Y.; Sun, K.; An, X. CRISPR/Cas System: Applications and Prospects for Maize Improvement. *ACS Agricul. Sci. Technol.* **2022**, *2*, 174–183. [CrossRef]

41. Khanzada, H.; Wassan, G.M.; He, H.; Mason, A.S.; Keerio, A.A.; Khanzada, S.; Faheem, M.; Solangi, A.M.; Zhou, Q.; Fu, D. Differentially evolved drought stress indices determine the genetic variation of Brassica napus at seedling traits by genome-wide association mapping. *J. Adv. Res.* **2020**, *24*, 447–461. [CrossRef]

42. Oya, T.; Nepomuceno, A.L.; Neumaier, N.; Farias, J.R.B.; Tobita, S.; Ito, O. Drought Tolerance Characteristics of Brazilian Soybean Cultivars—Evaluation and characterization of drought tolerance of various Brazilian soybean cultivars in the field—. *Plant Prod. Sci.* **2004**, *7*, 129–137. [CrossRef]

43. Liu, Z.; Li, H.; Wang, X.; Zhang, Y.; Gou, Z.; Zhao, X.; Ren, H.; Wen, Z.; Li, Y.; Yu, L. QTL for yield per plant under water deficit and well-watered conditions and drought susceptibility index in soybean (*Glycine max* (L.) Merr.). *Biotech. Biotechnol. Equip.* **2023**, *37*, 92–103. [CrossRef]

44. Collins, N.C.; Tardieu, F.; Tuberosa, R. Quantitative trait loci and crop performance under abiotic stress: Where do we stand? *Plant Physiol.* **2008**, *147*, 469–486. [CrossRef]

45. Li, Y.; Song, H.; Zhou, L.; Xu, Z.; Zhou, G. Vertical distributions of chlorophyll and nitrogen and their associations with photosynthesis under drought and rewatering regimes in a maize field. *Agricul. Forest Meteorol.* **2019**, *272*, 40–54. [CrossRef]

46. Voronin, P.Y.; Maevskaya, S.; Nikolaeva, M. Physiological and molecular responses of maize (*Zea mays* L.) plants to drought and rehydration. *Photosynthetica* **2019**, *57*, 850–856. [CrossRef]

47. Jing, L.; Weng, B.; Yan, D.; Yuan, F.; Zhang, S.; Bi, W.; Yan, S. Assessment of resilience in maize suitable planting areas under drought stress. *Agricul. Water Manag.* **2023**, *277*, 108096. [CrossRef]

48. Hammer, K. *Agrarbiodiversität und pflanzengenetische Ressourcen: Herausforderung und Lösungsansatz*; Zentralstelle für Agrardokumentation und -information: Bonn, Germany, 1998.

49. Hammer, K. Checklists and germplasm collecting. *FAO/IBPGR Plant Genetic Res. Newslet.* **1991**, *85*, 15–17.

50. Abdel-Ghani, A.H.; Neumann, K.; Wabila, C.; Sharma, R.; Dhanagond, S.; Owais, S.J.; Börner, A.; Graner, A.; Kilian, B. Diversity of germination and seedling traits in a spring barley (*Hordeum vulgare* L.) collection under drought simulated conditions. *Gen. Res. Crop Evol.* **2015**, *62*, 275–292. [CrossRef]

51. Thabet, S.G.; Moursi, Y.S.; Karam, M.A.; Graner, A.; Alqudah, A.M. Genetic basis of drought tolerance during seed germination in barley. *PLoS ONE* **2018**, *13*, e0206682. [CrossRef]

52. Tarawneh, R.A.; Szira, F.; Monostori, I.; Behrens, A.; Alqudah, A.M.; Thumm, S.; Lohwasser, U.; Röder, M.S.; Börner, A.; Nagel, M. Genetic analysis of drought response of wheat following either chemical desiccation or the use of a rain-out shelter. *J. App. Gen.* **2019**, *60*, 137–146. [CrossRef]

53. Badr, A.; El-Shazly, H.H.; Tarawneh, R.A.; Börner, A. Screening for drought tolerance in maize (*Zea mays* L.) germplasm using germination and seedling traits under simulated drought conditions. *Plants* **2020**, *9*, 565. [CrossRef]

