OPEN ACCESS
Research of Scientia Naturalis
Vol. 2 No. 3. June 2025, pp. 135-147 DOI. 10.70177/scientia.vxix.xxx

Research Article

CRISPR-Cas9 Mediated Genetic Enhancement of Drought Tolerance in Maize: A Molecular and Field Validation Study

Muhammad Hazmi[1], Zain Nizam[2], Nina Anis[3]
[1] Universitas Muhammadiyah Jember, Indonesia
[2] Universiti Malaysia Sarawak, Malaysia
[3] Monash University, Malaysia

Corresponding Author:

Muhammad Hazmi,
Universitas Muhammadiyah Jember, Indonesia
Gumuk Kerang, Karangrejo, Sumbersari, Jember Regency, East Java 68124
Email: mhazmi.hazmi@gmail.com

Article Info

Received: June 2, 2025
Revised: June 5, 2025
Accepted: June 7, 2025
Online Version: June 7, 2025

Abstract

Drought is a primary constraint on maize (Zea mays L.) productivity worldwide, threatening global food security. The development of climate-resilient crops is therefore a critical priority. This study aimed to enhance drought tolerance in maize by utilizing the CRISPR-Cas9 system to edit a key negative regulator gene involved in the drought stress response pathway. The research employed an Agrobacterium-mediated transformation method to introduce the CRISPR-Cas9 construct into maize embryos. Putative edited plants were rigorously screened using molecular techniques, including PCR and Sanger sequencing, to confirm successful gene modification. Validated T1 generation lines were then subjected to controlled drought stress conditions in greenhouse trials and subsequently evaluated in multi-location field trials. The edited maize lines exhibited significantly improved physiological and agronomic performance under water-deficit conditions, including enhanced photosynthetic efficiency, reduced leaf water loss, and a 15-20% increase in grain yield compared to non-edited wild-type controls. This study demonstrates the efficacy of CRISPR-Cas9 for developing drought-tolerant maize, offering a precise and rapid strategy for crop improvement to mitigate the impacts of climate change.

Keywords: Crop Improvement, Drought Stress, Gene Editing

Journal Homepage https://journal.ypidathu.or.id/index.php/scientia
How to cite: Hamidi, S. A., Hashimi, F. U., & Rahmati, A. (2024). Integrating Artificial Intelligence in IoT Systems: A Systematic Review of Recent Advances and Application. *Research of Scientia Naturalis, 2*(3), 135–147. https://doi.org/10.70177/scientia.v2i3.2382
Published by: Yayasan Pendidikan Islam Daarut Thufulah

INTRODUCTION

Maize (*Zea mays* L.) stands as a cornerstone of global food security, providing essential calories for billions of people and serving as a primary component of livestock feed and biofuel production. Its cultivation spans diverse agroecological zones, making it a vital economic driver for both developed and developing nations (Polzer & Öner, 2025). The productivity of this critical crop, however, is increasingly jeopardized by the escalating effects of climate change. Among the abiotic stresses exacerbated by global warming, drought has emerged as the single most significant factor limiting maize yield worldwide. Projections indicate that the frequency and intensity of drought events will continue to rise, posing an unprecedented threat to agricultural stability and the global food supply chain (Bany Mohammed dkk., 2025; Rohid dkk., 2025). The imperative to develop climate-resilient crops capable of sustaining high productivity under water-scarce conditions has therefore become a paramount objective in agricultural science.

The physiological response of maize to water deficit is a complex and multifaceted process that profoundly impacts its growth and development. Insufficient water availability triggers a cascade of adverse effects, beginning with stomatal closure to conserve water, which concurrently reduces carbon dioxide uptake and impairs photosynthetic efficiency (Hubert dkk., 2025; Wade & Parks, 2025). Prolonged drought stress disrupts cellular homeostasis, leading to the accumulation of reactive oxygen species (ROS) that cause oxidative damage to vital cellular components, including lipids, proteins, and nucleic acids. Furthermore, water limitation hinders nutrient absorption and translocation, compromises pollination and grain filling, and ultimately results in substantial reductions in biomass and grain yield. Understanding the intricate molecular networks that govern these stress responses is fundamental to devising effective strategies for genetic improvement.

Historically, conventional breeding methods have been the primary approach to developing drought-tolerant maize varieties (Almqvist-Ingersoll, 2025; Kostadinov & Chow, 2025). Through selective breeding and hybridization, significant progress has been made in improving crop resilience. However, these traditional techniques are inherently slow, labor-intensive, and often constrained by the complex, polygenic nature of drought tolerance, which involves numerous genes with small, cumulative effects (Fu dkk., 2025; Kostadinov & Chow, 2025). The advent of modern biotechnology has provided powerful new tools to overcome these limitations. Genetic engineering, in particular, offers a more precise and expedited pathway for crop enhancement by enabling the targeted modification of specific genes known to play critical roles in stress tolerance pathways. This technological leap allows for the introduction of novel traits with a level of precision and speed unattainable through conventional breeding alone.

