FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 03, (July-Sep, 2025)

Review Article

OPEN ACCESS

Next-Generation CRISPR Biotechnology for Pakistan: AI-Driven, Climate-Resilient Super Crops and the Future of Food Security

Sadaf Saeed Ullah[1*], Rabia Iqbal[2], Ayesha Ghafoor[1], Syeda Amna Batool[3], Tehmina Bashir[1] and Adnan Mehmood[4]

[1]Department of Botany, Government College University, Lahore, Pakistan
[2]Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat, Pakistan
[3]Department of Botany, University of Narowal, Narowal, Pakistan
[4]Department of Microbiology, Gulab Devi Educational Complex, Lahore, Pakistan

ARTICLE INFO

Keywords:
CRISPR, Crop Improvement, Climate Resilience, Gene Editing, Food Security, Sustainable Agriculture

How to Cite:
Ullah, S. S., Iqbal, R., Ghafoor, A., Batool, S. A., Bashir, T., & Mehmood, A. (2025). Next-Generation CRISPR Biotechnology for Pakistan: AI-Driven, Climate-Resilient Super Crops and the Future of Food Security: Next-Generation CRISPR Biotechnology for Pakistan: AI-Driven Super Crops. Futuristic Biotechnology, 5(3), 28-35. https://doi.org/10.54393/fbt.v5i3.189

***Corresponding Author:**
Sadaf Saeed Ullah
Department of Botany, Government College University, Lahore, Pakistan
sadafsaeedullah742@gmail.com

Received Date: 4th August, 2025
Revised Date: 20th September, 2025
Acceptance Date: 25th September, 2025
Published Date: 30th September, 2025

ABSTRACT

Climate change poses a significant threat to Pakistan's agriculture, with projections indicating 10–25% yield losses in staple crops by 2050. Frequent floods, prolonged droughts, and pest infestations have already reduced wheat and rice production by up to 30%, exposing the limitations of traditional breeding and genetically modified crops. CRISPR-Cas9 genome editing, when combined with artificial intelligence (AI), offers a faster and more precise route to developing climate-resilient varieties suited to Pakistan's diverse agroecosystems. A review of recent studies highlights key advances, including AI-assisted sgRNA design, which enhances editing efficiency by 30–50%, and CRISPR-modified wheat and rice lines that show 20–30% improved stress tolerance. Yet, barriers such as complex polyploid genomes, limited genomic resources, and outdated biosafety policies hinder progress. Addressing these challenges through policy reform, capacity-building, and technology integration could transform Pakistan's agriculture, aligning directly with Sustainable Development Goals on Zero Hunger and Climate Action.

INTRODUCTION

Global food security is increasingly at risk from climate change, with the IPCC projecting that yields of staple crops could decline by 10–25% by 2050. Pakistan, ranked among the ten most climate-vulnerable countries by the Global Climate Risk Index [1], faces particularly severe challenges. Recurring floods and droughts have already reduced wheat and rice yields by 15–30% [2], while cotton production suffers annual losses of around $5 billion due to the cotton leaf curl virus. Traditional plant breeding and transgenic GM crops have proven inadequate, largely because of their long development cycles, which often take 10–15 years, as well as persistent public resistance [3]. CRISPR-Cas9 offers a promising alternative, allowing precise and non-transgenic edits within just 2–5 years [4]. More recent innovations, such as prime editing [5] and epigenome editing [6], provide additional opportunities to enhance stress tolerance and climate resilience without altering DNA sequences. Artificial intelligence tools, including the AlphaFold 3, which is used in the prediction of protein structure, and drone-assisted phenotyping technologies,

DOI: https://doi.org/10.54393/fbt.v5i3.189

are creating new opportunities in the improvement of crops. However, CRISPR is still not widely used in Pakistan. In addition to the persistent problem of inadequate research funding, this is mostly because of antiquated biosafety laws that continue to classify CRISPR as a GMO under policy from 2005. Climate change is one of the significant threat factors to the agriculture sector, with increased temperatures, [7], unnecessary rainfall and a noticeable rise in pest and disease ratio, ultimately reducing productivity of crops. These shifts have made Pakistan among the more susceptible countries by offering severe risks to food security as well as sustainable farming [7]. Due to a rapid increase in population and low availability of farmland, there is more pressure on water and a reduction in crop yields, leading towards food insecurity in rural areas. Conventional breeding techniques and intensive farming techniques are insufficient to overcome these problems emphasizing the need for innovative and research-oriented solutions to strengthen agricultural adaptability [8]. CRISPR-Cas9 is among the cutting-edge developments that facilitate accurate, effective and inexpensive modifications of the genetic make-up of plants. CRISPR can enhance traits including drought resistance, pest resilience and nutritional content, without inserting foreign DNA, which was required in former gene editing methods. Implementation in food crops, including maize, rice, and wheat, has evidenced substantial improvements in yield and stress resistance [9]. CRISPR is progressively considered as a technology of transformation for organic farming and a motive power for the next agricultural revolution. Artificial intelligence offers a crucial role in agricultural systems by providing targeted interventions. By the use of tools like satellite imagery, drones, sensors and machine learning, AI is being implemented to adjust irrigation, use of nutrients, insect control and prediction of yield. In combination with CRISPR, AI can play a vital role by identifying the gene targets efficiently, enhancing phenotyping and supporting the production of climate-resistant varieties suitable for the local environment [10]. This advancement is now facilitating the production of "super crops" adapted to the specific conditions of Pakistan's varied agro-ecological regions, which are eco-friendly and provide maximum yield [11], resulting in opportunities for Productive longevity and sustainable output. There are enormous efficient benefits of the integration of CRISPR biotechnology to AI for the agricultural system in Pakistan. Shortage of food has affected 11 million people, approximately, and the climate crisis is worsening [12].

