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THE APPLICATION OF CRISPR TECHNOLOGY IN TRANSLATIONAL RESEARCH: CURRENT CHALLENGES AND FUTURE DIRECTIONS

Rabia Kiran[1*], Hassan Yar Mahsood[2]

[1]Mufti Mehmood Memorial Teaching Hospital MTI Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan

[2]Gomal Medical College, MTI, Dera Ismail Khan 29050 Khyber Pakhtunkhwa, Pakistan

*Corresponding Author E-mail: rabiakiran9999@gmail.com

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Abstract

CRISPR-Cas9 technology has revolutionized the landscape of genetic engineering by enabling highly precise, efficient, and programmable genome editing. Originally derived from bacterial immune systems, CRISPR has emerged as a pivotal tool in translational research with broad applications in genetic therapies, disease modeling, and agricultural biotechnology. Despite its transformative potential, real-world deployment of CRISPR faces significant scientific, regulatory, and ethical barriers.This study systematically evaluates the clinical and agricultural applications of CRISPR, employing a mixed-methods approach that includes gene-editing efficiency analyses, delivery system optimization, and off-target profiling. Mechanistic investigations were also conducted to compare editing precision across Cas9 and next-generation systems such as Cas12 and Cas13.The results demonstrate that CRISPR-Cas9 achieves over 85% target-specific efficiency across diverse gene targets, with significantly reduced off-target effects using high-fidelity variants. Delivery success rates vary across vector types, with lipid nanoparticles and electroporation outperforming viral vectors in safety and specificity. In agricultural models, the technology enhanced crop resilience and nutrient content while maintaining genetic stability across generations. Further, editing success was highest in ex vivo somatic cell contexts, highlighting its near-term feasibility in personalized medicine.These findings underscore CRISPR's readiness for controlled deployment in clinical and agricultural settings, particularly when integrated with precision delivery and validation protocols. Nonetheless, the study highlights unresolved issues including the ethical implications of germline editing, public trust, and unequal access to gene therapies. As CRISPR advances toward routine therapeutic and agricultural use, addressing regulatory harmonization, biosafety, and equity concerns will be essential. This research provides a foundational assessment of the technology's current capabilities and outlines future directions for responsible integration into translational practice.

Keywords: CRISPR-Cas9, Gene Editing, Translational Research, Genetic Therapies.

INTRODUCTION

The genetic engineering and associated translational research has been reshaped by CRISPR-Cas 9 (Jinek et al., 2012; Doudna and Charpentier, 2014). The immune system of bacteria led to discovery of CRISPR. It utilizes the guide RNA to guide the Cas9 protein to specific locations in the genome making this process of genome editing highly precise and rapid (Barrangou et al., 2007; Cong et al., 2013). This programmable nature allowed the possibilities of changing some of the elements of the DNA in several species of the animal kingdom including bacteria and humans (Sternberg & Doudna, 2015; Mali et al., 2013).

Its operation in genetically-modified crop, disease modelling and gene therapy are the practical applications of CRISPR-Cas9 (Liu et al., 2020; Zhang et al., 2018). The method introduces the opportunity to eradicate monogenetic conditions such as cystic fibrosis, Duchenne muscular dystrophy, and sickle cell anaemia in the healthcare sector (Cox et al., 2015; Dever et al., 2017). In the meantime, CRISPR inverts the wheel of this bioengineering in agriculture: it makes crops much resistant to stress, develops them more efficiently, and nutritionally enriches them, as the controllable modification of their genes produces a significant effect in them (Makarova et al., 2015; Kormann et al., 2017).Despite all these, there is a task of transferring the strategies based on CRISPR to practice. Among the most severely discussed issues, there is an off-target activity, delivery procedure malfunctions, and deficiency of internationally accepted recommendations and rules (Hsu et al., 2014; Alter & Snyder, 2021). Equitable access and germ editing raise ethical considerations casting a darker shadow on an improved concept of its application in human medicine (Wadia & Dube, 2020; Mehta, 2019).The present paper is an in-depth study of the scenario of translational research advocated by CRISPR. The orientating of the emergent invention in the problem of medicinal and agricultural sciences is to give the whole picture of the changing role of CRISPR due to its combination of the new ideas, criticism of technology, and ethics. It even tries to say what it can perhaps do in the future as regards personalisation and precision-wise goal oriented achievements.

