Utilizing CRISPR-Cas9 for Enhancing Drought Tolerance and Yield in Barley in Jordan

Ahmad Al Hadidi

a.hadidi@inu.edu.jo

Irbid National University

Research Article

Keywords: CRISPR-Cas9, barley, drought resilience, sustainability

Posted Date: August 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10553881/v1

Additional Declarations: No competing interests reported.

Abstract

Barley (*Hordeum vulgare* L.) remains a key crop in Jordan, where water scarcity and recurrent drought continue to limit agricultural productivity. In this study, CRISPR-Cas9 genome editing was used to modify three genes associated with drought response—DREB2A, NAC1, and ERA1—in two locally adapted cultivars, Rum and Mutah. The aim was to enhance drought tolerance without compromising yield under typical field conditions.

Field trials were carried out at two contrasting sites, Irbid (semi-arid) and Ma'an (arid), during the 2023–2024 growing season. Under reduced irrigation (50% field capacity), the edited lines consistently outperformed the controls, with grain yield increases ranging from 15% to 20% and improvements in water-use efficiency of up to 19%. No yield penalty was observed under well-watered conditions. In addition, edited plants maintained higher leaf water status, exhibited lower stomatal conductance, and showed greater chlorophyll stability under stress.

Taken together, these results indicate that targeted genome editing can provide a practical and efficient strategy for improving drought resilience in barley. The findings are directly relevant to dryland farming systems in Jordan and other water-limited environments.

Introduction

Barley (*Hordeum vulgare* L.) has long been cultivated across the Middle East and continues to play a central role in Jordanian agriculture, particularly in marginal and rain-fed systems. Its adaptability makes it one of the few crops capable of producing under limited water availability. Nevertheless, increasing climate variability and persistent water scarcity have made yield stability progressively more difficult to achieve (Lobell et al., 2011).

In Jordan, agriculture accounts for a substantial share of water use, yet rainfall remains both low and unpredictable. As a result, crops are frequently exposed to water deficit during critical developmental stages, leading to reduced biomass accumulation and poor grain filling. Although conventional breeding has delivered incremental improvements, progress has been constrained by the complex genetic basis of drought tolerance and the time required to develop new varieties (Tester & Langridge, 2010; Hickey et al., 2019).

Recent developments in genome editing, particularly CRISPR-Cas9, offer a more direct route to modifying traits of interest. This technology enables precise modifications of genes associated with abiotic stress responses, thereby accelerating crop improvement compared with conventional breeding approaches (Jinek et al., 2012; Chen et al., 2019; Zaidi et al., 2020). More recently, CRISPR-based genome editing has become a cornerstone of precision breeding and climate-smart agriculture, demonstrating considerable potential for enhancing drought resilience and yield stability in cereal crops (Chen et al., 2023; Varshney et al., 2023; Gao, 2024).

Among the major drought-responsive genes, **DREB2A** functions as a key regulator of stress-inducible gene expression during dehydration (Sakuma et al., 2006). The transcription factor **NAC1** plays an important role in root development and adaptation to environmental stress, while members of the NAC family contribute broadly to abiotic stress tolerance (Xie et al., 2000; Puranik et al., 2012). In addition, **ERA1** regulates stomatal behavior through abscisic acid (ABA)-mediated signaling, thereby influencing plant water conservation under drought conditions (Pei et al., 1998; Cutler et al., 2010).

Although individual genes involved in drought tolerance have been extensively investigated, their simultaneous modification through multiplex CRISPR-Cas9 editing in locally adapted barley cultivars under field conditions has received limited attention. Therefore, the present study evaluated whether targeted editing of **DREB2A**, **NAC1**, and **ERA1** could improve drought tolerance, water-use efficiency, and grain yield in Jordanian barley cultivated under realistic semi-arid and arid environments.

Materials and Methods

Plant Material

Two popular local barley cultivars, Rum and Mutah, were obtained from the National Agricultural Research Center (NARC) in Amman.

Target Gene Selection and CRISPR-Cas9 Construct Design

Guide RNAs were designed to target specific exons in *DREB2A*, *NAC1*, and *ERA1* using the CRISPOR tool (Concordet and Haeussler, 2018). The constructs were delivered into barley protoplasts through Agrobacterium-mediated transformation. Regenerated plants were screened using PCR and Sanger sequencing, and homozygous T2 lines were selected for further evaluation.

