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Engineering Drought Tolerance in Crops Using CRISPR Cas systems

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Abstract

Drought stress is one of the most considerable threats to global agricultural food security, causing yield losses worldwide. Therefore, the search for effective genetic and molecular methods for developing cultivars that are tolerant or resistant to harsh environments has been more intensive over the last decades. Apart from time-consuming conventional breeding techniques, biotechnologists are now investigating modern genome editing tools for engineering tolerance and resistance to various biotic and abiotic stresses in crops. Various genetic engineering techniques such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were developed based on the discovery of the DNA structure. However, these methods have limitations, with ZFNs being prone to errors due to their limited base pair recognition, and TALENs requiring a complex protein engineering process and struggling to cleave methylated DNA. In recent years, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) and its alternatives have gained popularity in plant biotechnology. Out of the genome editing techniques mentioned earlier, CRISPR/Cas9 is becoming more popular because it's faster and easier to use. Given that drought is now a significant threat to global agriculture due to the drying of arable lands, this review focuses on how we can use CRISPR genome editing to enhance crop tolerance to drought stress and explores its future potential.

Keywords

CRISPR/Cas9; drought; DNA; endonuclease; gene; genome editing; sgRNA

Introduction

In recent years, the global scenario of drought has emerged as a pressing concern, with its far-reaching effects rippling across various regions. Scientists and breeders have acknowledged drought as an environmental hazard. It is defined as a long period of precipitation decline, such as a season or a year, and happens in practically all climatic zones, including both high and low-rainfall places (1). This phenomenon has cast a shadow over agricultural landscapes worldwide, leading to significant consequences for both crop productivity and food security (1, 2). As we investigate this critical issue, we will first investigate the extent and geographical areas affected by drought, backed up by credible citations. Following that, we will delve into quantitative data to shed light on how drought affects crop productivity and, ultimately, its implications for global food security, all supported by rigorous research and factual evidence (3).

Plants acquired morphological and physiological adaptations, as well as signaling pathways that evoke biochemical and molecular processes to survive various stress conditions, to tolerate these harsh climatic conditions (4). Among these stresses, drought is an unavoidable element that exists in a variety of situations with no discernible bounds and no warning, hindering plant biomass production, quality, and energy (5). Drought is the climatic condition when the water level in the soil is low or absent. In this situation, the plants are not able to grow and develop fully and in this case, not only the profits of farmers but also the economy of the entire country suffer significantly. For instance, The plants confront challenges to their full growth and development in this scenario. As a result, both farmer profits and the national economy suffer significant consequences. For decades, the methods of conventional breeding have been quite successful in creating drought-tolerant wheat, rice, maize, soybean, and other crops, yet most of those crops did not possess high productivity traits concurrently (6). At this point, transgenic methods can implement and combine all the necessary traits in one single implement and combine all the necessary traits in one crop line.

CRISPR/Cas9 is like a genetic tool that starts by breaking the DNA in a specific spot. Then, there are two ways the cell can fix it: one is like gluing the broken ends together (non-homologous end-joining), and the other is like swapping out puzzle pieces (homologous recombination). While the cell is fixing things, it can also make changes to the DNA. Sometimes, when gluing things together, some pieces are lost or added (deletions and insertions), and when swapping pieces, it can change a few puzzle pieces (base substitutions) (7) (Fig.1). CRISPR/Cas9 system is the most widely used in a bacterial immune system against the invasion of foreign DNA (8). This editing mechanism comprises a Cas9 endonuclease and a single guide RNA (sgRNA) molecule of about 20 nucleotides complementary to the DNA of the target gene (9). This RNA confers target specificity, and the Cas9 enzyme makes a double-stranded break on the target DNA. Subsequently, these breaks are repaired by the plant DNA repair system, leading to the emergence of new mutations, gene knockout, or loss of protein function (10). In recent years, this highly efficient genome editing tool has been frequently used in different fields of life sciences – in test systems and biosensors (11,12); in biomedicine and drug