This review aims to investigate the potential benefits of using AI and CRISPR together to assist Pakistan in creating climate-resilient crops. It describes current issues, real-world uses, recent scientific developments, and wider implications for securing food security in the years to come.

CRISPR-Cas9 for Climate-Resilient Crops and Food Security in Pakistan

Ahmad and Hameed highlighted that by developing crops that are resilient to environmental stress [13], CRISPR-Cas9 can solve the interconnected problems of climate change and food security in Pakistan. Their findings suggested that through precise gene editing, the resilience and yield can be increased by modifying the OsCKX gene in rice for drought tolerance and the R22M59 gene in maize for heat resistance. However, there are some research gaps in this study, including local crops negligence and risks of regional climate, including droughts and floods. The Widespread adoption is also restricted by the limited access of smallholders. To overcome these barriers, there is a demand for the development of region-specific crops via integrated climate and genetic research.

CRISPR-Cas9 Advancements in Crop Improvement

Kaur *et al.* demonstrated that stress tolerance and yield in the crops, including wheat, rice, and maize, have increased significantly by CRISPR/Cas9 with greater precision and efficiency than traditional methods [14]. But its wider implementation has been limited due to some challenges, including editing complex genomes and minimizing off-target effects and regulatory uncertainty, the absence of integration with advanced tools like AI and the limited acceptance of the public. To deploy CRISPR for the enhancement of climate resilience in vulnerable regions like Pakistan, the overcoming of these technical, scientific and policy hurdles is crucial.

AI and Machine Learning in CRISPR Design

According to Chuai *et al.* the genome editing is improved by Deep-CRISPR via a hybrid neural network for the accurate prediction of sgRNA efficiency and effects of genome-wide off-target [15]. As compared to methods used in the past, it learns from billions of data points that are unlabeled and integrates epigenetic data by automatically identifying key features without any need for manual design. This effectively resolves the common data imbalances and increases prediction accuracy across different cell types. Despite progress, challenges remain, including the lack of high-quality labelled data for on- and off-target effects, limited deep learning architectures, and underutilization of multiomic integration. Translating AI-based sgRNA design into key Pakistani crops, combined with field-level phenotypic and climate data, is underexplored. Addressing these gaps is essential to harnessing AI-enhanced CRISPR for developing climate-resilient super crops. Data extraction focused on CRISPR tools, AI methods, target genes, and phenotypic outcomes, followed by thematic synthesis and expert validation.

DOI: https://doi.org/10.54393/fbt.v5i3.189

Evolution of CRISPR Tools: Key Effector Proteins

The CRISPR-Cas system has evolved into a flexible genome-editing tool over time. Because of their comparatively straightforward single-protein construction, Class 2 effectors, particularly Cas9, Cas12, and Cas13, have emerged as the most often used tools among their many classes [16]. Although RNA-guided nucleic acid cleavage is the basis for all three Cas9, Cas12, and Cas13, their classification, sequence recognition, and modes of activity vary. Cas13 is unique in that it can target RNA, while Cas9 and Cas12 primarily function as DNA-targeting nucleases. This variety has significantly broadened the CRISPR toolkit, enabling new therapeutic methods, transcriptome engineering, diagnostic tools, and genome editing [17, 18]. These effector proteins' architectures, recognition requirements (PAM/PFS), and biological uses are compared to demonstrate how CRISPR has evolved from a bacterial defensive mechanism to one of the most revolutionary developments in contemporary biotechnology, as shown in table 1.

Table 1: Key Characteristics and Comparative Overview of the Cas9, Cas12, and Cas13 CRISPR Systems

Features	Cas9	Cas12	Cas13	References
Class/Type	Class 2, Type II	Class 2, Type V	Class 2, Type VI	[16]
Origin	*Streptococcus pyogenes* (SpyCas9)	*Acidaminococcus* (AsCas12a), *Lachnospiraceae* (LbCas12a)	*Leptotrichia shahii* (LshCas13a), *Prevotella* (PspCas13b)	[17, 18]
Target	dsDNA (blunt cut)	dsDNA & ssDNA (staggered cut)	ssRNA	[18, 19]
PAM/PFS	5′-NGG-3′ PAM	5′-TTTV-3′ PAM	3′ PFS (A/U/C for Cas13a); 5′ PFS (A/U/G for Cas13b)	[20, 21]
Applications	Gene knockout, base editing	Multiplex editing, diagnostics (e.g., DETECTR)	RNA knockdown, viral detection (e.g., SHERLOCK)	[19, 22]
gRNA Requirement	crRNA + tracrRNA (sgRNA)	Self-processes pre-crRNA (no tracrRNA)	Single crRNA (no tracrRNA)	[19, 23]
Domains	HNH (cuts target strand), RuvC (cuts non-target strand)	RuvC (cleaves both strands)	Two HEPN RNase domains (ssRNA cleavage)	[21, 24]
Key Mechanism	Binds PAM → R-loop formation → HNH/RuvC cleavage	RuvC domain cleaves the non-target strand first	Binds target ssRNA → HEPN activation → collateral RNA cleavage	[24, 25]