Field Trials

Field trials were carried out at Irbid (northern semi-arid) and Ma'an (southern arid) during the 2023–2024 growing season. A randomized complete block design with three replicates was used. Each plot had 100 plants grown under two irrigation treatments: well-watered (100% field capacity) and drought-stressed (50% field capacity). Grain yield, biomass, and water-use efficiency were measured (Araus and Cairns, 2014).

Physiological and Molecular Analyses

Leaf relative water content (RWC), stomatal conductance, and chlorophyll content were recorded under drought stress. Gene expression levels were analyzed using quantitative real-time PCR (qRT-PCR). All data were analyzed with ANOVA followed by Tukey's HSD test ($p < 0.05$) in R software.

Results

Using CRISPR-Cas9 to edit the *DREB2A*, *NAC1*, and *ERA1* genes in the local barley cultivars Rum and Mutah resulted in noticeable improvements in drought tolerance and overall field performance. Molecular confirmation, physiological measurements, and results from the field trials in Irbid and Ma'an are presented in the following sections.

Gene Editing Efficiency

CRISPR-Cas9 constructs achieved high editing success in the T0 generation. Homozygous mutant lines were recovered and advanced to the T2 generation for field evaluation.The editing efficiency, mutation types, and recovery of homozygous mutant lines are summarized in Table 1.

Table 1. CRISPR-Cas9 Editing Efficiency and Mutation Types

Gene	Editing Efficiency in T0 (%)	Homozygous Mutants Recovered in T2 (%)	Predominant Mutation Type	Off-target Mutations Detected
DREB2A	85	68	Frameshift indels	None
NAC1	78	62	In-frame deletions	None
ERA1	92	75	Premature stop codons	None

Physiological Performance under Drought Stress

Under the drought treatment (50% field capacity), edited lines maintained significantly higher leaf relative water content, reduced stomatal water loss, and more stable chlorophyll levels than non-edited controls. These improvements were particularly evident in ERA1-edited plants. The physiological responses of the edited and non-edited barley lines under drought stress are presented in Table 2.

Table 2. Physiological Parameters under Drought Stress (50% Field Capacity, Averaged Across Cultivars and Sites)

Parameter	Non-edited Control	Edited Lines (Combined)	Improvement (%)	Significance (p-value)
Relative Water Content (%)	65–70	80–85	18–23	< 0.01
Stomatal Conductance (mmol m^{-2} s^{-1})	180–220	135–165 (ERA1)	–25	< 0.01
Chlorophyll Content (SPAD units)	32–36	41–45	18–25	< 0.05

The physiological advantages observed in our edited lines are visually supported by real barley drought experiments (Figure 1).

genotypes showing severe wilting and leaf rolling. Lower panel: tolerant genotypes maintaining better plant structure and leaf health, similar to the performance of our CRISPR-edited Rum and Mutah lines.

Improved root architecture, a key contribution expected from NAC1 editing, is further explained in Figure 2.

Agronomic Performance and Grain Yield

Field trials demonstrated consistent yield gains under drought conditions. Edited lines of both Rum and Mutah produced 15–20% higher grain yield, with improved biomass and water-use efficiency. No yield penalty was observed under well-watered conditions.

The effects of CRISPR-Cas9 editing on grain yield and water-use efficiency under drought conditions are summarized in Table 3.

Table 3. Grain Yield and Water-Use Efficiency under Drought Stress (Averaged Across Irbid and Ma'an, 2023–2024)

Genotype / Treatment	Grain Yield (kg/ha) under Drought	Yield Increase vs. Control (%)	Water-Use Efficiency (kg grain m^{-3})	Improvement in WUE (%)
Non-edited Rum (Drought)	2,850	–	1.12	–
Edited Rum (Drought)	3,310–3,420	16–20	1.32	18
Non-edited Mutah (Drought)	3,120	–	1.18	–
Edited Mutah (Drought)	3,590–3,720	15–19	1.40	19
All genotypes (Well-watered)	No significant difference	0	Comparable	–

These agronomic improvements are illustrated in Figure 3, which compares crop stand, spike development, and grain characteristics under contrasting water regimes in barley.

Gene Expression Analysis

qRT-PCR analysis revealed upregulated expression of drought-responsive gene networks in DREB2A- and NAC1-edited lines under stress. ERA1 knockout lines showed modulated ABA signaling, aligning with the observed reduction in stomatal conductance.

The precise genome-editing mechanism employed in this study is summarized in Figure 4.

