IMPROVING CROP DROUGHT TOLERANCE THROUGH GENOME EDITING TECHNOLOGY

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Abstract

The increase in the frequency and intensity of drought due to recent climate changes significantly affects agriculture. Addressing this challenge requires concerted research efforts to develop new crop varieties with increased tolerance to this abiotic stress. Genome editing technology is the latest approach in this direction. This review summarizes recent applications of genome editing in improving drought tolerance in various crops. The CRISPR/Cas9 technique is the newest and most advanced genome editing tool that gives exceptional versatility to develop improved varieties, thus facilitating their cultivation in drought conditions without compromising production.

Key words**: **CRISPR/Cas9, drought, gene of interest, genome-edited crops

INTRODUCTION

Researchers believe that by the end of 2050 there will be 10 billion inhabitants, therefore, to ensure food security, it will be necessary to increase crop production by over 60% (Jaganathan et al., 2018). According to experts, by the end of 2050, about half of the cultivated land globally will suffer from a severe water shortage (Mushtaq et al., 2018).

Although insufficient rainfall is usually the main stressor caused by drought, evaporation of water from the soil caused by high temperatures and wind further aggravates drought. Current climate change is affecting food production by inducing large yield losses (Soare et al., 2019; Nițu et al., 2022; Dunăreanu et al., 2023; Constantinescu et al., 2024).

The use of traditional breeding methods, based mainly on spontaneous and artificially induced mutations, demonstrated the possibility of increasing crop production and tolerance of drought. However, these traditional methods have a major drawback, namely the long time required to obtain improved varieties.

The development of genetic engineering has allowed the modification of the plant's genome and their faster development.

The first genetically modified plants obtained by introducing foreign DNA into the genome of a plant were cultivated in 1996 on 1.7 million hectares. This technology has developed very rapidly, reaching 190.4 million hectares cultivated with genetically modified plants in 2019 (ISAAA, 2019), but their commercialization and use in several countries have been limited by ethical and legislative issues related to safety and environmental health (Bonea, 2023; 2024).

At the beginning of the 21st century, researchers created and used DNA-based markers making it possible to generate new varieties of agricultural crops and also to develop genomic editing technology.

Genome editing or gene editing is a new technology that uses artificially created sequence-specific nucleases (SSNs) to introduce targeted and precise mutations into crops.

Compared to transgenic crops, genome-edited crops have only a few nucleotide changes similar to the variations found in naturally occurring populations.

Because no foreign DNA is introduced into ribonucleoproteins, mutants developed by genome editing, particularly by the CRISPR/Cas technique, are not transgenic organisms and are not subject to GMO regulations (Joshi et al., 2020).
The purpose of this review is to explore the use of CRISPR/Cas9-mediated genome editing technology in the development of drought-tolerant crops that can maintain their productivity under this stress, thereby ensuring food security.

MATERIALS AND METHODS

In this work, a systematic search of articles from several databases, namely PubMed, SinceDirect, ResearchGate, Google Scholar, etc., was carried out, in order to identify some relevant and up-to-date references for the applications of genome editing in the improvement of crops for tolerance to drought. The adverse effects of drought on plants were also briefly presented.

RESULTS AND DISCUSSIONS

Harmful effects of drought on plants

As a result of current climate change, drought has become one of the most uncontrolled and unpredictable abiotic stressors that continuously limit crop production.
This situation is exacerbated by the increase in food demand due to population growth, thus underscoring the need for agricultural innovations.
The adverse effects of drought on plants shown in summary (Figure 1) are manifested through physiological, morphological, biochemical and molecular disturbances (Nadeem et al., 2019; Sami et al., 2021).