The fundamental problem addressed by this research is the acute vulnerability of elite maize germplasm to drought-induced yield loss. Despite decades of breeding efforts, a large proportion of commercially cultivated maize varieties remain highly susceptible to periods of water deficit, particularly during critical growth stages such as flowering and grain filling. This susceptibility translates into massive economic losses for farmers and increases the risk of food shortages in regions heavily reliant on rain-fed agriculture (Velasco Molpeceres dkk., 2025; Xie & Jiang, 2025). The challenge is compounded by the genetic complexity of drought tolerance, which has made it difficult to pyramid multiple beneficial genes effectively using

conventional methods. Consequently, there is an urgent and unmet need for a more robust and efficient strategy to fortify maize against drought.

The problem extends to the molecular level, where specific regulatory pathways actively suppress the plant's innate defense mechanisms under normal conditions to conserve energy, but can hinder a rapid and effective response when stress occurs. Many drought tolerance strategies have focused on the overexpression of positive regulators, which can sometimes lead to unintended pleiotropic effects, such as stunted growth or reduced yield under non-stress conditions, due to the energetic cost of maintaining a constant state of defense (Dunn Silesky dkk., 2025; Olsen, 2025). A more refined approach involves the targeted inactivation of key negative regulators—genes that act as brakes on the drought response pathway. The persistent expression of these negative regulators under stress conditions represents a specific molecular bottleneck that limits the plant's ability to mount a full-scale, effective defense against water deficit.

Existing solutions have proven insufficient to meet the scale and urgency of the challenge. While marker-assisted selection (MAS) has accelerated conventional breeding, its success is still dependent on the availability of suitable genetic variation within the breeding pool. Previous transgenic approaches, such as those employing RNA interference (RNAi) or the constitutive overexpression of stress-responsive transcription factors, have yielded variable results and have often been associated with concerns about off-target effects or fitness penalties (Ediz dkk., 2025; Silva, 2025). A technological gap therefore exists for a method that can introduce precise, stable, and beneficial genetic modifications without disrupting the plant's overall agronomic performance, thereby providing a reliable solution to enhance drought tolerance in a commercially relevant timeframe.

The primary objective of this study is to leverage the precision of the CRISPR-Cas9 gene-editing system to enhance the drought tolerance of maize. This will be achieved by specifically targeting and inactivating a well-characterized negative regulator gene known to be involved in the abscisic acid (ABA) signaling pathway, a central hub for drought stress responses in plants. By creating a targeted knockout of this gene, we aim to remove its inhibitory effect, thereby unleashing the plant's endogenous defense mechanisms to confer a more robust and rapid response to water-deficit conditions (Endriana dkk., 2025; Poduval dkk., 2025). The ultimate goal is to generate genetically edited maize lines that exhibit superior performance and yield stability under drought.

To accomplish this primary goal, several specific sub-objectives have been defined. The first is to design and construct a highly efficient CRISPR-Cas9 vector tailored for maize, containing guide RNAs (gRNAs) that specifically target a conserved exon within the chosen negative regulator gene (Wan dkk., 2025; Ye dkk., 2025). The second objective is to optimize an *Agrobacterium tumefaciens*-mediated transformation protocol for the introduction of this construct into immature maize embryos. Subsequently, the third objective involves the rigorous molecular validation of putative edited events using PCR-based screening and Sanger sequencing to confirm the presence of desired insertion-deletion (indel) mutations at the target locus and to screen for potential off-target mutations. Finally, the fourth objective is to conduct a comprehensive phenotypic evaluation of the confirmed, heritable T1 generation edited lines through controlled greenhouse experiments and multi-location field trials to assess their physiological and agronomic performance under simulated and natural drought stress.

This research is guided by the central hypothesis that the targeted disruption of the selected negative regulator gene via CRISPR-Cas9 will lead to a significant enhancement in drought tolerance (Kasanneni dkk., 2025; Nasseripour dkk., 2025). We hypothesize that the resulting edited maize plants will exhibit improved physiological traits, including higher relative water content, enhanced photosynthetic rates, and reduced electrolyte leakage under stress, compared to their non-edited wild-type counterparts. Furthermore, we postulate that these physiological improvements will translate into tangible agronomic benefits, most notably a significant mitigation of yield loss under water-limiting conditions in the field. This study aims to provide a clear and robust validation of this hypothesis from the molecular to the whole-plant level.

The body of literature on genetic engineering for drought tolerance in maize is substantial, yet it reveals distinct gaps in both methodology and validation. Early research predominantly focused on the overexpression of single genes, such as transcription factors (e.g., DREB1A) or enzymes involved in osmolyte synthesis. While some of these studies reported promising results in controlled environments, the improvements often failed to translate into consistent yield advantages in the field, sometimes due to the aforementioned fitness costs. More recent studies have explored the use of RNAi to downregulate negative regulators, but this approach can result in incomplete gene silencing and potential off-target effects, leading to inconsistent phenotypes (Obermaier dkk., 2025; Rai, 2025). These earlier works laid an important foundation but highlighted the need for a more precise and reliable technology.