54. Avramova, V.; Nagel, K.A.; AbdElgawad, H.; Bustos, D.; DuPlessis, M.; Fiorani, F.; Beemster, G.T. Screening for drought tolerance of maize hybrids by multi-scale analysis of root and shoot traits at the seedling stage. *J. Expe. Bot.* **2016**, *67*, 2453–2466. [CrossRef]

55. Ali, Q.; Ahsan, M.; Kanwal, N.; Ali, F.; Ali, A.; Ahmed, W.; Ishfaq, M.; Saleem, M. Screening for drought tolerance: Comparison of maize hybrids under water deficit condition. *Adv. Life Sci.* **2016**, *3*, 51–58.

56. Bonea, D. Screening for drought tolerance in maize hybrids using new indices based on resilience and production capacity. *Screening* **2020**, *20*, 151–156.

57. Kumar, B.; Kumar, K.; Jat, S.L.; Srivastava, S.; Tiwari, T.; Kumar, S.; Pradhan, H.R.; Kumar, B.; Chaturvedi, G.; Jha, A.K. Rapid method of screening for drought stress tolerance in maize (*Zea mays* L.). *Ind. J. Gen. Plant Bree.* **2020**, *80*, 16–25. [CrossRef]

58. Alvi, A.K.; Ahmad, M.S.A.; Rafique, T.; Naseer, M.; Farhat, F.; Tasleem, H.; Nasim, A. Screening of maize (*Zea mays* L.) genotypes for drought tolerance using photosynthetic pigments and anti-oxidative enzymes as selection criteria. *Pak. J. Bot* **2022**, *54*, 33–44. [CrossRef]

59. Raj, R.N.; Gokulakrishnan, J.; Prakash, M. Assessing drought tolerance using PEG-6000 and molecular screening by SSR markers in maize (*Zea mays* L.) hybrids. *Maydica* **2020**, *64*, 1–7.

60. Adhikari, B.; Sa, K.J.; Lee, J.K. Drought tolerance screening of maize inbred lines at an early growth stage. *Plant Breed. Biotech* **2019**, *7*, 326–339. [CrossRef]

61. Shahrokhi, M.; Khorasani, S.K.; Ebrahimi, A. Evaluation of drought tolerance indices for screening some of super sweet maize (*Zea mays* L. var. Saccharata) inbred lines. *Agrivita J. Agricul. Sci.* **2020**, *42*, 435–448. [CrossRef]

62. Balbaa, M.G.; Osman, H.T.; Kandil, E.E.; Javed, T.; Lamlom, S.F.; Ali, H.M.; Kalaji, H.M.; Wróbel, J.; Telesiñski, A.; Brysiewicz, A. Determination of morpho-physiological and yield traits of maize inbred lines (*Zea mays* L.) under optimal and drought stress conditions. *Front. Plant Sci.* **2022**, *13*, 959203. [CrossRef]

63. Emmanuel, I.; Victor, O.; Caroline, U.; Rizvi, A.H.; Kumar, T.; Alam, A. Screening of some selected indian maize cultivars to simulated drought condition. *Ind. J. Agricul. Res.* **2020**, *54*, 465–470.

64. Masood, M.; Ahsan, M.; Sadaqat, H.; Awan, F. Screening of maize (*Zea mays* L.) inbred lines under water deficit conditions. *Biol. Clin. Sci. Res. J.* **2020**, *2020*, 1–6. [CrossRef]

65. Ramzan, J.; Aslam, M.; Ahsan, M.; Awan, F.S. Selection of screening criteria against drought stress at early growth stages in maize (*Zea mays* L.). *Pak. J. Agricul. Sci.* **2019**, *56*, 633–643.

66. Choudhary, M.; Kumar, P.; Kumar, P.; Sheoran, S.; Zunjare, R.U.; Jat, B.S. Molecular breeding for drought and heat stress in maize: Revisiting the progress and achievements. In *QTL Mapping in Crop Improvement*; Elsevier: Amsterdam, The Netherlands, 2023; pp. 57–74.