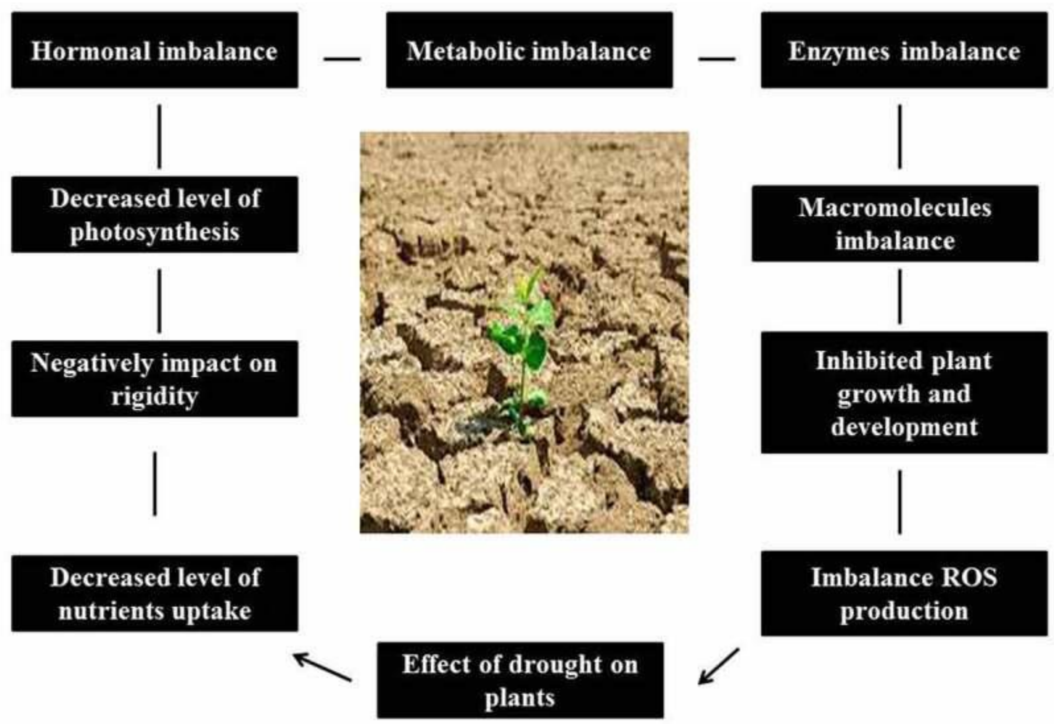

Figure 1. Effect of drought stress on plants
Source: Sami et al. (2021)

The lack of water in the soil modifies many vital processes in the plant inducing an osmotic stress. This osmotic stress leads to the excessive accumulation of reactive oxygen species (ROS) that trigger cellular oxidative damage, inhibit the activity of many enzymes, and disrupt cellular redox homeostasis (Abd-El-Mageed et al., 2023).
The most used SSNs, respectively CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats - associated endonuclease Cas9), TALENs (transcriptional activator-like effector

nucleases) and ZFNs (zinc finger nucleases) have shown the possibility of providing target gene modifications in plants (Figure 1).

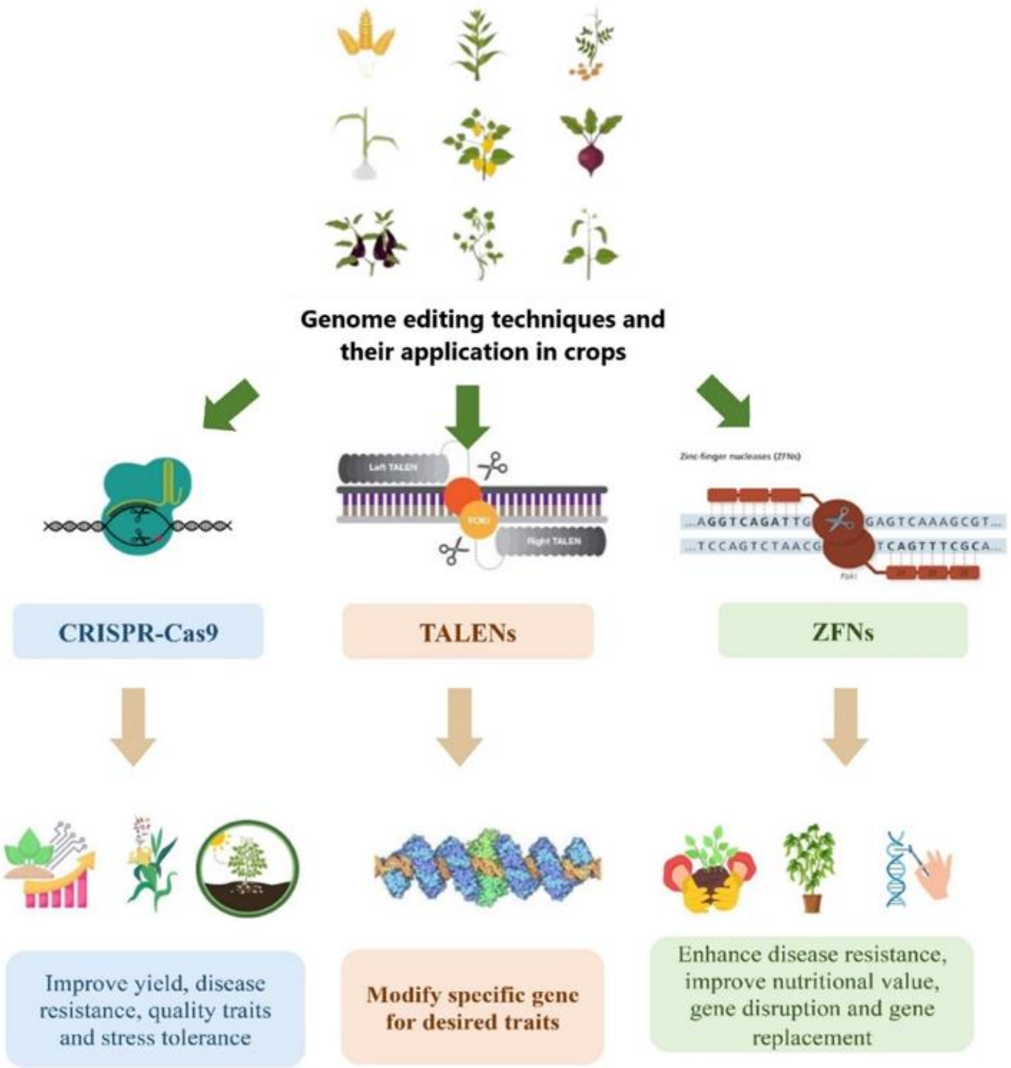

Figure 2. Genome editing in crops: editing techniques and their applications.
Source: adapted after Daniel et al. (2023)