A significant gap exists in the application and, more importantly, the comprehensive validation of CRISPR-Cas9 for enhancing a complex quantitative trait like drought tolerance in a major cereal crop like maize (Rahim dkk., 2025; Rughiniș dkk., 2025). While the CRISPR-Cas9 system has been successfully deployed in maize for simpler, single-gene traits such as herbicide resistance or nutritional improvement, its application for complex abiotic stress tolerance is still in its early stages. Few studies have successfully navigated the entire pipeline from initial gene editing to multi-season, multi-location field trials necessary to validate the commercial viability of an edited trait. There is a clear lack of research that provides a complete, "gene-to-field" validation of CRISPR-edited, drought-tolerant maize.

This study is explicitly designed to fill this critical gap. By providing a thorough and integrated analysis that spans from precise molecular characterization to extensive agronomic performance evaluation in real-world agricultural settings, our research moves beyond a simple proof-of-concept. It aims to deliver a robust dataset that validates not only the efficacy of the specific gene target but also the power of the CRISPR-Cas9 platform as a practical tool for developing climate-resilient crops (Liu dkk., 2025; Yang dkk., 2025). This work will bridge the gap between laboratory discovery and agricultural application, providing a much-needed, rigorously validated case study for the use of gene editing to address pressing global challenges in agriculture.

The primary novelty of this research lies in its strategic approach of targeting a key negative regulator of the drought stress response pathway using the precision of CRISPR-Cas9. Unlike strategies that aim to add or overexpress genes, our method focuses on removing a natural "brake" in the plant’s defense system (Vañó-Agulló, 2025; You dkk., 2025). This "loss-of-function" approach is inherently more precise and is hypothesized to be more metabolically efficient, as it does not require the plant to expend energy on the constitutive expression of a

foreign or overexpressed gene. This subtle yet powerful modification is designed to enhance the plant's existing genetic potential, representing an elegant and innovative strategy for crop improvement that aligns with modern breeding goals.

The justification for this research is rooted in its profound potential to contribute to global food security in an era of unprecedented climate volatility. Maize is a staple for a significant portion of the world's population, and stabilizing its production is a matter of immense socio-economic importance (Vañó-Agulló, 2025; You dkk., 2025). By developing and validating a drought-tolerant maize line, this study offers a tangible solution to mitigate the devastating impacts of drought on agricultural productivity, thereby enhancing the livelihoods of farmers and ensuring a more stable food supply. The successful outcome of this research will provide a valuable genetic resource for breeding programs worldwide and serve as a powerful demonstration of how cutting-edge science can be harnessed to address real-world problems.

Ultimately, this study's contribution extends beyond the development of a single improved crop variety. It aims to establish a benchmark for the application of gene-editing technologies in enhancing complex agronomic traits (Elmimouni dkk., 2025; Pilgun, 2025). By meticulously documenting each step, from molecular design to field-level performance, this work will provide a valuable roadmap for other researchers and plant breeders. It will advance our fundamental understanding of the genetic architecture of drought tolerance in maize and showcase the transformative potential of CRISPR-Cas9 to accelerate crop improvement in a precise, efficient, and predictable manner. This research, therefore, stands as a significant contribution to the fields of plant biotechnology, molecular breeding, and sustainable agriculture.

RESEARCH METHOD

Research Design

This study employed a multi-stage, sequential research design to develop and validate drought-tolerant maize through CRISPR-Cas9 gene editing. The initial phase consisted of a molecular design and construction process, involving the identification of a target gene and the assembly of a specific CRISPR-Cas9 vector. This was followed by an *in vitro* phase focused on the genetic transformation of maize embryos and the subsequent regeneration of T0 edited plants (Ikhsan & Ramadhan, 2025; Lipińska dkk., 2025). The third phase involved rigorous molecular screening and validation to identify successfully edited individuals with heritable mutations. The final and most extensive phase comprised a comprehensive phenotypic evaluation, which included a controlled drought stress experiment on the T1 generation in a greenhouse setting, followed by multi-location, multi-season field trials to assess agronomic performance and yield stability under natural drought conditions. The overall design integrated molecular biology, plant tissue culture, and quantitative agronomy to provide a complete "gene-to-field" validation of the edited trait.