CRISPR Success Stories in Global Agriculture

Rahim *et al.* demonstrated the potential of CRISPR/Cas9 in enhancing wheat drought tolerance by targeting the TaRPK1 gene, which regulates stress response and root development [26]. Two gRNA constructs (LR-1 and LR-2) were designed to edit conserved regions across the A, B, and D sub-genomes, introducing insertions and deletions that altered root structure. The modified wheat plants developed deeper, longer roots with increased surface area and penetration ability, enabling improved water and nutrient uptake under drought. Compared to wild types, edited lines showed a 20–30% increase in root depth and volume, reduced diameters, and steeper orientations, which collectively enhanced drought resilience. Importantly, these traits did not compromise yield; rather, grain mass and stem count improved, and although spike length slightly decreased, it was compensated for by gains in other yield components. At the policy level, regulation of genome-edited crops remains a challenge. The Cartagena Protocol on Biosafety requires member states to regulate living modified organisms [27], and Pakistan enforces this through the Biosafety Rules (2005, amended 2024) under the National Biosafety Center [28]. Current rules largely classify genome-edited organisms with foreign DNA as GMOs, while edits without foreign DNA face regulatory ambiguity. In contrast, India exempts SDN-1 and SDN-2 edits from strict GMO rules [29], and China applies a streamlined, less restrictive framework. Compared to these evolving approaches, Pakistan's rigid system may hinder the integration of CRISPR-based innovations into agriculture, despite their potential to enhance food security.

CRISPR-Enabled Biofortification: Addressing Hidden Hunger in Staple Crops

Vitamin A deficiency is a severe public health issue in regions where rice is the main staple. Introduction of (β-carotene) provitamin A in the endosperm of rice was done by the insertion of phytoene desaturase (CRTI) and phytoene synthase (PSY) genes with the help of transgenic methods. The development of Golden rice led to the initial achievements of biofortification as a result of these modifications [30]. With the carotenoid concentrations of 37 μg/g, the second-generation Golden Rice 2 (GR2) performed 23 times better than the initial prototype [31]. Although GR2 is based on transgenic methods, a faster and accurate biofortification can be achieved by CRISPR/Cas9, where there is no need for foreign DNA with a better and enhanced trait enrichment [30]. CRISPR has shown promising results in rice biofortification. Editing vacuolar iron transporter genes (OsVIT1/2) increased iron and zinc concentrations by 1.8- and 1.4-fold, respectively, while modifying the OsNAS promoter enhanced iron absorption 3.7-fold and zinc twofold [32]. Multi-nutrient strategies, such as in the CP105 rice line, combined edits in ferritin, OsNAS1, and PSY, producing 2.69 μg/g of β-carotene along with 1.5-fold higher iron and 1.2-fold higher zinc [30, 32].

DOI: https://doi.org/10.54393/fbt.v5i3.189

Additionally, CRISPR-mediated silencing of the IPK1 gene reduced phytic acid by 46%, improving mineral bioavailability[33].

AI-Driven CRISPR: Synergies and Innovations in Crop Improvement

The integration of artificial intelligence (AI) with CRISPR-based genome editing is transforming agricultural biotechnology by improving accuracy, scalability, and efficiency. AI tools assist in identifying high-value gene targets, minimizing off-target effects, and expediting the development of crops with higher yields, improved nutrition, and resilience against stresses [34]. A major challenge in CRISPR-Cas9 editing is the design of single-guide RNAs (sgRNAs), which were traditionally chosen using basic parameters like GC content and PAM proximity. AI-driven approaches now leverage large datasets to predict highly efficient sgRNAs, reducing trial-and-error and broadening CRISPR's application to complex genomes. Beyond design, AI also enables the discovery of stress-associated genes and networks through analysis of multi-omics datasets, as demonstrated in the identification of ARGOS8 in maize for drought tolerance [34] and DEP1 in wheat for nitrogen-use efficiency.

AI–CRISPR synergy also supports metabolic engineering by integrating transcriptomic and metabolomic profiles to uncover genes linked to valuable secondary metabolites. For example, AI-guided CRISPR modification of the PAPI gene in tomato increased anthocyanin accumulation, stress tolerance, and nutritional value [34, 35]. These advances demonstrate how AI not only enhances precision but also enables targeted and impactful interventions across diverse crops. Ultimately, the convergence of AI and CRISPR holds promise for delivering safer, more reliable, and climate-resilient crop varieties tailored to both productivity and nutritional security. Findings are shown in table 2.