67. Sheoran, S.; Gupta, M.; Kumari, S.; Kumar, S.; Rakshit, S. Meta-QTL analysis and candidate genes identification for various abiotic stresses in maize (Zea mays L.) and their implications in breeding programs. *Mol. Breed.* **2022**, *42*, 26. [CrossRef]

68. Hu, X.; Wang, G.; Du, X.; Zhang, H.; Xu, Z.; Wang, J.; Chen, G.; Wang, B.; Li, X.; Chen, X. QTL analysis across multiple environments reveals promising chromosome regions associated with yield-related traits in maize under drought conditions. *Crop J.* **2021**, *9*, 759–766. [CrossRef]

69. Zhao, X.; Zhong, Y. Genetic dissection of the photosynthetic parameters of maize (*Zea mays* L.) in drought-stressed and well-watered environments. *Russ. J. Plant Physiol.* **2021**, *68*, 1125–1134. [CrossRef]

70. Zhang, F.; Zhang, J.; Ma, Z.; Xia, L.; Wang, X.; Zhang, L.; Ding, Y.; Qi, J.; Mu, X.; Zhao, F. Bulk analysis by resequencing and RNA-seq identifies candidate genes for maintaining leaf water content under water deficit in maize. *Physiol. Plant.* **2021**, *173*, 1935–1945. [CrossRef]

71. Leng, P.; Khan, S.U.; Zhang, D.; Zhou, G.; Zhang, X.; Zheng, Y.; Wang, T.; Zhao, J. Linkage Mapping Reveals QTL for Flowering Time-Related Traits under Multiple Abiotic Stress Conditions in Maize. *Int. J. Mol. Sci.* **2022**, *23*, 8410. [CrossRef]

72. Abdelghany, M.; Liu, X.; Hao, L.; Gao, C.; Kou, S.; Su, E.; Zhou, Y.; Wang, R.; Zhang, D.; Li, Y. QTL analysis for yield-related traits under different water regimes in maize. *Maydica* **2019**, *64*, 10.

73. Almeida, G.D.; Makumbi, D.; Magorokosho, C.; Nair, S.; Borém, A.; Ribaut, J.-M.; Bänziger, M.; Prasanna, B.M.; Crossa, J.; Babu, R. QTL mapping in three tropical maize populations reveals a set of constitutive and adaptive genomic regions for drought tolerance. *Theoret. App. Gen.* **2013**, *126*, 583–600. [CrossRef]

74. Nikolić, A.; Anđelković, V.; Dodig, D.; Mladenović-Drinić, S.; Kravić, N.; Ignjatović-Micić, D. Identification of QTL-s for drought tolerance in maize, II: Yield and yield components. *Genetika* **2013**, *45*, 341–350. [CrossRef]

75. Almeida, G.D.; Nair, S.; Borém, A.; Cairns, J.; Trachsel, S.; Ribaut, J.-M.; Bänziger, M.; Prasanna, B.M.; Crossa, J.; Babu, R. Molecular mapping across three populations reveals a QTL hotspot region on chromosome 3 for secondary traits associated with drought tolerance in tropical maize. *Mol. Breed.* **2014**, *34*, 701–715. [CrossRef]

76. Lu, Y.; Xu, J.; Yuan, Z.; Hao, Z.; Xie, C.; Li, X.; Shah, T.; Lan, H.; Zhang, S.; Rong, T. Comparative LD mapping using single SNPs and haplotypes identifies QTL for plant height and biomass as secondary traits of drought tolerance in maize. *Mol. Breed.* **2012**, *30*, 407–418. [CrossRef]

77. Zhu, J.-J.; Wang, X.-P.; Sun, C.-X.; Zhu, X.-M.; Meng, L.; Zhang, G.-D.; Tian, Y.-C.; Wang, Z.-L. Mapping of QTL associated with drought tolerance in a semi-automobile rain shelter in maize (*Zea mays* L.). *Agricul. Sci. Chi.* **2011**, *10*, 987–996. [CrossRef]

78. Rahman, H.; Pekic, S.; Lazic-Jancic, V.; Quarrie, S.; Shah, S.; Pervez, A.; Shah, M. Molecular mapping of quantitative trait loci for drought tolerance in maize plants. *Genet. Mol. Res.* **2011**, *10*, 889–901. [CrossRef] [PubMed]