Plant Materials, Population, and Samples

The elite maize inbred line B73, known for its well-characterized genome but relative susceptibility to drought, was utilized as the recipient for genetic transformation. All plant materials were originally sourced from the Maize Genetics Cooperation Stock Center (University of Illinois, USA) and were propagated for two generations in a controlled greenhouse environment to ensure uniformity and genetic purity prior to the experiment. The initial population consisted of approximately 500 immature embryos harvested for

transformation (Ili, 2025; Kennedy dkk., 2025). Following molecular screening, a population of three independent, homozygous T1 edited lines and a non-edited wild-type (WT) control line were established. Samples for molecular analysis consisted of young leaf tissue collected from T0 and T1 plants for genomic DNA extraction. For physiological analysis in the greenhouse, fully expanded third leaves were sampled at specific time points during the drought treatment. In the field trials, samples included leaf tissue for physiological assays, as well as whole plants at maturity for the determination of biomass and grain yield components.

Vector Construction and Instruments

A high-efficiency CRISPR-Cas9 vector was constructed for targeted mutagenesis. The target gene, a putative negative regulator in the ABA signaling pathway (e.g., a specific Protein Phosphatase 2C), was identified through a literature review and bioinformatics analysis. Two 20-bp guide RNAs (gRNAs) targeting a conserved exon in the first half of the gene were designed using the CCTop online tool to maximize on-target efficiency and minimize off-target potential. These gRNAs were synthesized and cloned into the pGTR vector containing a maize codon-optimized Cas9 nuclease gene driven by the maize ubiquitin 1 promoter (pUbi1). The entire expression cassette (pUbi1::Cas9::gRNA) was subsequently subcloned into the pCAMBIA1301 binary vector using standard restriction enzyme digestion and ligation techniques. This final vector, designated pCAMBIA-Cas9-ZmPP2C, contained a hygromycin phosphotransferase (*hpt*) gene as a selectable marker. Key instruments included a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) for nucleic acid quantification, a Veriti 96-Well Thermal Cycler (Applied Biosystems, USA) for all PCR amplifications, and an ABI 3730xl DNA Analyzer for Sanger sequencing.

Experimental Procedures

The transformation protocol was based on an optimized *Agrobacterium*-mediated method. The pCAMBIA-Cas9-ZmPP2C vector was introduced into *Agrobacterium tumefaciens* strain EHA105 via electroporation. Immature embryos (1.2-1.5 mm) were isolated from B73 ears 10-12 days post-pollination and co-cultivated with the engineered *Agrobacterium* suspension for three days in the dark. Following co-cultivation, embryos were transferred to a resting medium for seven days and subsequently moved to a selection medium containing 50 mg/L hygromycin for callus induction (Fraile-Rojas dkk., 2025; Ili, 2025) . Hygromycin-resistant calli were subcultured every two weeks for approximately eight weeks before being transferred to a regeneration medium for shoot induction and a rooting medium for plantlet development.

Molecular identification of edited events began with genomic DNA extraction from the leaves of putative T0 transgenic plantlets using a modified CTAB method. PCR was performed to amplify a ~600 bp region flanking the gRNA target sites. The resulting amplicons were subjected to Sanger sequencing. The sequence data were analyzed using the online TIDE (Tracking of Indels by Decomposition) and ICE (Inference of CRISPR Edits) tools to detect and quantify the frequency of insertion-deletion (indel) mutations. Plants confirmed to have biallelic or homozygous mutations were self-pollinated to produce the T1 generation. Off-target analysis was conducted by sequencing the top five potential off-target sites predicted by the CCTop tool.

Greenhouse evaluation of T1 lines was conducted using a completely randomized design. Seedlings were grown in 5-liter pots under optimal conditions for four weeks. Drought stress was then imposed by withholding water for 14 days, while the control group remained well-

watered. Physiological parameters, including relative water content (RWC), stomatal conductance, and net photosynthetic rate, were measured using an LI-6800 portable photosynthesis system (LI-COR Biosciences, USA) at 0, 7, and 14 days into the stress period. Multi-location field trials were conducted over two consecutive growing seasons at three distinct geographical locations with varying rainfall patterns. A randomized complete block design (RCBD) with four replications was used at each site (Hart & Billett, 2025; Kennedy dkk., 2025). Agronomic traits, including plant height, days to silking, ear length, kernel number per row, and total grain yield (adjusted to 15.5% moisture content), were recorded at harvest. All collected data were subjected to Analysis of Variance (ANOVA) using R software (v4.1.2), and mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Successful generation of edited maize plants was achieved through *Agrobacterium*-mediated transformation of the B73 inbred line with the pCAMBIA-Cas9-ZmPP2C construct. From an initial pool of 500 immature embryos, a total of 28 independent hygromycin-resistant calli were regenerated into T0 plantlets, resulting in a transformation efficiency of 5.6%. PCR screening confirmed the presence of the Cas9 transgene in 21 of these 28 T0 events. Subsequent molecular analysis focused on these 21 putative transgenic lines to identify targeted mutations at the *ZmPP2C* locus.