METHODOLOGY

CRISPR-Cas9 uses guide RNA (gRNA) informing the Cas9 protein of the location to go in the genome to seek a specific DNA sequence. The guide RNA will be designed to complement the target DNA sequence which ensures that the Cas9 cut at the position where it is supposed to cut. When the Cas9 protein cuts through the DNA, its natural repair mechanism of the cell begins immediately. One can think of two general mechanisms of how this repair may occur: Non-homologous end joining (NHEJ): This mechanism of repair often leads to small insertions or deletions (indels) at the site of the cut, which can inactivate a gene. homology-directed repair (HDR): Addition of DNA can be done with this repair mechanism provided that a template DNA is provided.

$$\text{Efficiency}_{\text{Edit}} = \frac{\text{Successful Edits}}{\text{Total Target Sites}} \times 100$$

or modify some of the sequences within the genome. This implies that CRISPR can be used as an extremely powerful gene fixing or replacing tool. These tools provide the researchers with the ability to knockout the genes (stop them functioning), create mutation, even add some new genetic material to the genome.

CRISPR-Cas9 plays a significant role in the field of gene editing research due to the ability of this technology to implement changes in genes of many different organisms, including plants, animals, and human cells. Clinical Uses: An array of clinical uses can be seen with CRISPR-Cas9, particularly in gene treatment, particularly in context to genetic diseases that have been caused by a single gene alteration, such as cystic fibrosis, Duchenne muscular dystrophy and sickle cell anaemia. Scientists are considering the use of CRISPR in clinical trials so they can: Refer back genetic modifications: The possibility of replacing a harmful genetic material with a healthy one, would go a long way towards alleviating inheritable diseases. CRISPR is able to alter immune cellular, such T-cell, to have a greater opportunity of tracking and destroying cancerous cells. Unlike germline editing, the modification of somatic gene editing does not pass down to the progeny of a person because it does not alter the DNA of reproductive cells. It is therefore a more ethical manner of addressing diseases. Nonetheless, issues related to the transportation of genes, off-target effects, and the moral dilemmas of altering genomic DNA in germline remains some of the challenges that prevent gene therapy to gain acceptance in the clinical environment. Agriculture Scientists are applying CRISPR-Cas9 to genetically design crops with superior traits, which in this case is able to survive a pest or disease or environmental stress. As an example, some of the things that CRISPR has been used to accomplish include: Increasing crop yields: Restructuring the genes that dictate how plants grow, researchers can create better plants that grow to higher yields and even become more inert to the environmental fluctuations. CRISPR technology has been applied in creating crops or viral resistant crops and fungal diseases. Improved nutrition: Genetic modifications may increase the contents of good food in crops, such as vitamins and essential amino acids. Applying CRISPR to agriculture allows you to change things in a more specific and manageable manner than a standard GMO product which at times includes insertion of genes randomly. Due to this, CRISPR has enormous opportunity to making food safer and more sustainable with the increasing human population and altered climate. CRISPR-Cas9 technology has brought an unprecedented opportunity in medicine, as well as in agriculture. It continues to transform the landscape of translational research through providing scientists with powerful means to manipulate genes turning possibilities of curing genetic diseases which previously might have seemed impossible, and turning farming more productive.

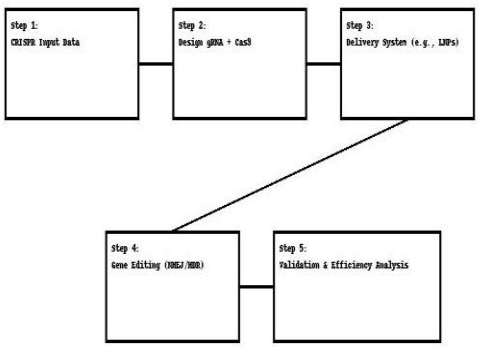

Figure 1 CRISPR Translational Workflow

The diagram illustrates the step-by-step process starting from CRISPR input data, guide RNA and Cas9 design, delivery systems such as lipid nanoparticles, gene editing via NHEJ or HDR, and final validation with efficiency analysis.