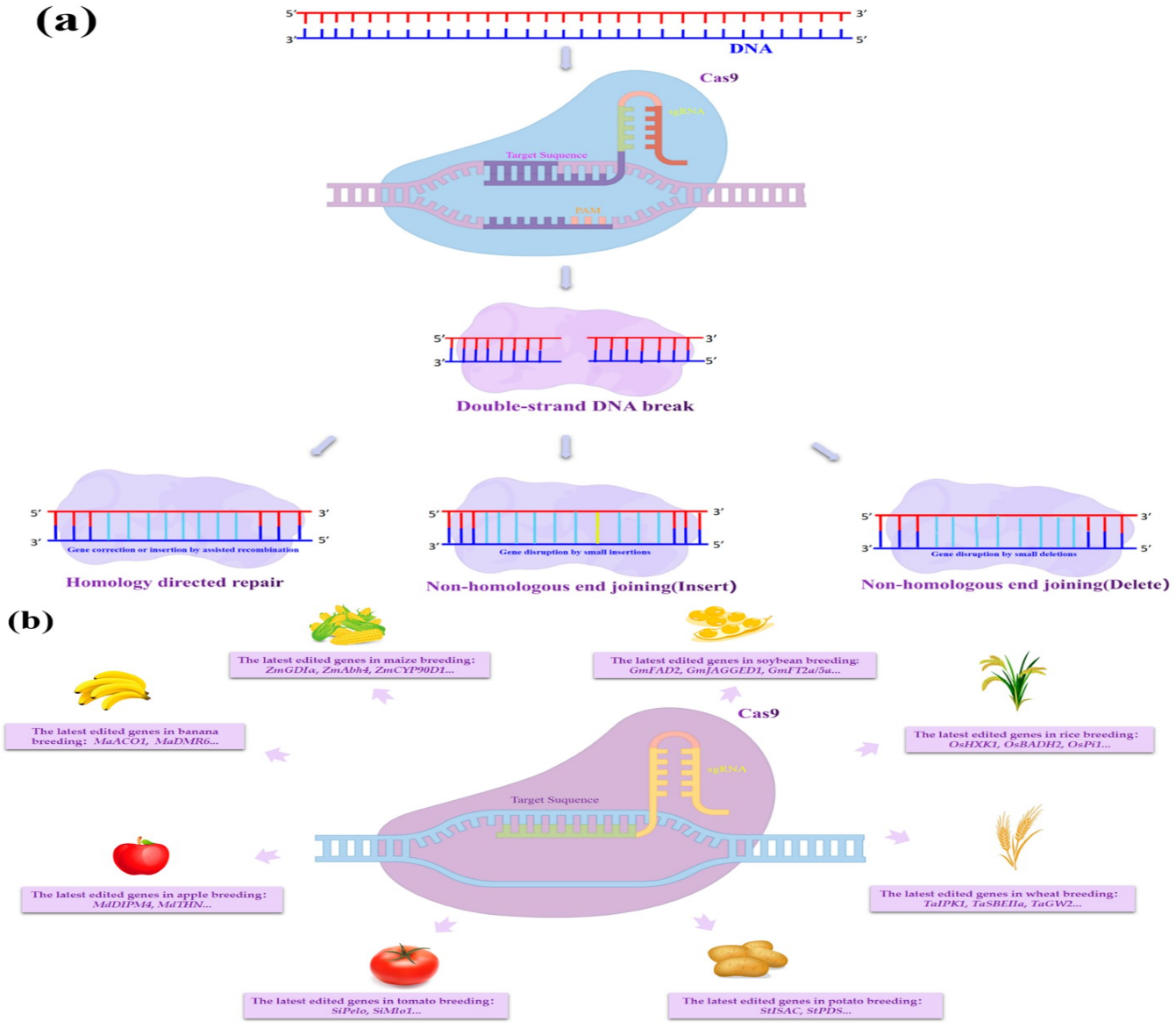

Fig. 1. CRISPR/Cas9 gene-editing technique and genome targeting: (a) CRISPR/Cas9-mediated DNA repair mechanism; (b) CRISPR/Cas9 targets numerous plant genomes. The figure was re-used from (7) with the permission of MDPI.