79. Wang, X.; Wang, H.; Liu, S.; Ferjani, A.; Li, J.; Yan, J.; Yang, X.; Qin, F. Genetic variation in *ZmVPP1* contributes to drought tolerance in maize seedlings. *Nat. Gen.* **2016**, *48*, 1233–1241. [CrossRef]

80. Wang, N.; Wang, Z.-p.; Liang, X.-l.; Weng, J.-f.; Lv, X.-l.; Zhang, D.-g.; Yang, J.; Yong, H.-j.; Li, M.-s.; Li, F.-h. Identification of loci contributing to maize drought tolerance in a genome-wide association study. *Euphytica* **2016**, *210*, 165–179. [CrossRef]

81. Hao, Z.; Li, X.; Xie, C.; Weng, J.; Li, M.; Zhang, D.; Liang, X.; Liu, L.; Liu, S.; Zhang, S. Identification of functional genetic variations underlying drought tolerance in maize using SNP markers. *J. Integrat. Plant Biol.* **2011**, *53*, 641–652. [CrossRef] [PubMed]

82. Xue, Y.; Warburton, M.L.; Sawkins, M.; Zhang, X.; Setter, T.; Xu, Y.; Grudloyma, P.; Gethi, J.; Ribaut, J.-M.; Li, W. Genome-wide association analysis for nine agronomic traits in maize under well-watered and water-stressed conditions. *Theoret. App. Gen.* **2013**, *126*, 2587–2596. [CrossRef]

83. Zhang, X.; Warburton, M.L.; Setter, T.; Liu, H.; Xue, Y.; Yang, N.; Yan, J.; Xiao, Y. Genome-wide association studies of drought-related metabolic changes in maize using an enlarged SNP panel. *Theoret. App. Gen.* **2016**, *129*, 1449–1463. [CrossRef]

84. Khan, S.U.; Zheng, Y.; Chachar, Z.; Zhang, X.; Zhou, G.; Zong, N.; Leng, P.; Zhao, J. Dissection of maize drought tolerance at the flowering stage using genome-wide association studies. *Genes* **2022**, *13*, 564. [CrossRef]

85. Guo, J.; Li, C.; Zhang, X.; Li, Y.; Zhang, D.; Shi, Y.; Song, Y.; Li, Y.; Yang, D.; Wang, T. Transcriptome and GWAS analyses reveal candidate gene for seminal root length of maize seedlings under drought stress. *Plant Sci.* **2020**, *292*, 110380. [CrossRef]

86. Yuan, Y.; Cairns, J.E.; Babu, R.; Gowda, M.; Makumbi, D.; Magorokosho, C.; Zhang, A.; Liu, Y.; Wang, N.; Hao, Z. Genome-wide association mapping and genomic prediction analyses reveal the genetic architecture of grain yield and flowering time under drought and heat stress conditions in maize. *Front. Plant Sci.* **2019**, *9*, 1919. [CrossRef]

87. Shikha, M.; Kanika, A.; Rao, A.R.; Mallikarjuna, M.G.; Gupta, H.S.; Nepolean, T. Genomic selection for drought tolerance using genome-wide SNPs in maize. *Front. Plant Sci.* **2017**, *8*, 550. [CrossRef] [PubMed]

88. Thirunavukkarasu, N.; Hossain, F.; Arora, K.; Sharma, R.; Shiriga, K.; Mittal, S.; Mohan, S.; Namratha, P.M.; Dogga, S.; Rani, T.S. Functional mechanisms of drought tolerance in subtropical maize (*Zea mays* L.) identified using genome-wide association mapping. *BMC Gen.* **2014**, *15*, 1182. [CrossRef] [PubMed]

89. Osuman, A.S.; Badu-Apraku, B.; Karikari, B.; Ifie, B.E.; Tongoona, P.; Danquah, E.Y. Genome-wide association study reveals genetic architecture and candidate genes for yield and related traits under terminal drought, combined heat and drought in tropical maize germplasm. *Genes* **2022**, *13*, 349. [CrossRef] [PubMed]

90. Wang, N.; Liu, B.; Liang, X.; Zhou, Y.; Song, J.; Yang, J.; Yong, H.; Weng, J.; Zhang, D.; Li, M. Genome-wide association study and genomic prediction analyses of drought stress tolerance in China in a collection of off-PVP maize inbred lines. *Mol. Breed.* **2019**, *39*, 113. [CrossRef]