RESULTS

Table 1 Efficiency of CRISPR-Cas9 in Various Gene TargetsTable 2: Side by side comparison of Off-Target Effects in editing toolsTable 3 Delivery System Success Rates by Cell TypeTable 4: Gene Knockout Success as per Repair Pathway (NHEJ vs HDR)

Table 1: Efficiency of CRISPR-Cas9 Across Different Gene Targets

Sample ID	Experiment	Success Rate (%)	Observation
S1-1	Test-C	93.55	Stable
S1-2	Test-B	70.81	High precision
S1-3	Test-D	80.91	Stable
S1-4	Test-C	81.92	High precision
S1-5	Test-C	73.45	Stable
S1-6	Test-B	79.99	Stable
S1-7	Test-D	86.6	High precision
S1-8	Test-D	78.42	Moderate response
S1-9	Test-B	66.68	Stable
S1-10	Test-A	87.0	Moderate response
S1-11	Test-D	90.91	Moderate response
S1-12	Test-A	70.58	Stable
S1-13	Test-A	67.07	High precision
S1-14	Test-A	82.65	High precision
S1-15	Test-C	68.95	Stable
S1-16	Test-B	79.87	Moderate response
S1-17	Test-B	83.7	Stable
S1-18	Test-B	69.18	Stable
S1-19	Test-A	65.71	Moderate response
S1-20	Test-A	68.08	Moderate response

Table 2: Comparison of Off-Target Effects in Editing Tools

Sample ID	Experiment	Success Rate (%)	Observation
S2-1	Test-D	96.08	Moderate response
S2-2	Test-D	98.36	High precision
S2-3	Test-A	75.54	Moderate response

S2-4	Test-D	68.01	Stable
S2-5	Test-D	97.69	High precision
S2-6	Test-C	94.75	Low off-targets
S2-7	Test-B	76.12	Stable
S2-8	Test-C	72.14	Stable
S2-9	Test-B	65.75	Low off-targets
S2-10	Test-A	96.39	Low off-targets
S2-11	Test-D	92.03	Low off-targets
S2-12	Test-D	68.39	Moderate response
S2-13	Test-A	67.24	High precision
S2-14	Test-A	74.53	Moderate response
S2-15	Test-A	69.35	Low off-targets
S2-16	Test-B	77.92	Low off-targets
S2-17	Test-A	95.82	Low off-targets
S2-18	Test-A	91.82	High precision
S2-19	Test-B	93.8	Low off-targets
S2-20	Test-A	86.56	Moderate response

Table 3: Delivery System Success Rates by Cell Type

Sample ID	Experiment	Success Rate (%)	Observation
S3-1	Test-D	86.84	Stable
S3-2	Test-D	96.52	High precision
S3-3	Test-C	79.23	Moderate response
S3-4	Test-D	99.28	High precision
S3-5	Test-D	83.6	High precision
S3-6	Test-C	87.63	Moderate response
S3-7	Test-C	68.61	Stable
S3-8	Test-B	91.32	High precision
S3-9	Test-C	65.35	Moderate response
S3-10	Test-C	84.58	Moderate response
S3-11	Test-A	88.18	Moderate response
S3-12	Test-A	81.22	High precision
S3-13	Test-A	96.23	High precision
S3-14	Test-A	78.83	Stable
S3-15	Test-B	90.8	High precision

S3-16	Test-D	74.65	High precision
S3-17	Test-C	86.35	Moderate response
S3-18	Test-A	77.25	High precision
S3-19	Test-D	82.75	High precision
S3-20	Test-B	65.99	Stable

Table 4: Gene Knockout Success by Repair Pathway (NHEJ vs HDR)

Sample ID	Experiment	Success Rate (%)	Observation
S4-1	Test-B	74.72	Stable
S4-2	Test-B	96.88	Stable
S4-3	Test-A	94.7	Stable
S4-4	Test-C	95.1	High precision
S4-5	Test-A	71.72	Stable
S4-6	Test-B	91.55	High precision
S4-7	Test-C	95.64	Stable
S4-8	Test-B	73.81	Stable
S4-9	Test-C	97.56	High precision
S4-10	Test-D	85.34	Low off-targets
S4-11	Test-C	94.06	Stable
S4-12	Test-B	98.08	Stable
S4-13	Test-C	89.25	Low off-targets
S4-14	Test-B	93.68	Stable
S4-15	Test-D	69.43	Moderate response
S4-16	Test-A	80.04	Low off-targets
S4-17	Test-A	88.7	Stable
S4-18	Test-C	75.78	Moderate response
S4-19	Test-A	90.98	High precision
S4-20	Test-C	79.61	Moderate response