Among all the tools, CRISPR-Cas9 is the most productive, accurate and fastest in improving tolerance to biotic and abiotic stresses. With this tool, genes are inserted by viral infection, agro-infiltration or pre-assembled Cas9-sgRNA protein ribonucleoprotein transformation into crops without transgenic imprinting (Mohamed et al., 2024).

CRISPR/Cas9-mediated genome editing for improved drought tolerance

As a result of the dynamic and complex nature of drought stress, genomic adaptation has proven to be the only approach to achieve drought tolerance (Sami et al., 2021).

After the discovery of genome editing tools, research was designed to edit genes involved in drought tolerance pathways, but also to increase public acceptance of genome-edited crops.

The efficiency, simplicity of SSN design and the ability to target multiple sites have made CRISPR/Cas technology a widely accepted genome editing tool by researchers (Joshi et al., 2020), while ZFN and TALEN have not have been widely used due to more complex procedures and lower efficacy (Zhang et al., 2018).

Recent studies have revealed the essential role of CRISPR/Cas9-mediated genome editing as a means to develop novel genotypes with drought tolerance in various crops.

Targeting genes of interest in vegetables (soybean, tomato, chickpea, etc.), cereals (rice, corn, wheat, etc.) and other crops using CRISPR-Cas9 can achieve various improvement objectives, drought tolerance and including increased production, through these improvements contributing to addressing global food security concerns.

The applications summarized in Table 1 represent the most recent successes in obtaining new cultivars with drought tolerance.

Table 1. Examples of genes of interest targeted in genome editing technology (via CRISPR/Cas9) conferring improved drought tolerance for various crops

Crops	Gene of interest	References
Tomato *(Solanum lycopersicum*)	*SlLBD40*	Liu et al. (2020)
	SlARF4	Chen et al. (2021)
	SlGT30	Lv et al. (2024)
	SlDEA1	Saikia et al. (2024)
Soybean (*Glycine max*)	*GmMYB118*	Zhong et al. (2022)
	GmHsps_p23-like	Zhang et al. (2022)
Chickpea (*Cicer arietinium*)	*At4CL, AtRVE7*	Badhan et al. (2021)
Potato *(Solanum tuberosum*)	*StFLORE*	Gonzales et al. (2021)
Grapevine (*Vitis vinifera*)	*VvEPFL9-1*	Clemens et al. (2022)
Rice (*Oryza sativa*)	*OsPYL9*	Usman et al. (2020)
	OsERA1	Ogata et al. (2020)
	OsPUB67	Qin et al. (2020)
	OsDST	Santosh-Kumar et al. (2020)
	OsADR3	Li et al. (2021)
	OsWRKY5	Lim et al. (2022)
	OsSAP	Park et al. (2022)
	OsEF1A	Gu et al. (2023)
	OsPUB7	Kim et al. (2023)
	OsSAPK3	Lou et al. (2023)
Maize (*Zea mays*)	*ZmMYB114*	Liu et al. (2020)
	ZmSRL5	Pan et al. (2020)
	ZmPYL8	Xu et al. (2020)
	ZmLRT	Zhang et al. (2022)
	ZmTCP14	Jiao et al. (2023)
	ZmGA20ox3	Liu et al. (2023)
Wheat (*Triticum aestivum*)	*TaSal1*	Abdallah et al. (2022)
	TaGW2	Li et al. (2024)
Rapeseed (*Brassica napus*)	*BnaA6.RGA*	Wu et al. (2020)
	BnPUB18 and BnPUB19	Lingu et al. (2023)
Tabacco (*Nicotiana tabacum)*	*NtPOD63 L*	Xu et al. (2023)

CONCLUSIONS

Drought tolerance is an important trait that influences the yield of most crops. Agriculture is currently facing frequent droughts as a result of climate change, so targeted and rapid breeding methods are urgently needed to accelerate plant improvement.

In recent decades, genome editing techniques have made significant contributions to alleviating this abiotic stress. CRISPR/Cas9 genome editing technique has immense potential in developing crops with improved resistance, proving to be an efficient tool in maintaining food security.

In addition, crops developed using CRISPR/Cas9 can be more easily accepted by society because they do not contain foreign genetic material transferred from other organisms.

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