

Advances in CRISPR for Abiotic Stress Response in Cash Crops and Application of AI in it

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Abstract:

Abiotic stresses are adverse effects on organisms caused by environmental factors (temperature, light, moisture, salinity, heavy metals, etc.), which result in changes of organisms at the physiological, biochemical and molecular levels and affect their growth and development. Abiotic stress is one of the most important environmental stresses faced by organisms and an important factor affecting agricultural production and ecosystem stability. Cash crops are of great importance to national production and people's life. At present, with the development of gene editing technology, some progress has been made in applying CRISPR/Cas9 technology to the study of abiotic stress resistance response in cash crops, but a systematic summary is lacking. Meanwhile, AI technology, which is a technology that uses computer systems to simulate and extend human intelligence, can provide new perspectives and methods for the field of plant science and improve the efficiency of design, prediction, optimisation and validation of CRISPR technology. In this review, we introduced the research progress of CRISPR technology in the response of economic crops to abiotic stresses and the application of AI in it, and analysed the advantages and shortcomings of AI technology in assisting CRISPR technology. This review provided new perspectives and methods for the response of plants to abiotic stresses, and provided new ideas and solutions for dealing with the challenges of agriculture, such as global climate change, pests and diseases.

Keywords:

Cash Crops, Gene Editing, Crispr Cas9, Resistance to Abiotic Factors, AI

1. Introduction

In 1973, the first bacteria that could resist antibiotics through gene editing was born, opening the way for gene editing technology. In 2012, with the discovery of the CRISPR-Cas9 protein, gene editing technology entered a phase of rapid development. As an immune boon for bacteria against viral attacks that evolved during the evolution of prokaryotes, the CRISPR was found to be able to excise the harmful gene loci of viruses, and after the discovery of the Cas protein, the gene

editing technology gradually matured. At present, CRISPR/Cas technology has been widely used in gene editing due to its higher precision and better specificity recognition compared to prokaryotic gene editing. Currently, gene editing technology has significant potential, although the side effects of gene editing are still poorly understood.

CRISPR/Cas gene editing technology has great potential for development in the area of abiotic stress resistance. Effective gene editing has begun to be applied on a large scale in some cash crops, and the technology plays an outstanding role in terms of flexibility, efficiency, and production cost. As the role of AI in CRISPR gene editing is becoming more and more significant, gene editing technology combined with the application of AI to study the role of abiotic stress resistance in cash crops has shown great potential.

2. Effects of Abiotic Stresses on Cash Crops

Abiotic factors, including extreme weather, flooding, salinity, ultraviolet radiation, windy weather and mineral deficiencies, have a serious impact on crop production. According to statistics, salinity in irrigated agricultural land has increased by 37 per cent over the past two decades (1990-2013). In addition, droughts have become more severe due to global warming [1]. Increased seeding density in modern agriculture has also increased competition for water and nutrients from cash crops [2]. With global warming and the effects of the hole in the ozone layer, there has been a significant increase in the intensity of ultraviolet light at the Earth's surface, despite partial evidence that the ozone space is being repaired [3,4]. In recent years, global warming has led to increased climate instability, and there is an urgent need for cash crops to become more resistant to abiotic stresses. Gene editing technology is becoming a breakthrough point to address this issue.

2.1. Drought stress

Drought stress is one of the most serious abiotic stresses affecting crop yield [5]. Drought stress leads to inadequate water uptake in crops, affecting photosynthesis and plant growth [5]. The use of AI-assisted CRISPR technology can improve crop tolerance to drought stress, such as increasing root development, regulating osmotic pressure homeostasis, activating stress signalling pathways, etc. [5]. For example, Shi et al. knocked out a gene *ZmRAVL1*, which negatively regulates root growth, in maize using CRISPR technology, which resulted in a significant increase in root length and surface area of transgenic maize, thus improving maize's resistance to drought stress [6].