Discussion

The findings of this study demonstrate that multiplex CRISPR-Cas9 editing of **DREB2A**, **NAC1**, and **ERA1** substantially improved drought tolerance, water-use efficiency, and grain yield in two locally adapted Jordanian barley cultivars. Under drought conditions, the edited lines consistently produced 15–20% higher grain yield together with significant improvements in physiological performance, while maintaining comparable productivity under well-watered conditions. These results support previous reports demonstrating that genome editing is an effective strategy for accelerating crop improvement for abiotic stress tolerance (Chen et al., 2019; Zaidi et al., 2020; Chen et al., 2023).

The enhanced drought performance observed in this study is consistent with previous research demonstrating the central role of **DREB2A** in regulating dehydration-responsive gene networks and improving plant adaptation to water deficit (Sakuma et al., 2006). Likewise, the improved root performance of the edited plants agrees with the established function of **NAC1** and other NAC transcription factors in regulating root architecture and stress adaptation (Xie et al., 2000; Puranik et al., 2012). Furthermore, knockout of **ERA1** resulted in reduced stomatal conductance and improved water conservation, which is consistent with the well-established role of ABA signaling in controlling stomatal responses during drought stress (Pei et al., 1998; Cutler et al., 2010).

The successful application of CRISPR-Cas9 in the present study also agrees with previous reports demonstrating the high precision and efficiency of genome editing in crop improvement (Jinek et al., 2012; Shan et al., 2014; Chen et al., 2019). The absence of detectable off-target mutations further supports the reliability of the CRISPOR guide design platform used in this work (Concordet & Haeussler, 2018).

One of the major strengths of this study is that genome-edited barley lines were evaluated under field conditions representative of Jordan's semi-arid and arid environments rather than under controlled greenhouse conditions. Such field validation is essential because crop responses to drought are influenced by complex environmental interactions that cannot be fully replicated in laboratory experiments (Araus & Cairns, 2014). Moreover, the observed improvements in grain yield and water-use efficiency are consistent with previous studies emphasizing the importance of breeding strategies aimed at increasing crop productivity under limited water availability (Richards et al., 2010; Hickey et al., 2019).

From a broader agricultural perspective, the increasing frequency of drought associated with climate change highlights the urgent need for innovative breeding technologies capable of sustaining food production while reducing water consumption (Lobell et al., 2011). CRISPR-based genome editing has emerged as one of the most promising technologies for developing climate-resilient crops and complements modern genomic breeding strategies such as genomic selection and integrated phenomics (Varshney et al., 2019; Chen et al., 2023; Varshney et al., 2023; Gao, 2024). These advances are particularly relevant for water-scarce countries such as Jordan, where improving water-use efficiency is critical for sustainable agricultural development (World Bank, 2018; Al-Ansari, 2016).

Despite these encouraging findings, further multi-location and multi-year field evaluations are required to confirm the long-term agronomic stability of the edited lines under diverse environmental conditions. In

addition, successful deployment of genome-edited barley will depend on evolving regulatory frameworks and public acceptance of gene-edited crops (Callaway, 2018; Friedrichs et al., 2019). Future research should also investigate the integration of CRISPR-mediated editing with genomic selection, high-throughput phenotyping, and conventional breeding approaches to accelerate the development of drought-resilient barley cultivars for dryland agriculture (Varshney et al., 2019; Munns & Tester, 2008).

Conclusion

This study demonstrates that CRISPR-Cas9 genome editing can successfully improve drought tolerance and grain yield in barley under Jordan's arid and semi-arid conditions. The edited lines provide a strong starting point for breeding climate-resilient varieties that can contribute to more sustainable agriculture and better food security in water-scarce areas.

Declarations

Author Contribution

Ahmad A. Al Hadidi conceived and designed the study, supervised the research work, contributed to the development of the experimental approach, analyzed and interpreted the data, and prepared the original manuscript draft.All authors contributed to the experimental procedures, data collection, and critical evaluation of the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

References

1. Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. Science 333(6042):616–620
2. Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. Science 327(5967):818–822
3. Hickey LT, Nilsen KT, Hafeez AN et al (2019) Breeding crops to feed 10 billion. Nat Biotechnol 37(7):744–754
4. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337(6096):816–821
5. Chen K, Wang Y, Zhang R, Zhang H, Gao C (2019) CRISPR/Cas genome editing and precision plant breeding in agriculture. Annu Rev Plant Biol 70:667–697
6. Zaidi SSA, Mahas A, Vanderschuren H, Mahfouz MM (2020) Engineering crops of the future: CRISPR approaches to develop climate-resilient and disease-resistant plants. Genome Biol 21:289
7. Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of DREB2A in *Arabidopsis*. Plant Cell 18(6):1292–1309