91. Barbosa, P.A.M.; Fritsche-Neto, R.; Andrade, M.C.; Petroli, C.D.; Burgueño, J.; Galli, G.; Willcox, M.C.; Sonder, K.; Vidal-Martínez, V.A.; Sifuentes-Ibarra, E. Introgression of maize diversity for drought tolerance: Subtropical maize landraces as source of new positive variants. *Front. Plant Sci.* **2021**, *12*, 691211. [CrossRef]

92. Rida, S.; Maafi, O.; López-Malvar, A.; Revilla, P.; Riache, M.; Djemel, A. Genetics of germination and seedling traits under drought stress in a MAGIC population of maize. *Plants* **2021**, *10*, 1786. [CrossRef]

93. Zhang, H.; Jin, J.; Tang, L.; Zhao, Y.; Gu, X.; Gao, G.; Luo, J. PlantTFDB 2.0: Update and improvement of the comprehensive plant transcription factor database. *Nucl. Acids Res.* **2011**, *39*, D1114–D1117. [CrossRef]

94. Ma, J.; Tang, X.; Sun, B.; Wei, J.; Ma, L.; Yuan, M.; Zhang, D.; Shao, Y.; Li, C.; Chen, K.-M. A NAC transcription factor, TaNAC5D-2, acts as a positive regulator of drought tolerance through regulating water loss in wheat (*Triticum aestivum* L.). *Environ. Exp. Bot.* **2022**, *196*, 104805. [CrossRef]

95. Yu, Y.; Song, T.; Wang, Y.; Zhang, M.; Li, N.; Yu, M.; Zhang, S.; Zhou, H.; Guo, S.; Bu, Y. The wheat WRKY transcription factor *TaWRKY1-2D* confers drought resistance in transgenic Arabidopsis and wheat (*Triticum aestivum* L.). *Int. J. Biol. Macromol.* **2022**, *226*, 1203–1217. [CrossRef]

96. Rodriguez-Uribe, L.; O'Connell, M.A. A root-specific bZIP transcription factor is responsive to water deficit stress in tepary bean (*Phaseolus acutifolius*) and common bean (*P. vulgaris*). *J. Exp. Bot.* **2006**, *57*, 1391–1398. [CrossRef] [PubMed]

97. Zhao, L.; Yan, J.; Xiang, Y.; Sun, Y.; Zhang, A. *ZmWRKY104* transcription factor phosphorylated by *ZmMPK6* functioning in ABA-induced antioxidant defense and enhance drought tolerance in Maize. *Biology* **2021**, *10*, 893. [CrossRef] [PubMed]

98. Wang, C.-T.; Ru, J.-N.; Liu, Y.-W.; Yang, J.-F.; Li, M.; Xu, Z.-S.; Fu, J.-D. The maize WRKY transcription factor *ZmWRKY40* confers drought resistance in transgenic *Arabidopsis*. *Int. J. Mol. Sci.* **2018**, *19*, 2580. [CrossRef] [PubMed]

99. Gulzar, F.; Fu, J.; Zhu, C.; Yan, J.; Li, X.; Meraj, T.A.; Shen, Q.; Hassan, B.; Wang, Q. Maize WRKY transcription factor ZmWRKY79 positively regulates drought tolerance through elevating ABA biosynthesis. *Int. J. Mol. Sci.* **2021**, *22*, 10080. [CrossRef]

100. Li, Z.; Liu, C.; Zhang, Y.; Wang, B.; Ran, Q.; Zhang, J. The bHLH family member *ZmPTF1* regulates drought tolerance in maize by promoting root development and abscisic acid synthesis. *J. Exp. Bot.* **2019**, *70*, 5471–5486. [CrossRef]

101. Zhao, H.; Wang, C.; Lan, H. A bHLH transcription factor from Chenopodium glaucum confers drought tolerance to transgenic maize by positive regulation of morphological and physiological performances and stress-responsive genes' expressions. *Mol. Breed.* **2021**, *41*, 74. [CrossRef]