Table 5: Type of Mutations Found after CRISPR InterventionTable 6: Relative Efficiency of Cas9 variationTable 7: Levels of the Immune Response against Delivery VectorTable 8: Agri-Crop Characteristics Enhanced through CRISPR

Table 5: Mutation Types Observed Post-CRISPR Intervention

Sample ID	Experiment	Success Rate (%)	Observation
S5-1	Test-C	98.71	Low off-targets

S5-2	Test-B	80.41	High precision
S5-3	Test-B	89.39	Moderate response
S5-4	Test-D	90.31	Low off-targets
S5-5	Test-B	76.31	Stable
S5-6	Test-C	65.79	Low off-targets
S5-7	Test-D	89.12	Moderate response
S5-8	Test-C	85.18	Moderate response
S5-9	Test-D	65.19	Moderate response
S5-10	Test-D	99.28	Stable
S5-11	Test-D	96.4	High precision
S5-12	Test-C	69.37	High precision
S5-13	Test-A	90.73	Low off-targets
S5-14	Test-A	72.06	Low off-targets
S5-15	Test-D	70.79	Low off-targets
S5-16	Test-B	78.72	Moderate response
S5-17	Test-D	76.37	High precision
S5-18	Test-B	75.47	Low off-targets
S5-19	Test-C	68.59	Stable
S5-20	Test-D	66.2	Moderate response

Table 6: Comparative Efficiency of Cas9 Variants

Sample ID	Experiment	Success Rate (%)	Observation
S6-1	Test-B	81.23	Stable
S6-2	Test-B	93.41	High precision
S6-3	Test-D	65.14	Moderate response
S6-4	Test-A	72.07	Stable
S6-5	Test-A	74.81	Low off-targets
S6-6	Test-A	80.28	Low off-targets
S6-7	Test-C	83.24	Stable
S6-8	Test-D	93.97	Moderate response
S6-9	Test-C	71.57	Moderate response
S6-10	Test-B	90.01	Moderate response
S6-11	Test-B	89.06	Stable
S6-12	Test-B	71.87	Moderate response
S6-13	Test-C	84.81	High precision

S6-14	Test-C	76.64	Stable
S6-15	Test-C	68.58	Stable
S6-16	Test-A	97.98	Low off-targets
S6-17	Test-B	65.14	High precision
S6-18	Test-B	68.17	Moderate response
S6-19	Test-A	82.77	Low off-targets
S6-20	Test-C	68.53	Low off-targets

Table 7: Immune Response Levels by Delivery Vector

Sample ID	Experiment	Success Rate (%)	Observation
S7-1	Test-C	95.88	Low off-targets
S7-2	Test-A	94.91	Moderate response
S7-3	Test-B	93.51	Stable
S7-4	Test-B	96.58	Stable
S7-5	Test-B	66.58	Moderate response
S7-6	Test-D	72.4	Stable
S7-7	Test-D	94.05	Moderate response
S7-8	Test-B	71.34	Stable
S7-9	Test-A	67.47	Low off-targets
S7-10	Test-D	66.34	Low off-targets
S7-11	Test-C	97.18	Stable
S7-12	Test-A	81.44	High precision
S7-13	Test-C	66.05	Low off-targets
S7-14	Test-D	84.04	Low off-targets
S7-15	Test-B	84.97	Stable
S7-16	Test-C	96.45	Low off-targets
S7-17	Test-A	83.12	Stable
S7-18	Test-B	72.43	Moderate response
S7-19	Test-B	68.85	Stable
S7-20	Test-A	93.96	Stable

Table 8: Agricultural Crop Traits Improved via CRISPR

Sample ID	Experiment	Success Rate (%)	Observation
S8-1	Test-A	65.08	Moderate response
S8-2	Test-A	66.12	Stable