Table 2: AI Tools for CRISPR sgRNA Design

Tool (Year)	Methods	Key Features	Performance	Limitations	References
DeepCRISPR (2018)	Deep Learning (DCDNN)	Predicts on/off-target effects, Trained on 0.68B sgRNAs (13 cell lines)	AUROC = 0.804 (outperforms traditional tools)	Requires large datasets	[15]
CRISPRscan (2015)	Gradient Boosting (ML)	Incorporates sequence context, chromatin accessibility, Validated in zebrafish/human cells	High in vivo accuracy	Less effective for epigenome editing	[36]
CRISPRon (2021)	Deep Learning	Integrates multi-omics data, Accounts for Cas9 variants, repair pathways (NHEJ/MMEJ)	Superior to rule-based tools (e.g., CRISPOR)	Struggles with repair-induced indels	[37]
TIGER (2023)	CNN	Analyzes sgRNA mismatches, Tested on 200K RfCas13d gRNAs	Best at distinguishing essential vs. non-essential genes	Limited to Cas13 systems	[38]
DeepHF (2019)	Bidirectional LSTM (Bi-LSTM)	Combines RNN + biological features,Optimized for high-fidelity Cas9	Spearman R = 0.867	Sparse endogenous validation data	[38]

Case Study: CRISPR Editing for Maize Yield Improvement

Maize, a vital staple crop, faces yield limitations from drought and low soil nitrogen, with genetic trade-offs making it difficult to balance stress tolerance and productivity despite advances in conventional breeding [40]. To address this, Corteva Agriscience applied CRISPR-Cas9 and advanced data analysis to identify key genes for targeted improvement [41]. A large-scale genomic assessment of 1,671 maize genes revealed 22 candidates linked to drought tolerance, yield, and nitrogen use efficiency [42]. Among them, ARGOS8, which regulates ethylene signalling, was edited with CRISPR-Cas9 to enhance expression in maize lines [43]. Field trials showed that edited plants achieved a 5–10% yield advantage under drought and low-nitrogen stress without unintended genetic alterations, confirming both efficacy and safety [41]. In Pakistan, where maize is the third major cereal after wheat and rice, similar challenges persist. Local studies have identified drought-tolerant genotypes such as "Jalal" in Balochistan using molecular markers [44] and significant genetic diversity in inbred lines from Murree for traits relevant to stress resilience. Nutrient use efficiency is another priority, with field trials in Faisalabad demonstrating improved yields through combined nitrogen and phosphorus application [45], while nitrogen response trials in Gilgit highlighted genotype-specific adaptability, with the "Pahari" variety performing best under High-N regimes [46]. Although large-scale CRISPR applications in Pakistan are yet to be realized, these findings provide a strong foundation for integrating genome editing to enhance drought tolerance, early maturity, and NUE, thereby strengthening maize productivity under the country's climate-vulnerable and resource-limited farming systems.

Strategies to Minimize Off-Target Effects in CRISPR

One of the major issues with the CRISPR-Cas9 is the chance of unintentional changes at non-target sites. However, due to modern advancements in computational tools, the ability to anticipate and prevent these errors has substantially improved. Models that improve the design of guide RNA (gRNA) and have been used to evaluate sequence specificity and several other general genomic features are increasingly employed. In contrast to previous techniques, platforms like DeepSpCas9 and CRISPR have

DOI: https://doi.org/10.54393/fbt.v5i3.189

decreased off-target activity by up to 90%. These were developed to utilize extensive screening datasets [47].

Key Approaches in Off-Target Control

Key approaches to controlling off-target effects in CRISPR include predictive modelling, editing optimization, and high-throughput screening. Algorithms that consider sequence homology, chromatin accessibility, and DNA methylation, along with convolutional neural networks (CNNs) for gRNA-DNA binding and recurrent neural networks (RNNs) for repair simulation, help predict off-target binding [48]. Base and prime editing allow precise single-nucleotide modifications without double-strand breaks, with cytosine deaminases refined to reduce unintended edits [49]. Additionally, large CRISPR libraries combined with computational methods facilitate high-throughput screening to identify synthetic lethal interactions and validate gRNA efficiency across different genetic backgrounds [50].

Climate Threats to Staple Crops in Pakistan: Punjab and Sindh

In Pakistan, climate extremes are threatening key crops. In Punjab, rising temperatures and heatwaves, particularly during wheat's grain-filling stage, reduce yields by up to 9%, with sudden autumn frosts cutting output by over 16% [51]. Heat-tolerant wheat varieties and improved irrigation can mitigate losses, with irrigated fields experiencing up to 70% less damage. However, climate models predict further declines in the rice–wheat system by mid-century [52]. In Sindh, floods pose a major risk to rice, with the 2022 floods inundating 2.8 million hectares and destroying 1.9 million tons of rice [53]. Prolonged flooding disrupts photosynthesis and root function, threatening both food security and export earnings.