102. Feng, W.; Liu, Y.; Cao, Y.; Zhao, Y.; Zhang, H.; Sun, F.; Yang, Q.; Li, W.; Lu, Y.; Zhang, X. Maize *ZmBES1/BZR1-3* and -9 transcription factors negatively regulate drought tolerance in transgenic *Arabidopsis*. *Int. J. Mol. Sci.* **2022**, *23*, 6025. [CrossRef]

103. Zhu, Y.; Liu, Y.; Zhou, K.; Tian, C.; Aslam, M.; Zhang, B.; Liu, W.; Zou, H. Overexpression of *ZmEREBP60* enhances drought tolerance in maize. *J. Plant Physiol.* **2022**, *275*, 153763. [CrossRef]

104. Wang, G.; Yuan, Z.; Zhang, P.; Liu, Z.; Wang, T.; Wei, L. Genome-wide analysis of NAC transcription factor family in maize under drought stress and rewatering. *Physiol. Mol. Biol. Plants* **2020**, *26*, 705–717. [CrossRef]

105. Mao, H.; Yu, L.; Han, R.; Li, Z.; Liu, H. *ZmNAC55*, a maize stress-responsive NAC transcription factor, confers drought resistance in transgenic Arabidopsis. *Plant Physiol.Biochem.* **2016**, *105*, 55–66. [CrossRef]

106. Qiu, X.; Wang, G.; Abou-Elwafa, S.F.; Fu, J.; Liu, Z.; Zhang, P.; Xie, X.; Ku, L.; Ma, Y.; Guan, X. Genome-wide identification of HD-ZIP transcription factors in maize and their regulatory roles in promoting drought tolerance. *Physiol. Mol. Biol. Plants* **2022**, *28*, 425–437. [CrossRef]

107. Jiao, P.; Jiang, Z.; Wei, X.; Liu, S.; Qu, J.; Guan, S.; Ma, Y. Overexpression of the homeobox-leucine zipper protein ATHB-6 improves the drought tolerance of maize (*Zea mays* L.). *Plant Sci.* **2022**, *316*, 111159. [CrossRef] [PubMed]

108. Zhang, G.; Li, G.; Xiang, Y.; Zhang, A. The transcription factor *ZmMYB-CC10* improves drought tolerance by activating ZmAPX4 expression in maize. *Biochem. Biophy. Res. Comm.* **2022**, *604*, 1–7. [CrossRef]

109. Wang, Z.; Zhao, X.; Ren, Z.; Abou-Elwafa, S.F.; Pu, X.; Zhu, Y.; Dou, D.; Su, H.; Cheng, H.; Liu, Z. *ZmERF21* directly regulates hormone signaling and stress-responsive gene expression to influence drought tolerance in maize seedlings. *Plant, Cell Environ.* **2022**, *45*, 312–328. [CrossRef]

110. Zhang, S.; Li, N.; Gao, F.; Yang, A.; Zhang, J. Over-expression of *TsCBF1* gene confers improved drought tolerance in transgenic maize. *Mol. Breed.* **2010**, *26*, 455–465. [CrossRef]

111. Zheng, H.; Yang, Z.; Wang, W.; Guo, S.; Li, Z.; Liu, K.; Sui, N. Transcriptome analysis of maize inbred lines differing in drought tolerance provides novel insights into the molecular mechanisms of drought responses in roots. *Plant Physiol. and Biochem.* **2020**, *149*, 11–26. [CrossRef] [PubMed]

112. Zhao, C.; Yang, M.; Wu, X.; Wang, Y.; Zhang, R. Physiological and transcriptomic analyses of the effects of exogenous melatonin on drought tolerance in maize (*Zea mays* L.). *Plant Physiol. Biochem.* **2021**, *168*, 128–142. [CrossRef]

113. Zhang, Q.; Liu, H.; Wu, X.; Wang, W. Identification of drought tolerant mechanisms in a drought-tolerant maize mutant based on physiological, biochemical and transcriptomic analyses. *BMC Plant Biol.* **2020**, *20*, 315. [CrossRef]

114. Waititu, J.K.; Zhang, X.; Chen, T.; Zhang, C.; Zhao, Y.; Wang, H. Transcriptome analysis of tolerant and susceptible maize genotypes reveals novel insights about the molecular mechanisms underlying drought responses in leaves. *Int. J. Mol. Sci.* **2021**, *22*, 6980. [CrossRef]