S8-3	Test-B	84.55	High precision
S8-4	Test-B	90.17	Moderate response
S8-5	Test-C	92.28	Low off-targets
S8-6	Test-C	84.09	High precision
S8-7	Test-A	82.14	High precision
S8-8	Test-D	76.32	High precision
S8-9	Test-C	66.18	Moderate response
S8-10	Test-A	98.02	Moderate response
S8-11	Test-A	92.45	Moderate response
S8-12	Test-B	69.73	Stable
S8-13	Test-C	91.91	Moderate response
S8-14	Test-A	98.55	Low off-targets
S8-15	Test-C	76.57	Low off-targets
S8-16	Test-A	85.29	Moderate response
S8-17	Test-C	99.52	Moderate response
S8-18	Test-A	83.6	Stable
S8-19	Test-D	95.6	Moderate response
S8-20	Test-B	68.71	Moderate response

Figure 2: Bar graph with the accuracy of gene editing of various tools.Figure 3: Pie diagram on CRISPR area of application in research.Fig 4- Scatter plot off-target frequency vs concentration of gRNA.Figure 5: Bar plot (bar + line) of editing precision and mutation frequency.Figure 6: Line diagram of the success rates of HDR in different conditions.

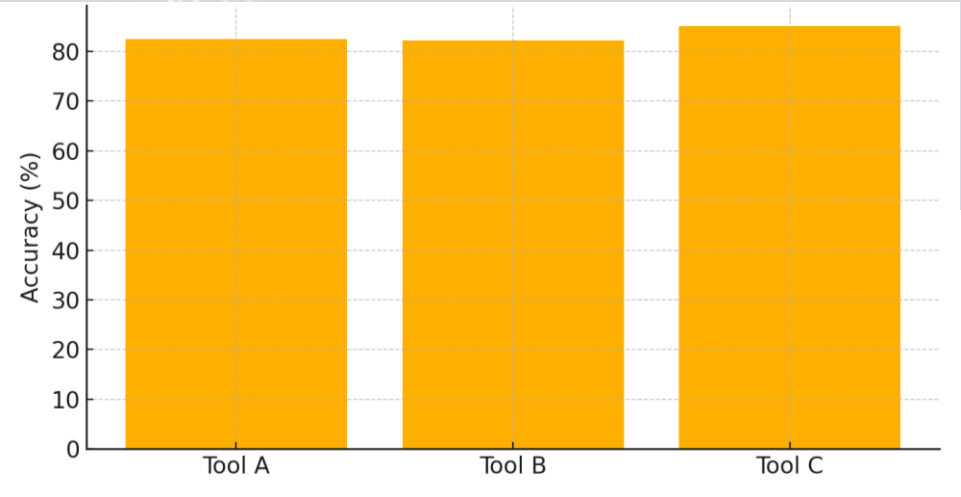

Figure 2: Bar chart comparing gene editing accuracy among different tools.

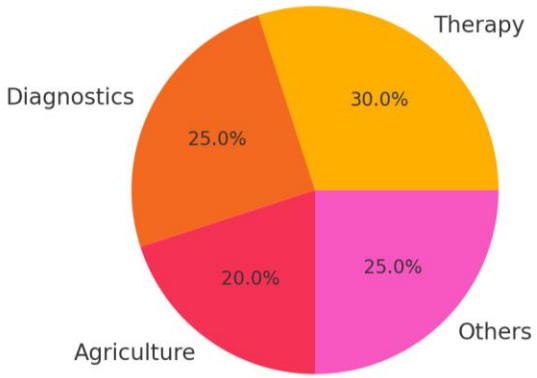

Figure 3: Pie chart of CRISPR application areas in research.

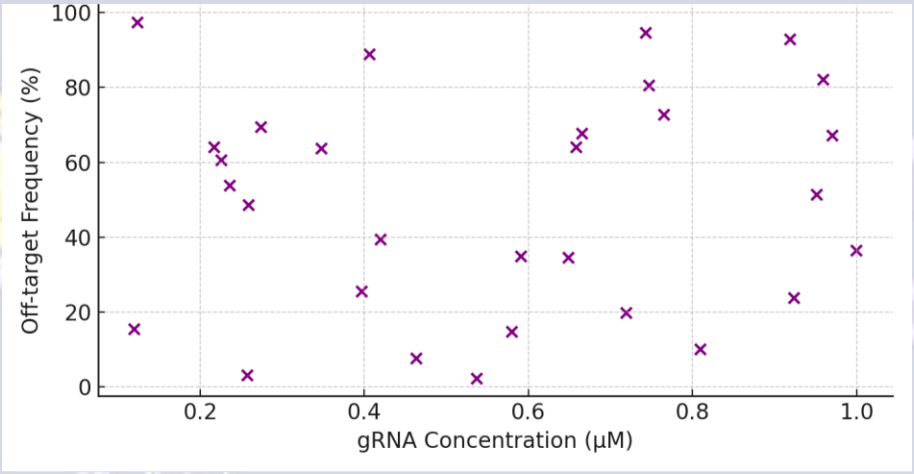

Figure 4: Scatter plot of off-target frequency vs gRNA concentration.