delivery (13,14); in plant breeding (8,10,15,16). Considering the undeniable success of the CRISPR/Cas9 system, several other types of Cas9 enzymes from other bacteria were introduced, such as SaCas9 (*Staphylococcus aureus* Cas9), StCas9 (*Streptococcus thermophiles* Cas9), and NmCas9 (*Neisseria meningitidis* Cas9) (17, 15, 16). The implementation of the CRISPR/Cas9 genome editing technique has significantly advanced global plant breeding (19). Based on the CRISPR system, monocot and dicot crop varieties were genetically engineered with improved yield traits, resistance to viral and bacterial diseases, salinity, heavy metals, extreme temperatures, drought, and other agronomically important traits (17). Application of the CRISPR/Cas9 method in agriculture opens new opportunities to provide global agriculture with transgene-free crops resistant/tolerant to different stresses (16); this review describes the application of this technology for engineering various crops tolerant to drought stress, investigation of the role of some genes in drought stress response; as well as our outlook on its future perspectives.

CRISPR-mediated improvement of crop drought tolerance

Several genetic engineering strategies targeting drought tolerance have been demonstrated thus far. These strategies represent the insertion of drought-resistance genes (14); alteration (12), gene silencing, and overexpression (of transcription factors, genes, and hormone pathways responsible for plant adaptation to drought (16, 19). Overexpression of genes and transcription factors participating in drought signaling may enhance plant drought tolerance (20). Drought tolerance can also be performed by silencing drought-sensitive genes and negatively regulated genes. For example, the CRISPR system was used to generate mutants in OPEN STOMATA 2 (*OST2*) gene coding a plasma membrane H+ ATPase enzyme responsible for stomatal activity in Arabidopsis. In the condition of dehydration, abscisic acid suppresses ATPase-mediated stomatal conductance (21). Using the CRISPR/Cas9 system combined with a truncated sgRNA, two mutations at the *OST2* locus with high efficiency (>32%) and no off-target mutations were created. The evaluation of ost2 mutants revealed an enhanced rate of stomatal closure and a lower rate of water loss compared with wild-type (19).

Several molecular studies have revealed that ABA functions as a central regulator of drought stress tolerance among abscisic acid (ABA), auxin, and brassinosteroid phytohormone signaling pathways (16, 22, 23, 24). Considering that *Arabidopsis thaliana* ENHANCED RESPONSE TO ABA1 (*ERA1*) gene regulates ABA signaling pathway and response to dehydration stress, Ogata and colleagues created frameshift mutations to explore *ERA1* gene function in rice plants using CRISPR/Cas9 system (25). osera1 mutant plants illustrated enhanced drought stress response, suggesting using this gene to improve drought tolerance in rice. Sucrose non-fermenting 1-related kinase 2 (SnRK2) is a family of plant-specific kinases regulating abscisic acid (ABA)-dependent abiotic stress signaling pathway. Particularly, the *SAPK9* gene, one of the rice *SnRK2s*, has been considered to be a key drought stress regulator in rice (26). Hence, the CRISPR/Cas9 system was applied to develop SAPK9 loss-of-function rice phenotypes. The results revealed that sapk2 mutants showed higher sensitivity to dehydration stress than wild-type plants, indicating that the *SAPK2* gene is one of rice's genes responsible for drought tolerance (27). These results suggest that all the genes participating in ABA signaling pathways are attractive candidates to improve plant tolerance to drought.

It is known that ethylene hormone plays an important role in various plant development mechanisms as well as in plant drought and heat tolerance. Under drought stress, the suppression of ABA induces the expression of ethylene-responsive factors (*ERFs*), and their overexpression has been associated with plant abiotic stress tolerance (16). The studies report that the family of auxin-regulated genes involved in organ size (*ARGOS*) genes negatively regulates the ethylene signaling pathway and confers higher yield under drought conditions (28, 29). Particularly, the maize *ARGOS8* gene negatively regulates ethylene response, which is one of the most important phytohormones regulating plant abiotic stress response (30). However, the wild-type *ARGOS8* gene expression is relatively low; researchers used CRISPR/Cas technology to either replace the native *ARGOS8* promoter with the *GOS2* promoter, which gives the *ARGOS8* gene higher expression, or to insert the *GOS2* promoter. The evaluation showed mutant lines had higher yields than wild-type ones under drought conditions (29). Kim et al. identified the upregulation of two genes related to drought stress response: wheat dehydration-responsive element binding protein 2 (*TaDREB2*) and wheat ethylene-responsive factor 3 (*TaERF3*) (31). It has been previously reported that overexpression of these genes in Arabidopsis, wheat, and barley increases plant drought tolerance (32, 33). The expression of CRISPR/Cas9 edited genes in wheat protoplast was evaluated under dehydration stress by qRT -PCR, and the analysis revealed that both *TaDREB2* and *TaERF3* are positive regulators of the drought stress response. These findings indicate that state-of-the-art technology could not only allow the creation of drought-resistant cultivars effectively but also provide higher yields to global agriculture.