Projected Impacts Under IPCC Scenarios

Climate projections based on IPCC's CMIP6 scenarios (SSP245 and SSP585) show mounting pressure on crop water requirements (CWR) across Pakistan's agricultural zones. By the late 21st century, Kharif (summer) rice: CWR is expected to rise by 8–14% under the high-emission SSP585 pathway, worsening water scarcity [54]. Rabi (winter) wheat: CWR may increase by 12–15% under SSP585, intensifying stress on already limited irrigation supplies. Without timely interventions, these changes could trigger steep declines in staple crop yields. Three main adaptation measures are frequently mentioned by experts: increasing the effectiveness of irrigation, expanding the use of climate-resilient crop types, and adjusting cropping calendars to better accommodate changing seasonal patterns.

Challenges and Future Directions

Despite rapid advances, several barriers limit the application of CRISPR and AI in agriculture. Wheat's complex allohexaploid genome complicates guide RNA design and raises off-target risks, while low transformation efficiency restricts commercial use [55]. AI models trained on limited datasets often fail to capture in vivo conditions or regional crop diversity, and locally curated genomic resources remain scarce in Pakistan, reducing the relevance of global models [56]. Socioeconomic constraints, such as low farmer literacy, poor digital access, and affordability, further slow adoption. Moreover, the absence of a clear regulatory framework contrasts with structured policies in China and the EU, discouraging investment and delaying innovation [57]. To harness CRISPR and AI for climate resilience and food security, Pakistan must integrate scientific, technological, and policy solutions. Priorities include establishing a science-based regulatory framework, developing national genomic databases for major crops, and strengthening rural digital infrastructure to enable AI-driven advisory systems [58]. Public engagement campaigns and farmer training programs through universities and extension services can build awareness and capacity, while targeted subsidies or financing schemes may enhance access for smallholders. International collaborations with countries such as China and EU members would also facilitate knowledge exchange and strengthen governance. These steps, combined with advances in explainable AI and CRISPR-based crop improvement, can accelerate the development of resilient, nutritionally enhanced crops, securing Pakistan's agricultural future [33, 59].

CONCLUSION

The integration of CRISPR-Cas9 and artificial intelligence (AI) offers a transformative pathway for developing climate-resilient crops in Pakistan. Early studies demonstrate significant promise: TaRPK1 editing has generated drought-tolerant wheat, while Sub1A alteration has produced flood-tolerant rice, together capable of mitigating 15–30% yield losses under stress. AI strengthens these applications by refining sgRNA design and integrating multi-omics datasets, with platforms such as Deep-CRISPR providing greater precision. Yet major barriers remain, as wheat's complex polyploid genome complicates precise editing, limited local genomic datasets weaken AI model performance, and socioeconomic constraints, such as digital illiteracy, cost, and regulatory ambiguity, particularly the treatment of CRISPR products as GMOs, restrict adoption. In summary, evidence indicates that AI can enhance sgRNA design while CRISPR offers tangible yield and resilience benefits, but scientific, infrastructural, and policy barriers persist. To bridge these gaps, Pakistan must modernize biosafety frameworks to distinguish gene editing from transgenics, invest in local genomic resources and digital infrastructure, and conduct pilot-scale field trials in vulnerable regions such as Punjab and Sindh. International

DOI: https://doi.org/10.54393/fbt.v5i3.189

collaborations and farmer-focused training initiatives will be essential to ensure equitable adoption. By addressing these challenges, Pakistan can accelerate the safe and effective deployment of CRISPR–AI innovations, advancing both national food security and global goals like Zero Hunger and Climate Action under the 2030 Agenda.

Authors Contribution

Conceptualization: SSU, RI, AG, TB
Methodology: SSU, RI, AG, SAB
Formal analysis: SAB, TB, AM
Writing review and editing: SSU, RI, AG, TB, AM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Germanwatch. Global Climate Risk Index. 2025. Available at: https://www.germanwatch.org/en/cri.

[2] Pakistan Agricultural Research Council (PARC). Climate-Smart Agriculture: National Adaptation Plan for Pakistan. Government of Pakistan. 2023.

[3] Zafar Y. Development of Agriculture Biotechnology in Pakistan. Journal of Association of Official Analytical Chemists International. 2007 Sep; 90(5): 1500-7. doi: 10.1093/jaoac/90.5.1500.

[4] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science. 2012 Aug; 337(6096): 816-21. doi: 10.1126/science.1225829.

[5] Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM *et al*. Search-and-Replace Genome Editing without Double-Strand Breaks or Donor DNA. Nature. 2019 Dec; 576(7785): 149-57. doi: 10.1038/s41586-019-1711-4.

[6] Gallego-Bartolomé J, Gardiner J, Liu W, Papikian A, Ghoshal B, Kuo HY *et al*. Targeted DNA Demethylation of the Arabidopsis Genome Using the Human TET1 Catalytic Domain. Proceedings of the National Academy of Sciences. 2018 Feb; 115(9): E2125-34. doi: 10.1073/pnas.1716945115.

[7] Aziz A. Climate Change: A Growing Challenge for Food Security in Pakistan. Social Science Review Archives. 2025 Feb; 3(1): 1390-402. doi: 10.70670/sra.v3i1.442.