2.2. Saline and alkaline stress

Salinity stress is defined as the presence of excessive soluble salts in the soil, which leads to the obstruction of water and nutrient uptake by crops and affects their physiological metabolism and growth and development. Using AI-assisted CRISPR technology, we can improve crop tolerance to salinity stress, such as reducing root salt uptake, increasing root salt excretion, regulating osmotic pressure balance, and protecting cell membrane stability. For example, Hoang et al. used CRISPR technology to knock out a gene involved in salt transport, *OsHKT1*, in rice; this resulted in a significant reduction in the root salt content of transgenic rice, which improved the resistance of rice to salinity stress [7].

2.3. Heat stress

With today's irreversible global warming trend, high temperature stress is becoming an increasingly critical stressor. When the ambient temperature exceeds the crop's optimal growth temperature, this can lead to impaired physiological metabolism and growth and development, which in turn affects the crop's pollen viability and fruit set. The use of AI-assisted CRISPR technology can improve crop tolerance to high temperature stress, such as increasing the expression of heat stress proteins, protecting the stability of the photosynthetic system, and regulating hormone balance. For example, Shen et al. knocked out a gene ICE1 involved in cold stress signal transduction in *Arabidopsis thaliana* using CRISPR technology, resulting in transgenic *Arabidopsis thaliana* showing higher pollen viability and fruit set under high temperature conditions [8].

2.4. Low-temperature stress

Low-temperature stress leads to physiological and metabolic disorders such as reduced cell membrane fluidity, decreased enzyme activity, and impaired signal transduction in plants, thus affecting plant growth, development, and yield [9]. In order to improve the resistance of plants to low-temperature stress, scientists knocked out a negative regulator OsMYB4 gene involved in cold signal transduction in rice using CRISPR/Cas9 technology, making it more sensitive to low-temperature stress, and analysed its transcriptome changes by deep learning methods, and discovered some new genes and pathways related to cold response. These new genes and pathways provide new targets for further improving cold resistance in rice [10].

3. Genetic Loci for Resistance to Abiotic Threats in Selected Cash Crops

In tomato, the SLNPR1 gene was experimentally shown to be regulatory for drought, responding to drought stress by overriding cell membrane oxidation and affecting gene transcription under stress. After compiling the gene using CRISPR-Cas9, mutants of SLNPR1 were found to develop drought intolerance, demonstrating that it mediates drought regulation [11]. In addition to this, tomato SIU6-2P4 can also drive sgRNA transcription and has enabled endogenous gene editing [12]. For the potato crop, the knockout of the potato S-RNase gene by CRISPR-Cas9 can transform breeding from a tetraploid clonal propagation platform to a self-compatible diploid breeding system. Enhanced sexual reproduction in potato allows for the multiplication of potato seeds several times over, ensuring the potential for genetic gain, allowing the potato crop to secure yields in harsher environments' which in turn promotes resistance to abiotic stress responses [13].

For other cash crops such as cauliflower bean relatives, due to the prevalence of drought stress, leaf drought tolerance candidate genes are significantly expressed in response to abiotic stresses during drought onset in cauliflower bean [14].

Under salt stress conditions, certain plants excrete excess salts accumulated in the body through salt glands, thus avoiding salt damage. Studies have shown that some plant bodies can excrete salt out of the body through salt glands [15], and some plants reduce salt uptake by reducing the permeability of root cells to Na ions. The thicker cuticle and waxy layer on the leaf surface of plants can protect the plant body from or reduce the damage caused by low temperature stress. Under normal conditions, the reactive oxygen radicals in plants are stabilised within a certain range and do not

cause damage to plants, but when plants are subjected to abiotic stress, the content of free radicals in the body is disturbed, and then the plants themselves recruit the appropriate antioxidant enzymes to resist the stress damage. These antioxidant enzymes mainly include superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). Stress increases the activity of these enzymes, effectively scavenging reactive oxygen radicals and reducing the extent of cellular damage. In addition, the plant body can produce osmoregulatory substances in response to external environmental stress. Osmoregulatory substances include inorganic ions and amino acids, among which proline is the most common. Proline is a water-soluble amino acid, which reduces the osmotic potential within the cell and prevents cell water loss. Betaine is also a better osmoregulator, maintaining the structure and function of protein molecules to remain stable. These regulatory effects on gene expression are inevitably the result of the combined action of multiple genes, and studies have shown that the amino acid activating enzymes have been found in abundance in mung bean and higher plants [16], while betaine, as a secondary product of plant metabolism, also enhances the plant's ability to cope with heavy metal contamination, and the study demonstrated that under chromium metal toxicity, betaine application significantly increased the root and leaf nutrient contents of two varieties' root and leaf nutrient content, which contributed to better growth and better tolerance of heavy metal chromium treated plants, thus reducing the uptake and translocation of heavy metal cadmium [17].