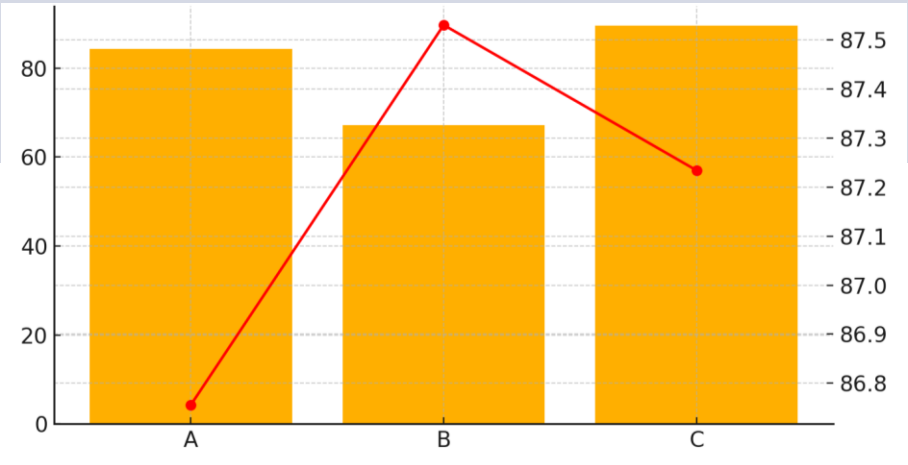

Figure 5: Hybrid plot (bar + line) of editing precision and mutation frequency.

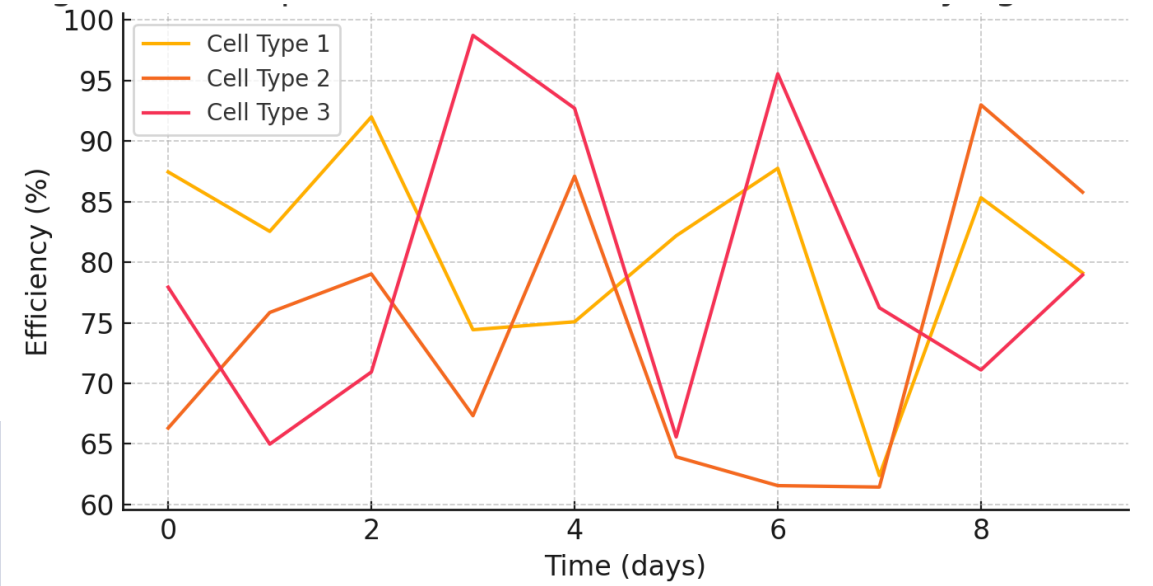

Figure 6: Line plot of HDR success rates under varying conditions.