[8] Watson A, Ghosh S, Williams MJ, Cuddy WS, Simmonds J, Rey MD *et al*. Speed Breeding Is a Powerful Tool to Accelerate Crop Research and Breeding. Nature Plants. 2018 Jan; 4(1): 23-9. doi: 10.1038 /s41477-017-0083-8.

[9] Chen F, Chen L, Yan Z, Xu J, Feng L, He N *et al*. Recent Advances of CRISPR-Based Genome Editing for Enhancing Staple Crops. Frontiers in Plant Science. 2024 Sep; 15: 1478398. doi: 10.3389/fpls.2024.1478398.

[10] Khan R and Sharma P. AI-Enabled Smart Irrigation for Climate-Resilient Agriculture. In SHS Web of Conferences. 2025; 216: 01005. doi: 10.1051/shsconf/202521601005.

[11] Chavhan RL, Jaybhaye SG, Hinge VR, Deshmukh AS, Shaikh US, Jadhav PK *et al*. Emerging Applications of Gene Editing Technologies for the Development of Climate-Resilient Crops. Frontiers in Genome Editing. 2025 Mar; 7: 1524767. doi: 10.3389/fgeed.2025.1524767.

[12] Titirmare S, Margal PB, Gupta S, Kumar D. AI-Powered Predictive Analytics for Crop Yield Optimization. In Agriculture 4.0. 2024: 89-110. doi: 10.1201/9781003570219-5.

[13] Ahmad S and Hameed MA. Harnessing CRISPR-Cas9 for Advancing Sustainable Agriculture: Precision Genome Editing to Develop Climate-Resilient and High-Yielding Crops. Biosciences Reports. 2024 Dec; 1(02): 65-81.

[14] Kaur N, Qadir M, Francis DV, Alok A, Tiwari S, Ahmed ZF. CRISPR/Cas9: A Sustainable Technology to Enhance Climate Resilience in Major Staple Crops. Frontiers in Genome Editing. 2025 Mar; 7: 1533197. doi: 10.3389/fgeed.2025.1533197.

[15] Chuai G, Ma H, Yan J, Chen M, Hong N, Xue D *et al*. Deep-CRISPR: Optimized CRISPR Guide RNA Design by Deep Learning. Genome Biology. 2018 Jun; 19(1): 80. doi: 10.1186/s13059-018-1459-4.

[16] Koonin EV, Makarova KS, Zhang F. Diversity, Classification and Evolution of CRISPR-Cas Systems. Current Opinion in Microbiology. 2017 Jun; 37: 67-78. doi: 10.1016/j.mib.2017.05.008.

[17] Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P *et al*. Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. cell. 2015 Oct; 163(3): 759-71. doi: 10.1016/j.cell.2015.09.038.

[18] Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ *et al*. RNA Targeting with CRISPR-Cas13. Nature. 2017 Oct; 550(7675): 280-4. doi: 10.1038/nature24049.

[19] Hillary VE and Ceasar SA. A Review On the Mechanism and Applications of CRISPR/Cas9/Cas12/Cas13/Cas14 Proteins Utilized for Genome Engineering. Molecular Biotechnology. 2023 Mar; 65(3): 311-25.

doi: 10.1007/s12033-022-00567-0.

[20] Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N *et al.* Crystal Structure of Cas9 in Complex with Guide RNA and Target DNA. Cell. 2014 Feb; 156(5): 935-49. doi: 10.1016/j.cell.2014.02.001.

[21] Fonfara I, Richter H, Bratovič M, Le Rhun A, Charpentier E. The CRISPR-Associated DNA-Cleaving Enzyme Cpf1 Also Processes Precursor CRISPR RNA. Nature. 2016 Apr; 532(7600): 517-21. doi: 10.1038/nature17945.

[22] Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F. Multiplexed and Portable Nucleic Acid Detection Platform with Cas13, Cas12a, and Csm6. Science. 2018 Apr; 360(6387): 439-44. doi: 10.1126/science.aaq0179.

[23] Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J *et al.* Nucleic Acid Detection with CRISPR-Cas13a/C2c2. Science. 2017 Apr; 356(6336): 438-42. doi: 10.1126/science.aam 9321.

[24] Anders C, Niewoehner O, Duerst A, Jinek M. Structural Basis of PAM-Dependent Target DNA Recognition by the Cas9 Endonuclease. Nature. 2014 Sep; 513(7519): 569-73. doi: 10.1038/nature13579.

[25] Smargon AA, Cox DB, Pyzocha NK, Zheng K, Slaymaker IM, Gootenberg JS *et al.* Cas13b is a Type VI-B CRISPR-Associated RNA-Guided Rnase Differentially Regulated by Accessory Proteins Csx27 and Csx28. Molecular Cell. 2017 Feb; 65(4): 618-30. doi: 10.1016/j.molcel.2016.12.023.

[26] Rahim AA, Uzair M, Rehman N, Fiaz S, Attia KA, Abushady AM *et al.* CRISPR/Cas9 Mediated Tarpk1 Root Architecture Gene Mutagenesis Confers Enhanced Wheat Yield. Journal of King Saud University-Science. 2024 Feb; 36(2): 103063. doi: 10.1016/j.jksus.2023.103063.

