



A Applications of CRISPR-Cas Genome Editing for Developing Climate-Resilient Crops

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Abstract

One of the biggest risks to world agriculture is climate change, which results in drastic drops in agricultural output because of salt, heat stress, drought, and new plant diseases. When it comes to creating crop varieties that can withstand stress, conventional breeding methods are frequently slower and less effective. CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats) genome editing technology has become a ground-breaking instrument for accurate and quick crop improvement. In key crops, CRISPR-Cas systems allow for the targeted alteration of genes linked to biotic and abiotic stress tolerance. The fundamentals of CRISPR-Cas technology are covered in this review, along with how it can be used to create crops like rice, wheat, soybeans, and chickpeas that are resistant to heat, drought, and disease. The paper also discusses recent developments in genome editing technology, the use of CRISPR in legume crops, and the ethical and legal issues surrounding genome-edited crops in India. Crop enhancement with CRISPR has great promise for future food security and sustainable agriculture in the face of climate change.

Keywords: CRISPR-Cas9, Genome Editing, Climate-Resilient Crops, Drought Tolerance, Heat Stress, Disease Resistance, Chickpea, Agricultural Biotechnology

1. Introduction

Climate change poses a serious threat to agriculture because of rising temperatures, erratic rainfall patterns, drought, salt, and the spread of plant diseases. Global agricultural growth, yield, and food security are all adversely impacted by these environmental pressures. Recent estimates indicate that crop productivity in important agricultural regions can be reduced by 20-40% due to climate-related pressures. In addition to requiring lengthy breeding cycles, traditional plant breeding techniques frequently introduce beneficial features with imprecise precision.

Plant genomes can now be specifically altered thanks to recent developments in biotechnology. CRISPR-Cas genome editing has emerged as one of these technologies' most potent and popular techniques for improving crops. CRISPR technology makes it possible to precisely modify stress-responsive genes linked to disease resistance, heat resistance, salinity tolerance, and drought tolerance.

CRISPR-Cas systems are more straightforward, economical, and successful than previous genome editing methods like Zinc Finger Nucleases (ZFNs) and TALENs. Several commercially significant

crops, such as rice, wheat, soybean, and chickpeas, have successfully benefited from CRISPR-mediated agricultural enhancement. The development of climate-resilient crops that can guarantee sustainable agricultural production in the face of shifting environmental conditions is made possible by this technique.

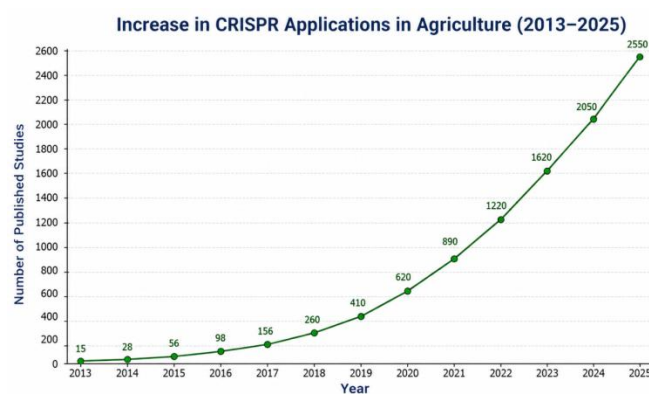


Figure1. Increasing research interest in CRISPR applications for crop improvement.

2. Overview of CRISPR-Cas Technology

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, while Cas refers to CRISPR-associated proteins. CRISPR was originally discovered as part of the adaptive immune system in bacteria and archaea, where it protects against viral infections.