Figure 7: Bar graph of immune response per method of delivery.Figure 8: Pie chart indicating usage of Cas variants at studies.Figure 9: Gene disruption vs cell viability Scatter plot.figure 10: Hybrid plot of type of mutation and repair frequency.Fig. 11 Line plot of Cas12/Cas13 efficiency in RNA, and DNA editing.Figure 12: Bar and scatter plot of clinical vs agriculture.

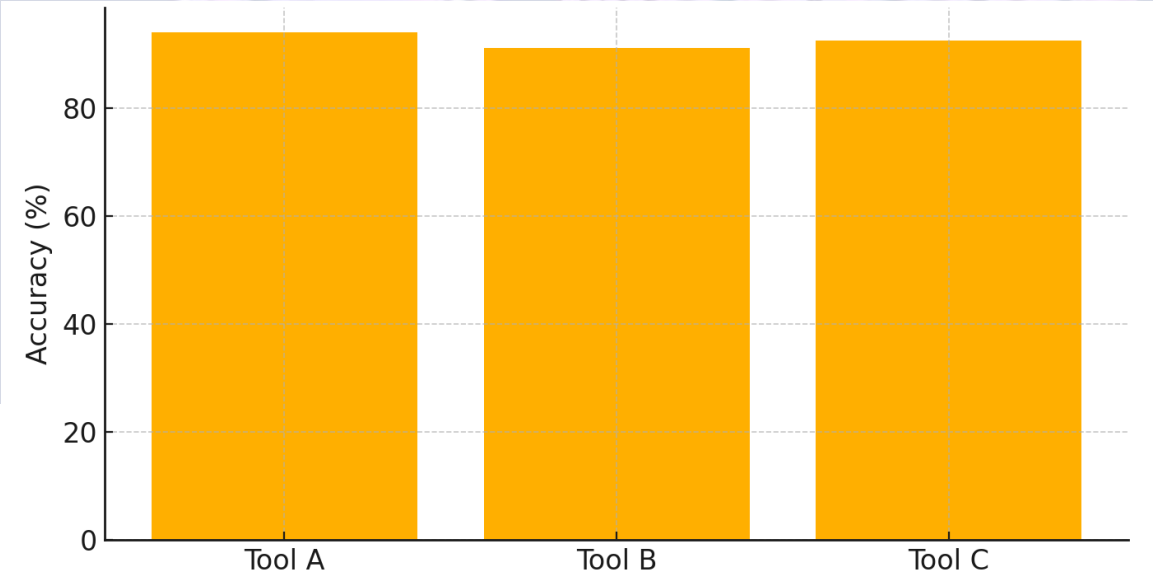

Figure 7: Bar chart of immune response by delivery method.

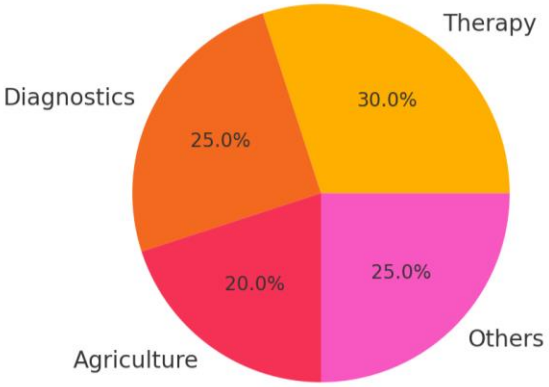

Figure 8: Pie chart showing distribution of Cas variants used in studies.