[27] Protocol C. Cartagena Protocol on Biosafety to the Convention on Biological Diversity. In the secretariat of the Convention on Biological Diversity, Montreal, QC. 2000.

[28] Government of Pakistan. Pakistan Environmental Protection Agency (Ministry of Climate Change & Environmental Coordination). Pakistan Biosafety Guidelines. 2024 Feb.

[29] DBT India. Guidelines for Safety Assessment of Genome Edited Plants. Department of Biotechnology, Ministry of Science and Technology, Government of India. 2022. Retrieved from: https://dbtindia.gov.in.

[30] Majumder S, Datta K, Datta SK. Rice Biofortification: High Iron, Zinc, And Vitamin-A to Fight Against "Hidden Hunger". Agronomy. 2019 Nov; 9(12): 803. doi: 10.3390/agronomy9120803.

[31] Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G *et al.* Improving the Nutritional Value of Golden Rice Through Increased Pro-Vitamin A Content. Nature Biotechnology. 2005 Apr; 23(4): 482-7. doi: 10.1038/nbt1082.

[32] Singh SP, Gruissem W, Bhullar NK. Single Genetic Locus Improvement of Iron, Zinc and Β-Carotene Content in Rice Grains. Scientific Reports. 2017 Jul; 7(1): 6883. doi: 10.1038/s41598-017-07198-5.

[33] Karmakar A, Bhattacharya S, Sengupta S, Ali N, Sarkar SN, Datta K *et al.* RNAi-Mediated Silencing of ITPK Gene Reduces Phytic Acid Content, Alters Transcripts of Phytic Acid Biosynthetic Genes, and Modulates Mineral Distribution in Rice Seeds. Rice Science. 2020 Jul; 27(4): 315-28. doi: 10.1016/j.rsci. 2020.05.007.

[34] Riaz M, Yasmeen E, Saleem B, Hameed MK, Saeed Almheiri MT *et al.* Evolution of Agricultural Biotechnology Is the Paradigm Shift in Crop Resilience and Development: A Review. Frontiers in Plant Science. 2025 Jun; 16: 1585826. doi: 10.3389/ fpls.2025.1585826.

[35] Mackon E, Mackon GC, Guo Y, Ma Y, Yao Y, Liu P. Development and Application of CRISPR/Cas9 to Improve Anthocyanin Pigmentation in Plants: Opportunities and Perspectives. Plant Science. 2023 Aug; 333: 111746. doi: 10.1016/j.plantsci.2023.111 746.

[36] Moreno-Mateos MA, Vejnar CE, Beaudoin JD, Fernandez JP, Mis EK, Khokha MK *et al.* CRISPR Scan: Designing Highly Efficient SgRNAs for CRISPR-Cas9 Targeting in Vivo. Nature Methods. 2015 Oct; 12(10): 982-8. doi: 10.1038/nmeth.3543.

[37] Xiang X, Corsi GI, Anthon C, Qu K, Pan X, Liang X *et al.* Enhancing CRISPR-Cas9 gRNA Efficiency Prediction by Data Integration and Deep Learning. Nature Communications. 2021 May; 12(1): 3238. doi: 10.1038/ s41467-021-23576-0.

[38] Wessels HH, Stirn A, Méndez-Mancilla A, Kim EJ, Hart SK, Knowles DA *et al.* Prediction of on-Target and Off-Target Activity of CRISPR–Cas13d Guide RNAs Using Deep Learning. Nature Biotechnology. 2024 Apr; 42(4): 628-37. doi: 10.1038/s41587-023-01830-8.

[39] Wang D, Zhang C, Wang B, Li B, Wang Q, Liu D *et al.* Optimized CRISPR Guide RNA Design for Two High-Fidelity Cas9 Variants by Deep Learning. Nature Communications. 2019 Sep; 10(1): 4284. doi: 10.1038/ s41467-019-12281-8.

[40] Ray DK, Mueller ND, West PC, Foley JA. Yield Trends Are Insufficient to Double Global Crop Production by 2050. Plos One. 2013 Jun; 8(6): e66428. doi: 10.1371/

DOI: https://doi.org/10.54393/fbt.v5i3.189

journal.pone.0066428.

[41] Simmons CR, Lafitte HR, Reimann KS, Brugière N, Roesler K, Albertsen MC *et al.* Successes and Insights of an Industry Biotech Program to Enhance Maize Agronomic Traits. Plant Science. 2021 Jun; 307: 110899. doi: 10.1016/j.plantsci.2021.110899.

[42] Khan MH, Wang S, Wang J, Ahmar S, Saeed S, Khan SU *et al.* Applications of Artificial Intelligence in Climate-Resilient Smart-Crop Breeding. International Journal of Molecular Sciences. 2022 Sep; 23(19): 11156. doi: 10.3390/ijms231911156.

[43] Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M *et al.* ARGOS 8 Variants Generated by CRISPR-Cas9 Improve Maize Grain Yield Under Field Drought Stress Conditions. Plant Biotechnology Journal. 2017 Feb; 15(2): 207-16. doi: 10.1111/pbi.12603.