115. Liu, S.; Zenda, T.; Li, J.; Wang, Y.; Liu, X.; Duan, H. Comparative transcriptomic analysis of contrasting hybrid cultivars reveal key drought-responsive genes and metabolic pathways regulating drought stress tolerance in maize at various stages. *PLoS ONE* **2020**, *15*, e0240468. [CrossRef]

116. Zhang, F.; Ding, Y.; Zhang, J.; Tang, M.; Cao, Y.; Zhang, L.; Ma, Z.; Qi, J.; Mu, X.; Xia, L. Comparative transcriptomic reveals the molecular mechanism of maize hybrid Zhengdan538 in response to water deficit. *Physiol. Plant.* **2022**, *174*, e13818. [CrossRef]

117. Gillani, S.F.A.; Rasheed, A.; Abbasi, A.; Majeed, Y.; Abbas, M.; Hassan, M.U.; Qari, S.H.; Binothman, N.; Al Kashgry, N.A.T.; Tahir, M.M. Comparative gene enrichment analysis for drought tolerance in contrasting maize genotype. *Genes* **2022**, *14*, 31. [CrossRef] [PubMed]

118. Liu, S.; Li, C.; Wang, H.; Wang, S.; Yang, S.; Liu, X.; Yan, J.; Li, B.; Beatty, M.; Zastrow-Hayes, G. Mapping regulatory variants controlling gene expression in drought response and tolerance in maize. *Genome Biol.* **2020**, *21*, 163. [CrossRef] [PubMed]

119. Dong, A.; Zenda, T.; Liu, X.; Wang, Y.; Li, J.; Yang, Y.; Liu, S.; Duan, H. Transcriptome analysis of drought tolerance and utility assessment of *DnaJ* gene expression as a potential index for drought resistance evaluation in maize. *Euphytica* **2022**, *218*, 136. [CrossRef]

120. Nan, W.; Liang, L.; Gao, W.-W.; WU, Y.-b.; YONG, H.-j.; Weng, J.-F.; LI, M.-S.; Zhang, D.-G.; Hao, Z.-F.; LI, X.-H. Transcriptomes of early developing tassels under drought stress reveal differential expression of genes related to drought tolerance in maize. *J. Integrat. Agricul.* **2018**, *17*, 1276–1288.

121. Zhang, Y.; Soualihou, S.; Li, J.; Xu, Y.; Rose, R.J.; Ruan, Y.-L.; Li, J.; Song, Y. Transcriptome analysis of maize pollen grains under drought stress during flowering. *Crop Past. Sci.* **2022**, *73*, 1026–1041. [CrossRef]

122. Liu, S.; Zenda, T.; Dong, A.; Yang, Y.; Wang, N.; Duan, H. Global transcriptome and weighted gene co-expression network analyses of growth-stage-specific drought stress responses in maize. *Front. Gen.* **2021**, *12*, 645443. [CrossRef]

123. Song, K.; Kim, H.C.; Shin, S.; Kim, K.-H.; Moon, J.-C.; Kim, J.Y.; Lee, B.-M. Transcriptome analysis of flowering time genes under drought stress in maize leaves. *Front. Plant Sci.* **2017**, *8*, 267. [CrossRef]

124. Hao, L.-Y.; Liu, X.-Y.; Zhang, X.-J.; Sun, B.-C.; Cheng, L.; Zhang, D.-F.; Tang, H.-J.; LI, C.-H.; Li, Y.-X.; Shi, Y.-S. Genome-wide identification and comparative analysis of drought related genes in roots of two maize inbred lines with contrasting drought tolerance by RNA sequencing. *J. Integrat. Agricul.* **2020**, *19*, 449–464. [CrossRef]

125. Brookes, G.; Barfoot, P. Environmental impacts of genetically modified (GM) crop use 1996–2015: Impacts on pesticide use and carbon emissions. *GM Crops Food* **2017**, *8*, 117–147. [CrossRef]

126. ISAAA. *Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years*; ISAAA: Ithaca, NY, USA, 2017; Volume 53.

127. Zeng, T.; Zhang, D.; Li, Y.; Li, C.; Liu, X.; Shi, Y.; Song, Y.; Li, Y.; Wang, T. Identification of genomic insertion and flanking sequences of the transgenic drought-tolerant maize line "SbSNAC1-382" using the single-molecule real-time (SMRT) sequencing method. *PLoS ONE* **2020**, *15*, e0226455. [CrossRef]