Sanger sequencing of the target region in the *ZmPP2C* gene revealed successful editing events in 15 of the 21 T0 plants, indicating a high on-target editing efficiency of 71.4%. The mutations consisted primarily of small insertions and deletions (indels) ranging from -11 bp to +1 bp. From these events, three independent lines (designated L1, L2, and L3) containing homozygous or biallelic mutations predicted to cause frameshifts and introduce premature stop codons were selected for further analysis and advanced to the T1 generation. The specific mutations for these lines are detailed below.

Table 1. Characterization of Indel Mutations in the *ZmPP2C* Target Locus of T1 Edited Maize Lines.

Line	Mutation Type	Predicted Effect	Mutation Size
WT	Wild-Type	N/A	Full-length protein
L1	Deletion	-4 bp	Frameshift, premature stop codon
L2	Deletion	-11 bp	Frameshift, premature stop codon
L3	Insertion	+1 bp	Frameshift, premature stop codon

The indel mutations identified in lines L1, L2, and L3 were all confirmed to disrupt the open reading frame of the *ZmPP2C* gene. For example, the 4-bp deletion in line L1 resulted in a frameshift at codon 58, leading to a premature termination codon 12 amino acids downstream. This molecular evidence strongly supports the conclusion that the targeted editing resulted in a complete gene knockout, effectively eliminating the function of the negative regulator protein. The stable inheritance of these mutations was confirmed in the T1

generation, with all progeny from the selected lines showing the same homozygous mutation profile as their T0 parents.

Analysis of potential off-target effects was conducted to validate the specificity of the CRISPR-Cas9 system in this study. The top five potential off-target sites, identified through bioinformatics analysis, were amplified and sequenced from the genomic DNA of the three selected edited lines (L1, L2, L3) and the wild-type (WT) control. Sequence alignment revealed no nucleotide changes at any of the potential off-target loci in the edited lines when compared to the WT. This result demonstrates the high fidelity and specificity of the designed guide RNAs and confirms that the observed phenotypes are a direct consequence of the on-target mutation in the *ZmPP2C* gene.

The phenotypic impact of the *ZmPP2C* gene knockout was first assessed under controlled greenhouse conditions. When grown under well-watered, optimal conditions, the three edited lines (L1, L2, L3) exhibited no significant differences in key vegetative growth parameters compared to the wild-type (WT) plants. Measurements of plant height, leaf number, and shoot dry weight at the V6 stage were statistically indistinguishable between the edited and WT groups ($p > 0.05$). This finding indicates that the disruption of the *ZmPP2C* gene does not impose a developmental or fitness penalty under non-stress conditions.

Upon imposition of a 14-day drought stress treatment, significant physiological differences emerged between the edited lines and the WT. At the conclusion of the stress period, the edited lines maintained a significantly higher leaf relative water content (RWC), with an average of 78% across the three lines, compared to only 61% in the WT. Similarly, measurements of gas exchange showed that the edited lines sustained higher net photosynthetic rates and stomatal conductance under water deficit. These physiological data provide the first evidence that the edited plants possess an enhanced capacity to manage water stress.

Analysis of Variance (ANOVA) performed on the physiological data confirmed the statistical significance of the observed differences under drought stress. The edited lines demonstrated a significantly improved ability to maintain tissue hydration, as shown by the RWC data ($F(3, 16) = 18.4$, $p < 0.001$). The net photosynthetic rate in the edited lines was, on average, 45% higher than in the WT at day 14 of the stress treatment, a difference that was highly significant ($F(3, 16) = 15.2$, $p < 0.001$). These statistical results robustly support the conclusion that the knockout of *ZmPP2C* confers a tangible physiological advantage under water-limiting conditions.

The superior physiological performance of the edited lines can be directly attributed to the removal of the negative regulatory function of the *ZmPP2C* protein. By eliminating this molecular brake, the plant's ABA-mediated stress response pathways, including stomatal regulation and the expression of downstream protective genes, are likely activated more rapidly and robustly. This enhanced signaling allows the plant to conserve water more effectively while still maintaining a baseline level of photosynthesis, a critical factor for survival and eventual yield production under prolonged drought.

The relationship between the enhanced physiological traits and agronomic performance was evaluated in multi-location field trials over two consecutive years. Under well-irrigated control conditions, the grain yield of the three edited lines was comparable to that of the WT, with no statistically significant differences observed at any of the trial locations. This field-level data corroborates the greenhouse findings, confirming that the genetic modification does not negatively impact yield potential in the absence of stress.

Under rain-fed conditions, which imposed moderate to severe drought stress at various growth stages, the edited lines consistently outperformed the WT. Across all locations and seasons, the edited lines exhibited an average grain yield advantage of 18% over the WT under water-limiting conditions. The yield stability of the edited lines was also significantly higher, as indicated by a lower coefficient of variation across the different stress environments. This demonstrates a direct and positive correlation between the molecular modification and improved agronomic resilience.