Some studies were conducted to examine the role of several genes that do not directly regulate drought stress signaling pathways (34, 35, 36). For instance, the CRISPR/Cas9 system was applied to create tomato non-expressers of pathogenesis-related gene 1 (npr1) mutant lines (16). Although this gene participates in the plant defense system, its alteration induced changes in plant drought response (34). The mutants with CRISPR/Cas9 mediated NPR1 loss of function demonstrated reduced tolerance to dehydration stress compared to wild-type tomato plants. The lateral organ boundaries domain (*LBD*) gene family plays a crucial role in plant organ development. To reveal the function of the tomato *LBD40* gene in drought stress response, Liu and his colleagues knocked out it using CRISPR/Cas9. Evaluation tests

revealed that LBD40 knockout mutants had lower water loss rates under dehydration stress compared to wild-type tomato plants (35). Same with auxin response factors (*ARFs*) - proteins responsible for various processes in plant development; however, their function in water deficit conditions was not clear. Thus, *ARF* genes were knocked out in tomato using CRISPR/Cas9. Mutant plants with loss of *SlARF4* function were more resistant to dehydration stress and had better rehydration ability (36). Therefore, down-regulation of such genes as *NPR, LBD40* or *ARFs*, and many others, which, apart from their main functions, are also responsible for drought response, could be capable of producing drought-tolerant crop varieties.

Moreover, a significant feature in drought stress management is creating plants with wider or rolled leaves, with reduced stomatal density and other morphological traits contributing to lower water loss rates and higher yields in water-deficit areas. Thus, semi-rolled leaf 1 (*SRL1*) and *SRL2* genes, which control various leaf phenotypes in rice, were modified using CRISPR/Cas9 technology to produce rice plants with rolled leaves (37). Mutant plants had several improved leaf traits including semi-rolled leaves, resulting in higher survival rates under dehydration conditions than wild-type plants. Another experiment was conducted to explore the function of drought and salt tolerance gene (*OsDST*) encoding a zinc finger transcription factor in indica rice cultivar (38). CRISPR/Cas9 mediated deletion of 184-305 region of *OsDST* gene led to wider leaves and lower density of stomas, which, in turn, improved plant tolerance to dehydration stress.

Conclusion and Future Prospects

Drought, being one of the other environmental stress factors, is threatening the world's food production. The condition of dehydration affects all stages of plant development at biochemical, morphological, and physiological levels significantly decreasing crop productivity. Considering that millions of people suffer from food deficiency, scientists all over the world are striving to create crops resistant to manifold biotic and abiotic stress. Since transgenic plants are not widely accepted, new effective genome editing techniques have been developed, with the most popular of them being CRISPR/Cas9. To date, this genomic tool has proven to be rapid and accurate. Particularly, it has been found very effective in identifying, modifying, and delivering drought stress-related genes to plants. Over the past decade, many crops such as maize, wheat, rice, soybean, tomato, and others were CRISPR/Cas9 genome edited on several agronomically important traits. There are still some limitations to its unlimited application in plant improvement, with one of them being the cultivation and regeneration of genome-edited plants and the second one the control of off-target mutations. However, taking the opportunities of modern genomics and plant breeding into account, we consider those problems can now be overcome.

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Authors' contributions

LK and MA – wrote the manuscript; MM, AY, BM, NO, ZB, AM -collected and analyzed world literature, and prepared figures; MA – critically read and edited the manuscript, drafted subsections; ZB and IA -rigorously edited and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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