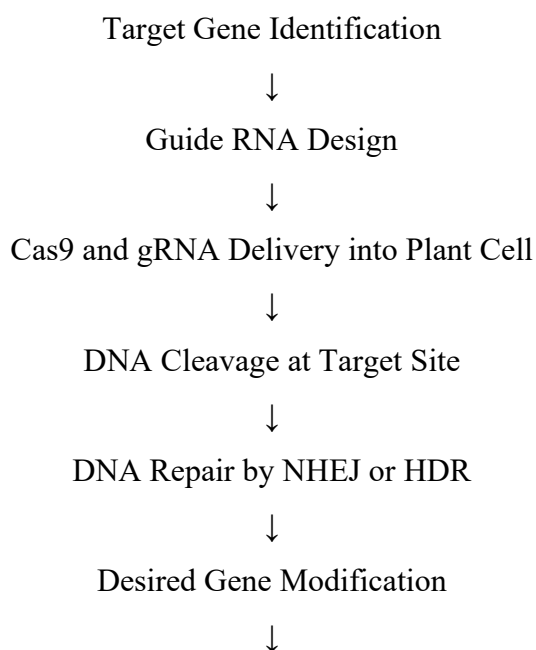
The CRISPR-Cas9 system mainly consists of:

1. Guide RNA (gRNA)
2. Cas9 nuclease enzyme
3. Target DNA sequence

The Cas9 enzyme is guided by the guide RNA to a particular DNA sequence in the genome. Double-stranded breaks are introduced at the target location by Cas9. Two main methods are used by the plant cell to repair these breaks:

- Non-Homologous End Joining (NHEJ)
- Homology Directed Repair (HDR)

Through the insertion, deletion, or change of genes brought about by these repair processes, scientists are able to increase crops' resistance to stress and disease.



Development of Climate-Resilient Crop
Flowchart 1: Basic mechanism of CRISPR-Cas9 genome editing in plants.

3. CRISPR for Drought Tolerance

One of the main abiotic factors lowering agricultural productivity globally is drought stress. Significant yield losses result from water deficiency's effects on photosynthesis, nitrogen uptake, root growth, and plant metabolism. Because drought resistance is regulated by several genes, conventional breeding for drought tolerance is laborious and complicated. By focusing on stress-responsive genes related to hormone communication, osmotic control, and root formation, CRISPR-Cas genome editing has become an effective method for enhancing drought resistance.

CRISPR-Cas9 has been used to modify genes including OsNAC, OsPYL, and DST in rice to enhance drought resistance, root architecture, and water-use efficiency. ABA-signaling pathway modification also improved stomatal control and decreased water loss during drought.

By turning on stress-responsive pathways, genome editing of transcription factors like TaDREB2 enhanced drought adaptation in wheat. Under moisture stress, CRISPR-edited wheat plants displayed

improved water absorption capacity and increased root development.

CRISPR-mediated editing of GmAIR genes enhanced drought tolerance, physiological stability, and antioxidant activity in soybeans. Under water stress, edited soybean lines showed improved osmotic balance and decreased oxidative damage.

Researchers are investigating the editing of dehydration-responsive genes in chickpeas, such as DREB and CAP2, to increase drought resistance and water-use efficiency. Enhancing root growth and nitrogen fixation efficiency under drought stress is another area of current research.

The potential of CRISPR technology for creating climate-resilient crops appropriate for sustainable agriculture under shifting environmental conditions is being further enhanced by recent developments like base editing and multiplex genome editing.

Table1. CRISPR Applications for Drought Tolerance

Crop	Target Gene	Trait Improved
Rice	OsNAC6	Drought tolerance
Wheat	TaDREB2	Water stress adaptation

Soybean	GmAIR genes	ABA-mediated drought resistance
Chickpea	CAP2	Dehydration tolerance

4. CRISPR for Heat Stress Tolerance

Crop productivity and grain quality are greatly impacted by global warming and rising temperatures. Heat stress reduces yield and impairs crop performance by interfering with critical physiological processes such as pollination, flowering, photosynthesis, and grain filling. Because heat resistance is regulated by several genes and intricate metabolic processes, conventional breeding for heat tolerance is challenging. An efficient method for altering heat-responsive genes and enhancing crop thermo tolerance is CRISPR-Cas genome editing.

Heat-sensitive genes linked to stress signaling pathways and grain formation in rice have been edited using CRISPR technology. Under high temperatures, these changes enhanced pollen viability, grain quality, and yield stability.

CRISPR-Cas9 has been utilized to target stress-responsive transcription factors and heat shock proteins (HSPs) in wheat in order

to improve thermo tolerance and lessen oxidative damage brought on by heat stress. Wheat plants that had been edited demonstrated enhanced cellular defense and increased temperature tolerance.