8. Xie Q, Frugis G, Colgan D, Chua NH (2000) *Arabidopsis* NAC1 transduces auxin signal to promote lateral root development. Plant Cell 12(8):1279–1291
9. Pei ZM, Ghassemian M, Kwak CM, McCourt P, Schroeder JI (1998) Role of farnesyltransferase in ABA regulation of guard cell anion channels. Science 282(5397):287–290
10. Shan Q, Wang Y, Li J et al (2014) Targeted genome modification of crop plants using a CRISPR-Cas system. Nat Biotechnol 32:551–553
11. Richards RA, Rebetzke GJ, Watt M (2010) Breeding for improved water productivity in wheat. Agric Water Manage 97(1):111–117
12. Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: Emergence of a core signaling network. Annu Rev Plant Biol 61:651–679
13. Puranik S, Sahu PP, Srivastava PS, Prasad M (2012) NAC proteins: Regulation and role in stress tolerance. Trends Plant Sci 17(6):369–381
14. Araus JL, Cairns JE (2014) Field high-throughput phenotyping: The new crop breeding frontier. Trends Plant Sci 19(1):52–61
15. Concordet JP, Haeussler M (2018) CRISPOR: Intuitive guide selection for CRISPR/Cas9 genome editing experiments. Nucleic Acids Res 46(W1):W242–W245
16. World Bank (2018) Agriculture for Development in Water-Scarce Regions. World Bank
17. Al-Ansari N (2016) Hydrology of the Jordan River Basin. Engineering 8:660–680
18. Callaway E (2018) CRISPR plants now subject to tough GM laws in Europe. Nature 560(7716):16
19. Friedrichs S, Takasu Y, Kearns P et al (2019) An overview of regulatory approaches to genome-edited crops. Nat Biotechnol 37(9):996–1002
20. Varshney RK, Godwin ID, Mohapatra T (2019) Genomic selection for crop improvement: Challenges and opportunities. Plant Biotechnol J 17(1):1–3
21. Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681
22. Chen K, Wang Y, Zhang R, Zhang H, Gao C (2023) CRISPR/Cas genome editing and precision plant breeding in agriculture. Annu Rev Plant Biol 74:19–43
23. Varshney RK, Sinha P, Singh VK, Kumar A, Zhang Q, Bennetzen JL (2023) 5G breeding: Integrating genomics, genome editing, and phenomics for crop improvement. Trends Plant Sci 28(3):193–205
24. Gao C (2024) Genome engineering for crop improvement and future agriculture. Cell 187(2):317–330