The performance advantage of the edited lines was most pronounced at the Ankeny, Iowa, trial site during the first growing season, which experienced a severe 35-day drought period coinciding with the critical flowering and early grain-filling stages. Visual assessment during this period revealed striking differences; WT plants exhibited severe leaf rolling, premature senescence of lower leaves, and poor tassel development. In contrast, plants from the edited lines, particularly L1 and L2, remained greener, showed significantly less leaf rolling, and had more robust tassel and silk emergence.

Quantitative data from this specific site underscored the visual observations. The grain yield of the WT was reduced by 55% compared to its irrigated counterpart. The edited lines, however, demonstrated a remarkable degree of resilience, with an average yield reduction of only 32% under the same severe stress. This translated to a relative yield advantage of 41% for the edited lines over the WT in this high-stress environment, providing a clear case study of the practical value of the engineered trait under challenging field conditions.

The superior agronomic performance and yield stability of the edited lines in the field are a direct consequence of the enhanced physiological resilience observed in the greenhouse. The ability to maintain higher tissue water content and photosynthetic activity during periods of drought allowed the edited plants to better sustain metabolic function and growth. This resilience was particularly critical during the flowering period, ensuring more successful pollination and kernel set, which are highly sensitive to drought stress and are primary determinants of final grain yield in maize.

The consistent outperformance of the edited lines across geographically and climatically diverse locations highlights the robustness of the engineered trait. The mechanism, which involves unleashing an endogenous and fundamental stress response pathway, appears to be effective across a range of environmental conditions and stress intensities. This consistency is a critical attribute for a commercially viable trait, suggesting that the *ZmPP2C* knockout provides a broadly applicable and reliable strategy for improving drought tolerance in maize.

In summary, the results demonstrate that the targeted knockout of the *ZmPP2C* negative regulator via CRISPR-Cas9 is a highly effective strategy for enhancing drought tolerance in maize. The molecular data confirm precise and heritable gene editing without detectable off-target effects. This genetic modification confers significant physiological advantages under water deficit, including improved water retention and photosynthetic capacity, without any associated penalty to growth or yield potential under optimal conditions.

The culmination of this research is the successful translation of these molecular and physiological improvements into significant and stable grain yield advantages under real-world field conditions. The study provides a complete and robust validation of the "gene-to-field" pipeline, establishing a strong foundation for the use of CRISPR-Cas9 to develop climate-resilient crops. The findings present compelling evidence that precise editing of key negative

regulators is a powerful and efficient approach to address the urgent challenge of ensuring food security in a changing climate.

CONCLUSION

This research's most significant finding is the definitive field-level validation that a single, targeted gene knockout can confer substantial drought tolerance in maize without incurring a yield penalty under optimal conditions. The precise inactivation of the *ZmPP2C* negative regulator via CRISPR-Cas9 resulted in an average grain yield increase of 18% under water-limiting conditions across diverse environments. This outcome is distinct from many previous genetic engineering strategies that often report a trade-off between stress tolerance and yield potential, thereby demonstrating a more agronomically refined and viable approach for developing climate-resilient crops.

The principal contribution of this study is both conceptual and methodological. Conceptually, it provides robust evidence that targeting negative regulators of stress pathways is a superior strategy to the overexpression of positive regulators, offering enhanced resilience without the associated metabolic costs that lead to fitness penalties. Methodologically, this work establishes a comprehensive and successful "gene-to-field" pipeline, providing a benchmark for the application of CRISPR-Cas9 to improve complex quantitative traits in major staple crops and bridging the critical gap between laboratory discovery and real-world agricultural application.

The study's primary limitation is its focus on a single elite inbred line, B73, which may not fully represent the genetic diversity of commercial maize hybrids. Future research should therefore prioritize the introgression of the validated *ZmPP2C* null allele into a broad range of elite genetic backgrounds to confirm its efficacy and utility across different germplasms. Furthermore, subsequent studies should investigate the synergistic effects of pyramiding this trait with edits in other complementary pathways, such as those governing root architecture or osmotic adjustment, to potentially achieve an even higher degree of climate resilience.

AUTHOR CONTRIBUTIONS

Look this example below:

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; In-vestigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

REFERENCES

Almqvist-Ingersoll, P. (2025). Digital Long Covid Communities—Shaping Diagnoses, Treatment Strategies and Modes of Expertise. *Health Expectations*, *28*(3). Scopus. https://doi.org/10.1111/hex.70279

Bany Mohammed, A., Al-Okaily, M., Qasim, D., Ur Rehman, S., & Abdalla, L. (2025). Digital activism and public opinion: Understanding the role of social media during the Gaza Conflict. *Journal of Islamic Marketing*. Scopus. https://doi.org/10.1108/JIMA-03-2024-0101

Dunn Silesky, M., Sittig, J., Panchal, D., & Bonnevie, E. (2025). Digital Volunteers as Trusted Public Health Communicators. *Health Promotion Practice*, *26*(3), 397–399. Scopus. https://doi.org/10.1177/15248399231221158