Genome editing in soybeans increased resistance to heat-induced oxidative stress and strengthened antioxidant defense mechanisms. Soybean lines that had undergone CRISPR modification showed enhanced physiological stability and greater survival in extended heat.

The efficiency of creating heat-tolerant crop varieties for climate-resilient agriculture is increasing thanks to recent developments in multiplex genome editing and precision breeding.

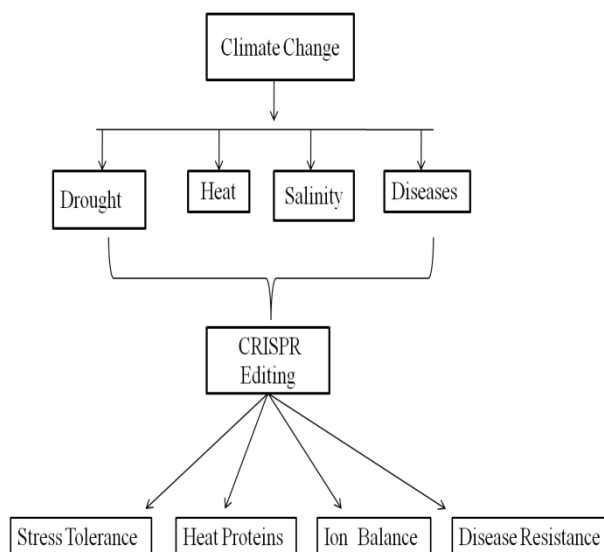


Figure2. Major climate-related stresses targeted using CRISPR genome editing in crops.

5. CRISPR for Disease Resistance

Global agricultural production and food security are severely hampered by plant diseases brought on by bacteria, fungus, and viruses. Chemical pesticides and traditional breeding are examples of conventional disease management techniques that are frequently costly, time-consuming, and ineffective against quickly evolving diseases. By precisely modifying susceptibility and defense-related genes, CRISPR-Cas genome editing has become a potent technique for creating crop varieties resistant to disease.

Xanthomonas oryzae-caused bacterial blight disease in rice has been effectively prevented by CRISPR-mediated editing of SWEET genes. Pathogens frequently take advantage of these genes during infection, and altering them lowers the risk of illness.

MLO gene genome editing increased wheat's resistance against powdery mildew. Without compromising plant development, CRISPR-edited wheat plants displayed

improved defense mechanisms and decreased fungal infection.

CRISPR technology has been applied to soybeans to increase their resistance to a number of fungal diseases and the soybean mosaic virus. Crop performance under pathogen attack was improved and disease resistance was increased by editing genes linked to immunity and stress.

In order to create resistance to Fusarium wilt, one of the most damaging diseases affecting chickpea output, researchers are investigating targeted genome editing techniques. Modification of defense-related genes with CRISPR may increase crop longevity and disease resistance in field settings.

All things considered, CRISPR technology provides a quick, accurate, and sustainable way to increase disease resistance in important crops and lessen reliance on chemical pesticides in agriculture.

Table2. Disease Resistance Developed Using CRISPR

Crop	Disease	Target Gene
Rice	Bacterial blight	SWEET genes
Wheat	Powdery mildew	MLO genes

Soybean	Mosaic virus	eIF genes
Chickpea	Fusarium wilt	Defense-related genes

6. CRISPR Applications in Legume Crops

Due to their capacity to fix nitrogen, legume crops are crucial for improving soil fertility, sustainable agriculture, and nutritional security. However, a number of diseases, heat stress, salinity, and drought have a major negative impact on legume output. A promising technological approach for enhancing stress tolerance, disease resistance, and yield attributes in legume crops is CRISPR-Cas genome editing.

Drought and heat stress brought on by climate change are significant obstacles to chickpea production, particularly in semi-arid areas. Researchers are targeting genes like DREB, CAP2, and ERF transcription factors with CRISPR technology to increase stress tolerance and drought adaptability. Drought tolerance, heat stress resistance, Fusarium wilt resistance, root architecture enhancement, and nitrogen-use efficiency are important target features in chickpeas. The development of climate-resilient chickpea varieties with increased productivity under stressful circumstances could be aided by genome editing.