4. Advantages and Limitations of Using CRISPR for Gene Editing

Traditional breeding methods using crossbreeding and natural breeding are poorly targeted, genetically unstable and take a long time to breed. At present, gene editing of some cash crops is a more feasible way, using CRISPR gene editing, through sgRNA to guide the nucleic acid endonuclease Cas to the target site, cut off the DNA double-stranded, based on this to achieve the goal of cutting and editing of genes. This approach can make the targeting action more precise, directed to change the host gene sequence, and target screening more efficient. However, due to the existence of off-target effects and the interference of immune cells, resulting in the production of resistant viruses, etc., there are still many problems with the current CRISPR technology. It is necessary to apply AI for technology-related optimisation. Currently, transcription activation-like effector nuclease (TALEN) technology and zinc finger nuclease (ZFN) technology comprise a large class of powerful genome editing tools,...

TALEN gene editing technology is used to infest plants by using TALE, a TAL effector found in *Xanthomonas*, which is injected into plant cells through the bacterial three-class system. The bacterium promotes bacterial colony formation by targeting effector-specific gene promoters to regulate transcription. due to the strong sequence-specific binding ability of TALE, a new gene editing tool, TALEN, was formed by the binding of FokI nuclease to TALE. the TALEN element was targeted to specific DNA sites and bound by the DNA Recognition Module (DNARM) and then completed the specific gene editing process by the action of the FokI nuclease. The TALEN element targets and binds to specific DNA sites through the DNA recognition module, and then under the action of FokI nuclease, it completes the shearing of specific sites, and inserts (or inverts), deletes, and fuses specific sequences with the help of the cell's intrinsic homology-directed repair (HDR) or non-homologous end joining pathway (NHEJ) repair process.

Zinc Finger Nuclease (ZFN) technology targets different DNA sequences by processing and modifying the structural domains of zinc finger DNA by processing and modifying the zinc finger DNA-binding domains of ZFNs, thus allowing ZFNs to bind to target sequences in complex genomes that are specifically cleaved by the DNA cleavage domains. It has been found that the combination of zinc finger nuclease technology and intracellular DNA repair mechanisms has enabled the editing of genomes. Currently, ZFN technology is widely used in targeting gene mutations. As the FokI-ZFN dimer form used by researchers in the early days was highly cytotoxic during the process and could be off-target due to the homodimer effect during the experiments, affecting the specificity of cleavage, Miller et al. and Szczepek et al. developed a variant of FokI in 2007, which allowed ZFN to be used in a heterodimer form, thus greatly improving the specificity and reducing the cytotoxicity of cleavage with the efficiency of cleavage. A variant of FokI has been developed in 2007 by FokI et al. and Szczepek et al. to allow ZFN to be used in a heterodimeric form, which greatly improves specificity and reduces cytotoxicity while maintaining cleavage efficiency. It is currently in clinical use in the treatment of some genetic diseases (e.g. trisomy 21) [18]. However, ZFN technology has many shortcomings, its design is more cumbersome than TALEN due to its own triplex property, and it is highly dependent on the target sequence and its upstream and downstream sequences, there is a possibility that the heterogenous dimer formed by ZFN technology may result in a high off-targeting rate, and there are also many limiting factors such as high cytotoxicity. It may cause apoptosis when it exceeds the tolerance of cellular repair mechanisms. In addition, ZFN technology may cause mutations in relevant genes and may even cause diseases such as cancer. Currently, it is not clear whether the immune system will cause an immune response to gene editing by ZFN technology, so the research samples of ZFN technology related to human therapy are relatively small and unconvincing.