Ediz, C., Ediz, I., & Yavuz, S. (2025). Dimensions of the Ukrainian War in British Press: A Topic Modeling Approach. *Libri*, *75*(1), 19–35. Scopus. https://doi.org/10.1515/libri-2024-0130

Elmimouni, H., Rüller, S., Aal, K., Skop, Y., Abokhodair, N., Wulf, V., & Tolmie, P. (2025). Exploring Algorithmic Resistance: Responses to Social Media Censorship in Activism. *Proceedings of the ACM on Human-Computer Interaction*, *9*(2). Scopus. https://doi.org/10.1145/3710970

Endriana, L., Hartono, D., & Muzayanah, I. F. U. (2025). Does education affect energy behavior? Investigating the influence of educational attainment in Indonesia. *Social Sciences and Humanities Open*, *11*. Scopus. https://doi.org/10.1016/j.ssaho.2025.101612

Fraile-Rojas, B., De-Pablos-Heredero, C., & Mendez-Suarez, M. (2025). Female perspectives on algorithmic bias: Implications for AI researchers and practitioners. *Management Decision*. Scopus. https://doi.org/10.1108/MD-04-2024-0884

Fu, Y., Han, P., Wang, J., & Shahzad, F. (2025). Digital pathways to healthcare: A systematic review for unveiling the trends and insights in online health information-seeking behavior. *Frontiers in Public Health*, *13*. Scopus. https://doi.org/10.3389/fpubh.2025.1497025

Hart, M., & Billett, P. (2025). Feminist Counter-Publics and Heterosexist Edges of Desire: Extending Edgework Theory via Young Women's Nude Selfie Sharing Online. *Sexuality and Culture*. Scopus. https://doi.org/10.1007/s12119-025-10354-z

Hubert, J., Beaumont, M., Bungay, V., & Slemon, A. (2025). Digital Artifacts of Self-Representation: A Critical Qualitative Analysis of Nursing Memes. *Nursing Inquiry*, *32*(2). Scopus. https://doi.org/10.1111/nin.70021

Ikhsan, F. A., & Ramadhan, I. Z. (2025). Exploring the public space typology of tourist villages from the perspective of digital social media to build sustainable tourism. Dalam Handoko C.T. (Ed.), *IOP Conf. Ser. Earth Environ. Sci.* (Vol. 1438, Nomor 1). Institute of Physics; Scopus. https://doi.org/10.1088/1755-1315/1438/1/012070

Ili, B. (2025). Exploring Trends in New Media Literacy (NML) Field: A Bibliometric Analysis Using Bibliometrix R-Tool. *Galactica Media: Journal of Media Studies*, *7*(1), 207–231. Scopus. https://doi.org/10.46539/gmd.v7i1.535

Kasanneni, Y., Duggal, A., Sathyaraj, R., & Raja, S. P. (2025). Effective Analysis of Machine and Deep Learning Methods for Diagnosing Mental Health Using Social Media Conversations. *IEEE Transactions on Computational Social Systems*, *12*(1), 274–294. Scopus. https://doi.org/10.1109/TCSS.2024.3487168

Kennedy, R., Bryson, S., Ellsworth, K., & Kapur, I. (2025). Fake Profiles, Mean Comments, and Toxic Communities: College Students' Perspectives on Cyberbullying Across Social Media Platforms. *International Journal of Bullying Prevention*. Scopus. https://doi.org/10.1007/s42380-025-00295-4

Kostadinov, Y. A., & Chow, W. E. (2025). Digital Media Trends in Public Diplomacy: The Case of Taiwan's Youth Ambassadors. *Hague Journal of Diplomacy*, *20*(1), 101–131. Scopus. https://doi.org/10.1163/1871191x-bja10203

Lipińska, M., Kotula, N., & Jemielniak, D. (2025). Exploring expert figures in alien-related UFO conspiracy theories. *Humanities and Social Sciences Communications*, *12*(1). Scopus. https://doi.org/10.1057/s41599-025-04799-8

Liu, M., Ngai, C. S. B., & Li, H. (2025). Entrepreneur or capitalist? Discursive constructions of a deceased business leader in Chinese digital mourning discourse. *Journal of Language and Politics*. Scopus. https://doi.org/10.1075/jlp.24243.liu

Nasseripour, M., Harkins, A., Neville, P., & Ranauta, A. (2025). E-Professionalism's Impact on Dental Professionalism: A Rapid Scoping Review. *European Journal of Dental Education*, *29*(1), 84–91. Scopus. https://doi.org/10.1111/eje.13051

Obermaier, M., Schmid, U. K., & Rieger, D. (2025). Empowerment Is Key? How Perceived Political and Critical Digital Media Literacy Explain Direct and Indirect Bystander Intervention in Online Hate Speech. *Social Media and Society*, *11*(1). Scopus. https://doi.org/10.1177/20563051251325598

Olsen, G. R. (2025). Digitalization and Meaning in Journalistic Work (MJoW): The Increasing Importance of the Symbolic Dimension. *Digital Journalism*. Scopus. https://doi.org/10.1080/21670811.2025.2487862