CRISPR technology has been widely used to improve the fatty acid composition, disease resistance, flowering control, and drought tolerance of soybeans. Drought tolerance and physiological response under water stress were improved by editing GmAIR genes. Additionally, scientists are investigating genome editing techniques to enhance the nutritional value and yield of soybeans.

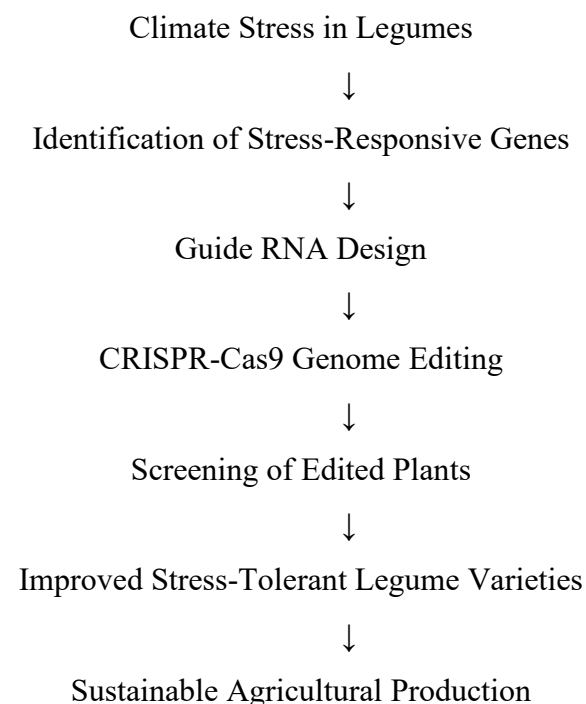
Other legume crops including lentils, cowpeas, and pigeon peas are seeing an increase in CRISPR applications. Genome editing is being investigated for drought tolerance, pod borer resistance, and increased production in pigeon peas. Improving heat tolerance, insect resistance, and nutrient enhancement are the main goals of cowpea research. CRISPR technology has the potential to enhance seed quality features, fungal disease resistance, and salinity tolerance in lentils.

All things considered, CRISPR-Cas technology presents a great deal of promise for creating climate-resilient legume crops that can support food security, sustainable agriculture, and enhanced nutritional quality in the face of shifting environmental conditions.

Table3. CRISPR Applications in Legume Crops

Legume Crop	Target Trait	Important Genes/Targets	Potential Outcome
Chickpea	Drought tolerance	DREB, CAP2	Improved water-use efficiency
Chickpea	Fusarium wilt resistance	Defense genes	Disease resistance
Soybean	Drought tolerance	GmAIR genes	Enhanced stress adaptation
Soybean	Viral resistance	eIF genes	Reduced disease incidence
Cowpea	Heat tolerance	Heat-shock proteins	Improved thermo tolerance
Lentil	Salinity tolerance	Ion transporter genes	Better salt adaptation

Flowchart 2: CRISPR Strategy for Legume Improvement



7. Recent Advances in CRISPR Technology

Recent developments in CRISPR technology have greatly increased the accuracy, effectiveness, and adaptability of plant genome editing. While more recent methods now enable more precise and targeted genetic alterations with fewer off-target effects, traditional CRISPR-Cas9 systems mostly produce double-stranded DNA breaks.

Base editing, which allows for the direct conversion of one DNA base into another without severing the entire DNA strand, is one significant development. Prime editing, a more accurate genome editing method that can add, remove, or replace particular DNA sequences, is another significant advancement. Additionally, scientists are employing multiplex genome editing to concurrently alter several genes linked to crop productivity, heat resistance, disease resistance, and drought tolerance.

The breadth of genome editing applications in plants has increased thanks to more recent CRISPR systems like CRISPR-Cas12 and CRISPR-Cas13, in addition to Cas9. For crop improvement initiatives, these technologies offer increased targeting

effectiveness and more extensive editing capabilities.