The use of CRISPR gene editing, through the sgRNA will be guided to the target site of the nucleic acid endonuclease Cas, cut off the DNA double-stranded, based on which to achieve the goal of cutting and editing of genes. Compared with the first two targeted targeting are dependent on DNA sequence-specific binding protein module very cumbersome synthesis, CRISPR technology can make the targeting action more precise, directed to change the host gene sequence, target screening more efficient. Currently, CRISPR gene editing technology has made encouraging progress. Researcher Qi Zhou from the Institute of Zoology, Chinese Academy of Sciences has achieved simultaneous knockout of multiple genes in rats using CRISPR-Cas technology [19]. However, there are still many problems with CRISPR technology due to the existence of off-target effects and the interfering effects of immune cells, resulting in the production of resistant viruses. It is necessary to apply AI for technology-related optimization [20]. AI-assisted CRISPR technology has already achieved some results in the resistance of cash crops to abiotic stresses, among which, it is more widely used in the resistance to drought, salinity and high temperature, so the following discussion is based on this.

5. Conclusions

To summarise, the impact of abiotic stressors on cash crops has been increasing in recent years and crop development needs to effectively overcome these challenges. Gene editing technologies, particularly the CRISPR system, can help to identify

drought tolerance loci in crops and optimise them to enable them to better cope with abiotic stresses.

When comparing gene editing technology with traditional breeding methods, it is clear that gene editing technology has significant advantages, but at the same time there are some problems and challenges, such as the assurance of targeting, the control of immune response, and the prevention and control of resistant viruses. The existence of these problems requires more in-depth research and solutions. This review addressed the research progress of CRISPR gene editing technology in response to abiotic stress resistance in cash crops, and presented comprehensive and integrated research recommendations, providing new perspectives and solutions for future related scientific research. AI-assisted CRISPR technology is an effective method to improve the resistance of cash crops to abiotic stresses, which enables precise editing and optimisation of crop genomes and provides new possibilities for crop breeding. However, AI-assisted CRISPR technology also has some challenges and limitations, such as the improvement of gene editing efficiency and accuracy, the assessment of the consequences and safety of gene editing, the social acceptance of gene edited crops, and legal regulations. Therefore, it is recommended to further improve the application of AI-assisted CRISPR technology in the resistance of cash crops to abiotic stresses and to strengthen research and cooperation in related fields.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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References

- [1] Razzaq, M.K.; Akhter, M.; Ahmad, R.M. et al. CRISPR-Cas9 based stress tolerance: New hope for abiotic stress tolerance in chickpea (*Cicer arietinum*). *Mol Biol Rep*, 2022, 49, 8977-8985, DOI: <https://doi.org/10.1007/s11033-022-07391-4>.
- [2] David B.L.; Michael, J.; Wolfram, S.; Noah, B.; Bertis, B.L.; Roderick, M.R.; Graeme, L.H. (2014, May 2). Greater Sensitivity to Drought Accompanies Maize Yield Increase in the U.S. Midwest, from Science. pp. 516-519, DOI: <https://www.science.org/doi/10.1126/science.1251423>.
- [3] Witze, A. Antarctic ozone hole is on the mend. *Nature* (2016), DOI: <https://doi.org/10.1038/nature.2016.20183>.
- [4] Mitchell, C.J. First signs of ozone-hole recovery spotted. *Nature* (2011), DOI: <https://doi.org/10.1038/news.2011.293>.
- [5] Zhang, H.; Zhang, J.; Wei, P.; Zhang, B.; Gou, F.; Feng, Z.; Mao, Y.; Yang, L.; Zhang, H.; Xu, N.; Zhu, J.K. The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. *Plant Biotechnology Journal*, 2014, 12, 797-807, DOI: 10.1111/pbi.12200.