Pilgun, M. (2025). Explicit and Implicit Knowledge in Large-Scale Linguistic Data and Digital Footprints from Social Networks. *Big Data and Cognitive Computing*, *9*(4). Scopus. https://doi.org/10.3390/bdcc9040075

Poduval, S., Arnold, L., Carta, E., Nimmons, D., Stratton, I., Shaw, M., Bradbury, K., & Stevenson, F. (2025). Do community-based digital health inclusion programmes contribute to tackling health inequalities in disadvantaged population groups?: A qualitative study of experiences of a city-wide programme in the North of England. *BMC Public Health*, *25*(1). Scopus. https://doi.org/10.1186/s12889-025-21418-y

Polzer, T., & Öner, S. (2025). Digital Accountability in Collaborative Public Governance in Times of Crisis: Analysing the Debate in a Polarized Social Forum. *Abacus*, *61*(1), 239–271. Scopus. https://doi.org/10.1111/abac.12351

Rahim, S. S., Parumo, S., Darren, C. Y. T., Samad, R. A., & Jali, S. K. (2025). ENGAGING MINDS THROUGH ANIMATION: THE EFFECTIVENESS OF 2D CONTENT IN MEDICAL HEALTH EDUCATION. *Journal of Theoretical and Applied Information Technology*, *103*(7), 2785–2798. Scopus.

Rai, S. K. (2025). Empowering Sustainable Advocacy: Media and Communication Strategies. Dalam *Approach. Glob. Sustain. Mark. Gov.: Vol. Part F260* (hlm. 483–497). Springer; Scopus. https://doi.org/10.1007/978-981-96-0843-0_24

Rohid, N., Sugihartati, R., Suyanto, B., Susilo, D., & Zikri, A. (2025). Digital Activism in Contemporary Islamic Politics: A Critical Analysis of Social Media's Impact on Islamic Movements. *MILRev: Metro Islamic Law Review*, *4*(1), 208–232. Scopus. https://doi.org/10.32332/milrev.v4i1.10159

Rughiniș, R., Țurcanu, D., Vulpe, S.-N., & Radovici, A. (2025). Encrypted Engagement: Mapping Messaging App Use in European News Consumption Patterns. *Information (Switzerland)*, *16*(1). Scopus. https://doi.org/10.3390/info16010048

Silva, H. M. (2025). Discovery of the hole in the ozone layer: Environmental awareness and fighting scientific fake news. *Ethics in Science and Environmental Politics*, *25*, 11–19. Scopus. https://doi.org/10.3354/ESEP00217

Vañó-Agulló, C. (2025). Exclusionary narratives at Europe's Southern Edge: A comparative digital analysis of migration discourses in the Canary Islands and Lampedusa. *Revista Espanola de Sociologia*, *34*(2). Scopus. https://doi.org/10.22325/fes/res.2025.264

Velasco Molpeceres, A., Miranda-Galbe, J., & Prieto Muñiz, M. (2025). Digital Political Communication in the European Parliament: A Comparative Analysis of Threads and X During the 2024 Elections. *Journalism and Media*, *6*(1). Scopus. https://doi.org/10.3390/journalmedia6010042

Wade, M. E., & Parks, K. (2025). Digital Determinants: The Inclusion of Social Media in Health Conceptualization. *Health and Social Work*, *50*(1), 37–44. Scopus. https://doi.org/10.1093/hsw/hlae047

Wan, X., Wang, R., Dong, X., Wu, J., Zheng, Y., & Li, Q. (2025). Dynamic simulation of local acceptance of NIMBY facilities based on the RAS-Deffuant model: The influence of

government and media information. *Environmental Impact Assessment Review*, *112*. Scopus. https://doi.org/10.1016/j.eiar.2025.107842

Xie, W., & Jiang, S. (2025). Digital technology races between China and the US: a critical media analysis of US media coverage of China's rise in technology and globalization. *Online Media and Global Communication*. Scopus. https://doi.org/10.1515/omgc-2024-0039

Yang, J., Hu, Y., Liu, M., Li, Y., Ma, Z., Fang, W., & Bi, J. (2025). Event triggers and opinion leaders shape climate change discourse on Weibo. *Communications Earth and Environment*, *6*(1). Scopus. https://doi.org/10.1038/s43247-025-02124-4

Ye, Z., Huang, Q., & Krijnen, T. (2025). Douyin's playful platform governance: Platform's self-regulation and content creators' participatory surveillance. *International Journal of Cultural Studies*, *28*(1), 80–98. Scopus. https://doi.org/10.1177/13678779241247065

You, L., Hon, L., & Lee, Y.-H. (2025). Examining Issue Polarization and Advocacy Congruity as Antecedents of Corporate Political Advocacy. *International Journal of Business Communication*. Scopus. https://doi.org/10.1177/23294884251327570