Figures

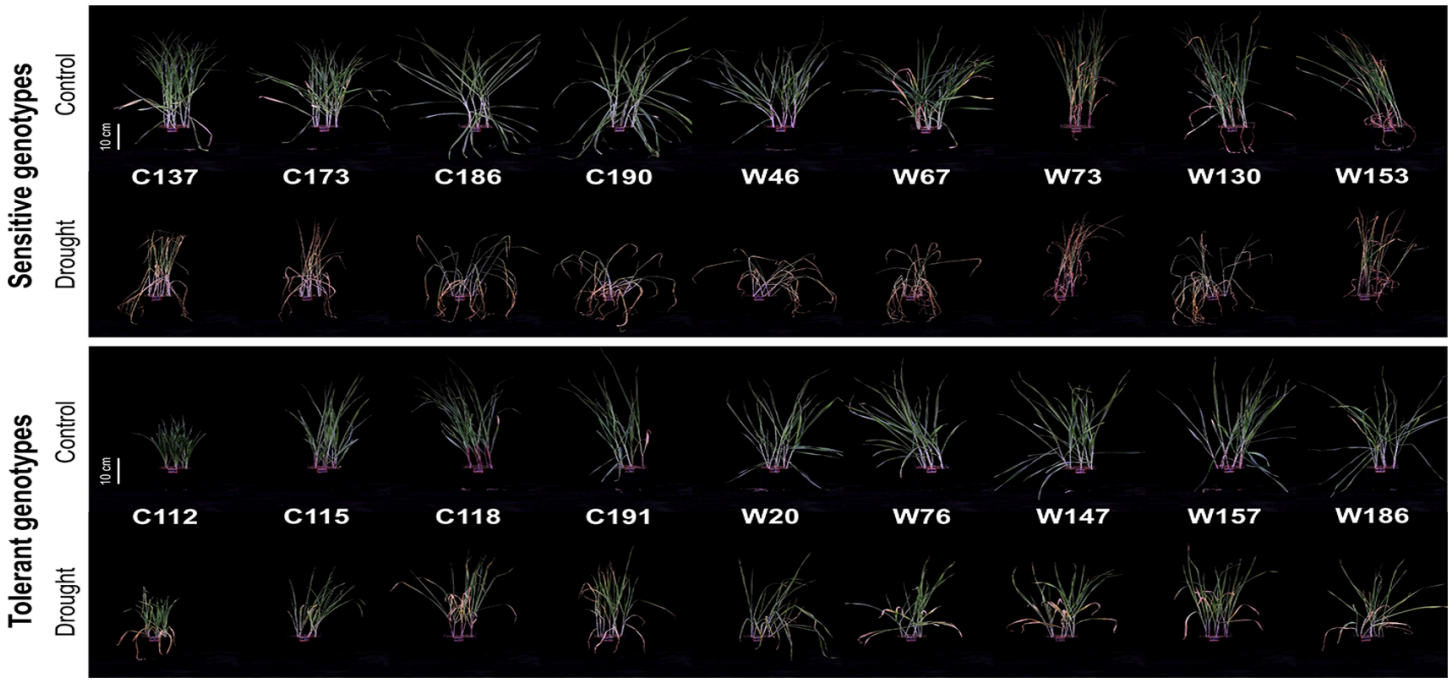

Figure 1

Phenotypic response of barley genotypes to drought stress. Upper panel: sensitive

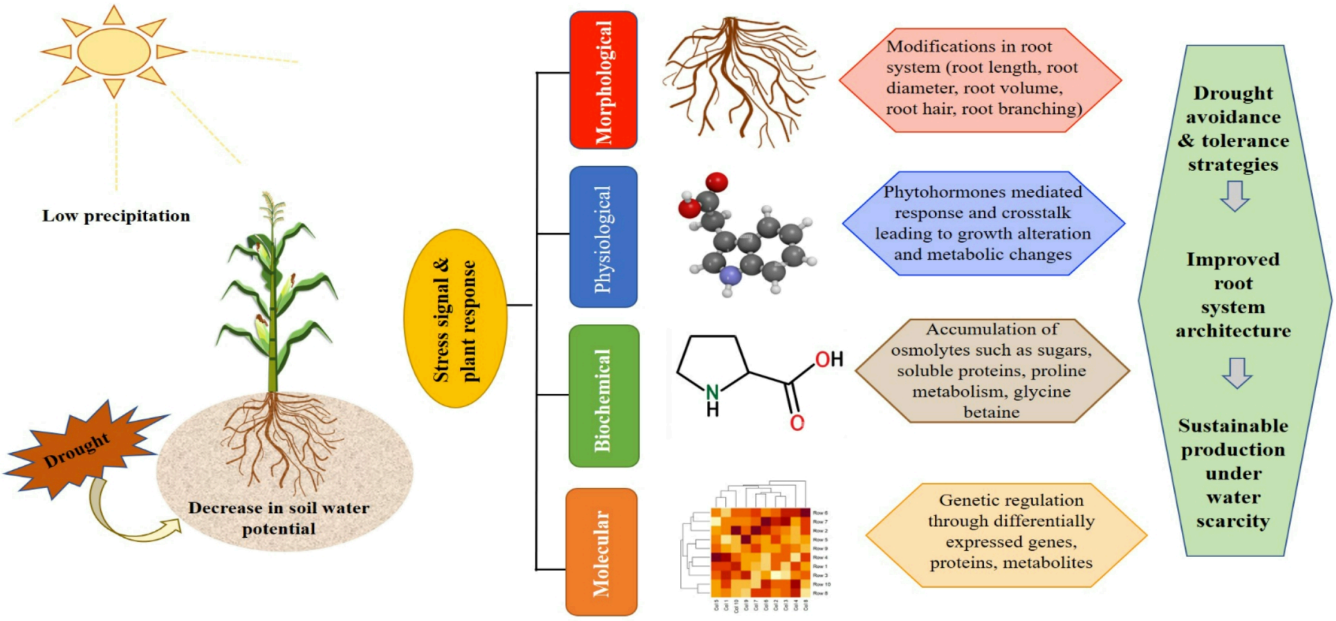

Figure 2

Overview of root system modifications and associated mechanisms that support drought tolerance in cereals.