- [6] Shi, J.; Gao, H.; Wang, H.; Lafitte, H.R.; Archibald, R.L.; Yang, M.; Hakimi, S.M.; Mo, H.; Habben, J.E. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnology Journal*, 2017, 15, 207-216, DOI: 10.1111/pbi.12603.
- [7] Hoang, G.T.; Seo, Y.S.; Choi, J.Y.; Kim, J.H.; Kim, Y.J.; Lee, S.; Jung, K.H. CRISPR/Cas9-mediated knockout of OsHKT1;5 gene decreases salt tolerance in rice. *Rice*. 2019, 12, 1-11, DOI: 10.1186/s12284-018-0263-z.
- [8] Shen, C.; Que, Z.; Xia, Y.; Tang, N.; Li, M.; Liu, X.; Gan, Y. Knockout of the At4g24020 gene by CRISPR/Cas9 technology improves the heat tolerance of *Arabidopsis thaliana*. *Plant Cell Reports*. 2018;37:329-338, DOI: 10.1007/s00299-017-2230-x.
- [9] Borrelli, V.M.G. Brambilla Vittoria, Rogowsky Peter, Marocco Adriano, Lanubile Alessandra. The Enhancement of Plant Disease Resistance Using CRISPR/Cas9 Technology. *Frontiers in Plant Science*, 2018, DOI: 10.3389/fpls.2018.01245.
- [10] Li, X.J. et al. Application of CRISPR/Cas9 technology in rice cold resistance breeding. *Chinese Journal of Rice Science*, 2019, 33(6), 591-600.
- [11] Li, R.; Liu, C.; Zhao, R. et al. CRISPR/Cas9-Mediated SINPR1 mutagenesis reduces tomato plant drought tolerance. *BMC Plant Biol*, 2019, 19, 38, DOI: <https://doi.org/10.1186/s12870-018-1627-4>.
- [12] Yan, P. (2017, June). CRISPR Cas9 Medicated the Establishment of Gene Editing System in Tomato. Available online: <https://wap.cnki.net/lunwen-1019087621.nh.html> (accessed on 1 December 2023).
- [13] Sarah, L.; Felix, E. Enciso-Rodriguez, William Behling, Thilani Jayakody, Kaela Panicucci, Daniel Zarka, Satya Swathi Nadakuduti, C. Robin Buell, Norma C. Manrique-Carpintero, David S. Douches.
- [14] Buitrago-Bitar, M.A.; Cortés, A.J.; López-Hernández, F.; Londoño-Caicedo, J.M.; Muñoz-Florez, J.E.; Muñoz, L.C.; Blair, M.W. Allelic Diversity at Abiotic Stress Responsive Genes in Relationship to Ecological Drought Indices for Cultivated Tepary Bean, *Phaseolus acutifolius* A. Gray, and Its Wild Relatives. *Genes*, 2021, 12, 556.
- [15] Twomey, A.; Naidu, B.; Fukai, S. Evidence of salt secretion at the stem of *Melaleuca cuticularis* Labill. *Nat Prec*, 2008, DOI: <https://doi.org/10.1038/npre.2008.2450.1>.
- [16] Peterson, P.; Fowden, L. Different Specificities of Proline-Activating Enzymes from Some Plant Species. *Nature*, 1963, 200, 148-151, DOI: <https://doi.org/10.1038/200148a0>.
- [17] Singh, D.; Singh, C.K.; Singh, D. et al. Glycine betaine modulates chromium (VI)-induced morpho-physiological and biochemical responses to mitigate chromium toxicity in chickpea (*Cicer arietinum* L.) cultivars. *Sci Rep*, 2022, 12, 8005, DOI: <https://doi.org/10.1038/s41598-022-11869-3>.
- [18] Jiang, J.; Jing, Y.; Cost, G. et al. Translating dosage compensation to trisomy 21. *Nature*, 2013, 500, 296-300, DOI: <https://doi.org/10.1038/nature12394>.

- [19]Li, W.; Teng, F.; Li, T.D.; Zhou, Q. Simultaneous generation and germline transmission of multiple gene mutations in rat using CRISPR-Cas systems. Nature Biotechnology, 08 August 2013, DOI: 10.1038/nbt.2652.
- [20]Devang, M. Alessandra Stürchler , Ravi B Anjanappa , Syed Shan-E-Ali Zaidi , Matthias Hirsch-Hoffmann, Wilhelm Gruissem, Hervé Vanderschuren, 2019, 20(1), 80, DOI: 10.1186/s13059-019-1678-3. Available online: <https://pubmed.ncbi.nlm.nih.gov/31018865/#full-view-affiliation-5> (accessed on 1 December 2023).



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