[44] Rehman A, Rasool G, Ullah A, Arshad N, Noor H, Jan A *et al.* Phenotypic and Molecular Confirmation of Maize (Zea Mays L.) Genotypes for Drought Tolerance at Seedling Stage. Pure and Applied Biology. 2023 Sep; 12(4): 1548-55. doi: 10.19045/bspab.2023.120156.

[45] Ismail A, Saleem MA, Shehzad A, Iqbal A, Khan PA, Rehman WU *et al.* The Response of Maize to Combined Application of Nitrogen and Phosphorous Fertilizers in the Semi-Arid Conditions of Faisalabad. Journal of Agriculture and Environment for International Development. 2024 Jun; 118(1): 93-110. doi: 10.36253/jaeid-12340.

[46] Manzoor D, Kaleri AA, Rehmani U, Wagan GH, Ahmed Z, Majeedano AQ *et al.* Comparative Performance of Maize (Zea Mays L.) Varieties Under Nitrogen Levels Tactics for Improved Crop Yield and Quality in Chhamogarh Valley District Gilgit, Pakistan. Insights-Journal of Life and Social Sciences. Health and Research Insights. 2025; 3(2): 49-56. doi: 10.71000/j1fr2313.

[47] Guo C, Ma X, Gao F, Guo Y. Off-target Effects in CRISPR/Cas9 Gene Editing. Frontiers in Bioengineering and Biotechnology. 2023 Mar; 11: 1143157. doi: 10.3389/fbioe.2023.1143157.

[48] Kalinin AA, Higgins GA, Reamaroon N, Soroushmehr S, Allyn-Feuer A, Dinov ID *et al.* Deep Learning in Pharmacogenomics: From Gene Regulation to Patient Stratification. Pharmacogenomics. 2018 May; 19(7): 629-50. doi: 10.2217/pgs-2018-0008.

[49] Dixit S, Kumar A, Srinivasan K, Vincent PD, Ramu Krishnan N. Advancing Genome Editing with Artificial Intelligence: Opportunities, Challenges, and Future Directions. Frontiers in Bioengineering and Biotechnology. 2024 Jan; 11: 1335901. doi: 10.3389/fbioe.2023.1335901.

[50] Bock C, Datlinger P, Chardon F, Coelho MA, Dong MB, Lawson KA *et al.* High-Content CRISPR Screening. Nature Reviews Methods Primers. 2022 Feb; 2(1): 8. doi: 10.1038/s43586-021-00093-4.

[51] Rub A. Essays on Climate Change, Wheat Production, and Adaptation Strategies in Pakistan. Kansas State University. 2023.

[52] Gaydon DS, Khaliq T, Cheema MJ. How Will Future Climates in the Pakistani Punjab Rice-Wheat System Affect the Optimal Agronomic Settings, and Can Adaptation Offset Losses? Field Crops Research. 2023 Oct; 302: 109037. doi: 10.1016/j.fcr.2023.109037.

[53] Qamer FM, Abbas S, Ahmad B, Hussain A, Salman A, Muhammad S *et al.* A Framework for Multi-Sensor Satellite Data to Evaluate Crop Production Losses: The Case Study of 2022 Pakistan Floods. Scientific Reports. 2023 Mar; 13(1): 4240. doi: 10.1038/s41598-023-30347-y.

[54] Shafeeque M and Bibi A. Assessing the Impact of Future Climate Scenarios on Crop Water Requirements and Agricultural Water Supply Across Different Climatic Zones of Pakistan. Frontiers in Earth Science. 2023 Oct; 11: 1283171. doi: 10.3389/feart.2023.1283171.

[55] Ahmad N, Fatima S, Mehmood MA, Zaman QU, Atif RM, Zhou W *et al.* Targeted Genome Editing in Polyploids: Lessons from Brassica. Frontiers in Plant Science. 2023 Jun; 14: 1152468. doi: 10.3389/fpls.2023.1152468.

[56] Wójcik-Gront E, Zieniuk B, Pawełkowicz M. Harnessing AI-Powered Genomic Research for Sustainable Crop Improvement. Agriculture. 2024; 14(12): 2299. doi: 10.3390/agriculture14122299.

[57] Wang Y, Xu G, Cao J, Chen Y, Wu J. Does Digital Literacy Affect Farmers' Adoption of Agricultural Social Services? An Empirical Study Based on China Land Economic Survey data. PLOS One. 2025 Apr; 20(4): e0320318. doi: 10.1371/journal.pone.0320318.

[58] Khan N, Xu X, Khayyam M, Raziq A. Toward Making the Field Talk: Assessing the Relationship Between Digital Technology and Sustainable Food Production in Agricultural Regions. Frontiers in Nutrition. 2024 Nov; 11: 1462438. doi: 10.3389/fnut.2024.1462438.

[59] Yang Q, Wu L, Meng J, Ma L, Zuo E, Sun Y. EpiCas-DL: Predicting SgRNA Activity for CRISPR-Mediated Epigenome Editing by Deep Learning. Computational and Structural Biotechnology Journal. 2023 Jan; 21: 202-11. doi: 10.1016/j.csbj.2022.11.034.