Additionally, CRISPR research is being combined with contemporary technologies like artificial intelligence (AI), bioinformatics, genomics, transcriptomics, and proteomics to find critical stress-responsive genes and hasten the development of climate-resilient crops. CRISPR technology is becoming more effective and promising for sustainable agriculture in the future because to these advancements.

8. Ethical and Regulatory Issues in India

CRISPR-Cas technology poses serious ethical, ecological, and biosafety issues in addition to its many benefits for crop enhancement. The possibility of off-target mutations unintentional modifications in the plant genome during editing is one of the main worries. Ecological issues, such as potential impacts on biodiversity and gene flow from modified crops to wild relatives, are also a cause for concern. The public's acceptance of genome-edited crops is still a problem because of worries about food safety, the effects on the environment, and moral issues.

National biotechnology and biosafety regulations govern genome-edited

crops in India. Organizations like the Indian Council of Agricultural Research (ICAR), the Department of Biotechnology (DBT), and the Genetic Engineering Appraisal Committee (GEAC) oversee regulations. These organizations assess the marketing, environmental impact, and safety of genetically modified and genome-edited crops.

For some genome-edited plants created using SDN1 and SDN2 techniques, where no foreign DNA is inserted into the crop genome, India recently established streamlined regulatory guidelines. While upholding biosafety standards and environmental protection, this regulatory easing is anticipated to speed up research and development of CRISPR-based climate-resilient crops.

9. Challenges and Limitations

Despite the fact that CRISPR-Cas technology has transformed crop development, a number of obstacles and restrictions continue to impede its widespread use in agriculture. Off-target genome editing, in which unintentional genetic changes may take place at non-target areas and may impact plant growth and stability, is one of the main worries. The low transformation efficiency in many

agricultural species, especially in legumes and other resistant plants, is another significant constraint that makes successful genome editing challenging.

The extensive application of CRISPR technology in underdeveloped nations is further constrained by high research and development expenses, the need for sophisticated laboratory facilities, and the need for highly qualified technical personnel. Commercialization of genome-edited crops is further hampered by regulatory ambiguity and varying biosafety regulations across nations. The acceptability of CRISPR-based agricultural products is further impacted by low public understanding and worries about food safety and environmental effects.

For sustainable agricultural uses, more research is therefore required to increase editing precision, minimize off-target effects, and create effective and affordable gene delivery technologies.

10. Future Perspectives

In the future, CRISPR-Cas technology is anticipated to play a significant role in climate-smart agriculture and sustainable crop enhancement. Rapid developments in genome editing are opening up new possibilities for the development of

crops that are more resistant to diseases, heat, salinity, and drought. Future studies will probably concentrate on multi-gene editing techniques to concurrently enhance a number of stress-related agricultural attributes.

Other cutting-edge methods for locating and altering crucial genes linked to crop productivity and climate resistance include precision breeding and AI-assisted genome editing. The creation of better crop varieties will proceed more quickly when CRISPR technology is combined with genomes, transcriptomics, proteomics, and other omics techniques.

Additionally, researchers are concentrating on underutilized and orphan crops that have gotten little scientific attention yet are crucial for regional food security. Enhancing these crops with CRISPR could have a big impact on environmental preservation, nutritional security, and sustainable agriculture.

All things considered, integrating CRISPR technology with contemporary breeding techniques and biotechnology tools has the potential to transform global agriculture and guarantee food security in the face of shifting climatic conditions in the upcoming decades.

11. Conclusion

Agricultural biotechnology has been transformed by CRISPR-Cas genome editing, which makes crop enhancement quick, accurate, and effective. Its enormous promise for climate-resilient agriculture is demonstrated by its applications in creating crops that are resistant to heat, disease, and drought. CRISPR-mediated genetic alterations have significantly improved major crops like rice, wheat, soybeans, and chickpeas. Genome editing presents excellent prospects for improving disease resistance, stress tolerance, nutritional quality, and sustainable production in legume crops. It is anticipated that further developments in CRISPR technology will improve global food security and sustainable agriculture in the face of shifting climate circumstances, despite current ethical, biosafety, and regulatory obstacles.

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