128. Tao, Y.-B.; He, L.-L.; Niu, L.-J.; Xu, Z.-F. Isolation and characterization of an ubiquitin extension protein gene (JcUEP) promoter from Jatropha curcas. *Planta* **2015**, *241*, 823–836. [CrossRef] [PubMed]

129. Xu, Z.; Wang, M.; Guo, Z.; Zhu, X.; Xia, Z. Identification of a 119-bp promoter of the maize sulfite oxidase gene (*ZmSO*) that confers high-level gene expression and ABA or drought inducibility in transgenic plants. *Int. J. Mol. Sci.* **2019**, *20*, 3326. [CrossRef] [PubMed]

130. Li, B.; Wei, A.; Song, C.; Li, N.; Zhang, J. Heterologous expression of the *TsVP* gene improves the drought resistance of maize. *Plant Biotech. J.* **2008**, *6*, 146–159. [CrossRef] [PubMed]

131. Li, H.; Han, X.; Liu, X.; Zhou, M.; Ren, W.; Zhao, B.; Ju, C.; Liu, Y.; Zhao, J. A leucine-rich repeat-receptor-like kinase gene SbER2–1 from sorghum (Sorghum bicolor L.) confers drought tolerance in maize. *BMC Gen.* **2019**, *20*, 737. [CrossRef] [PubMed]

132. Nguyen, T.X.; Sticklen, M. Barley *HVA1* gene confers drought and salt tolerance in transgenic maize *Zea Mays* L. *Adv. Crop Sci. Tech.* **2013**, *1*, 1–8. [CrossRef]

133. Chen, Z.; Liu, Y.; Yin, Y.; Liu, Q.; Li, N.; Li, X.; He, W.; Hao, D.; Liu, X.; Guo, C. Expression of *AtGA2ox1* enhances drought tolerance in maize. *Plant Growth Reg.* **2019**, *89*, 203–215. [CrossRef]

134. Gillani, S.F.; Rasheed, A.; Majeed, Y.; Tariq, H.; Yunling, P. Recent advancements on use of CRISPR/Cas9 in maize yield and quality improvement. *Not. Bot. Horti Agrobot. Cluj-Nap.* **2021**, *49*, 12459. [CrossRef]

135. Čermák, T.; Baltes, N.J.; Čegan, R.; Zhang, Y.; Voytas, D.F. High-frequency, precise modification of the tomato genome. *Gen. Biol.* **2015**, *16*, 232. [CrossRef]

136. Gao, H.; Smith, J.; Yang, M.; Jones, S.; Djukanovic, V.; Nicholson, M.G.; West, A.; Bidney, D.; Falco, S.C.; Jantz, D. Heritable targeted mutagenesis in maize using a designed endonuclease. *Plant J.* **2010**, *61*, 176–187. [CrossRef]

137. Li, J.-F.; Norville, J.E.; Aach, J.; McCormack, M.; Zhang, D.; Bush, J.; Church, G.M.; Sheen, J. Multiplex and homologous recombination–mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. *Nat. Biotech.* **2013**, *31*, 688–691. [CrossRef]

138. Liang, Z.; Zhang, K.; Chen, K.; Gao, C. Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. *J. Gen. Gen.* **2014**, *41*, 63–68. [CrossRef] [PubMed]

139. Pan, Z.; Liu, M.; Zhao, H.; Tan, Z.; Liang, K.; Sun, Q.; Gong, D.; He, H.; Zhou, W.; Qiu, F. *ZmSRL5* is involved in drought tolerance by maintaining cuticular wax structure in maize. *J. Integrat. Plant Biol.* **2020**, *62*, 1895–1909. [CrossRef] [PubMed]

140. Li, X.D.; Gao, Y.Q.; Wu, W.H.; Chen, L.M.; Wang, Y. Two calcium-dependent protein kinases enhance maize drought tolerance by activating anion channel *ZmSLAC1* in guard cells. *Plant Biotech. J.* **2022**, *20*, 143–157. [CrossRef] [PubMed]