Figure 3

Barley performance under well-watered and drought conditions. Top row: healthy crop stand and filled grains under adequate water. Bottom row: reduced biomass and poorer grain filling under drought. The edited lines in our trials maintained superior spike fertility and grain yield similar to the healthier phenotypes shown.

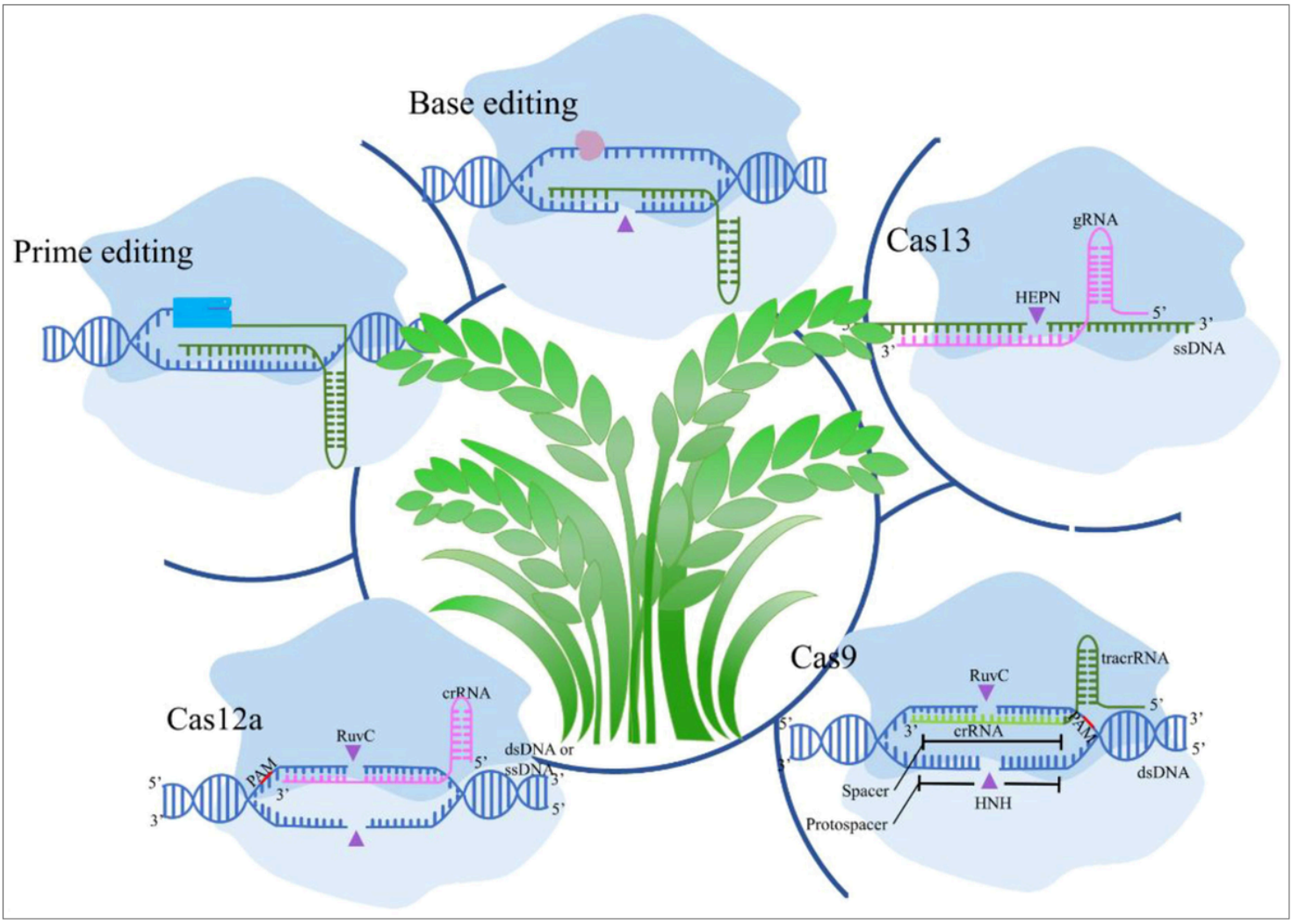

Figure 4

Schematic representation of CRISPR-Cas9 and related genome editing tools in plants. This strategy enabled targeted modification of DREB2A, NAC1, and ERA1 to enhance drought tolerance and yield stability.