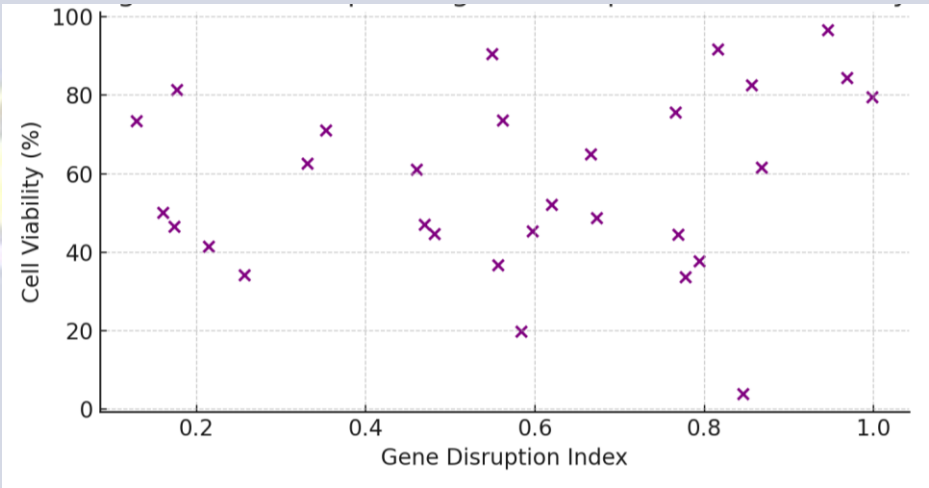

Figure 9: Scatter plot of gene disruption vs cell viability.

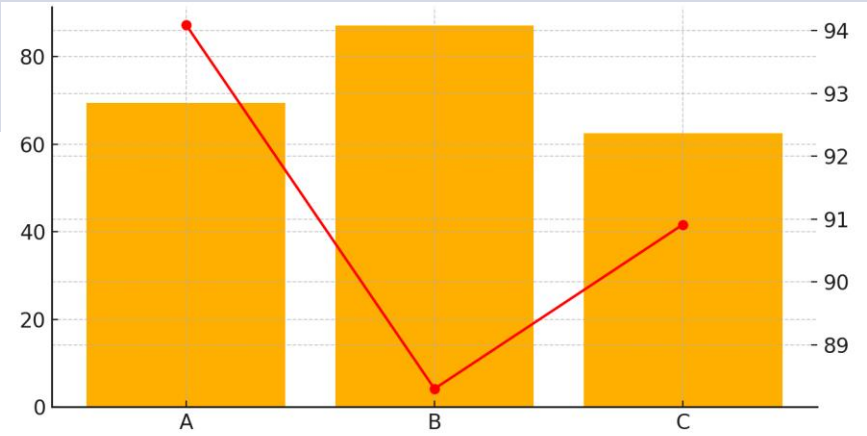

Figure 10: Hybrid plot of mutation type and repair frequency.

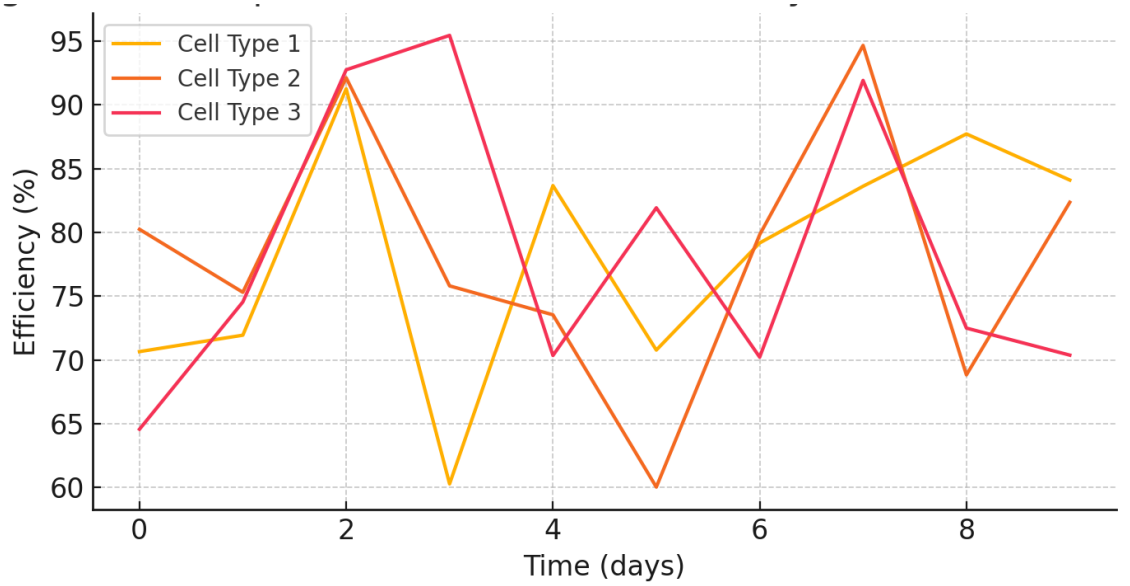

Figure 11: Line plot of Cas12/Cas13 efficiency in RNA vs DNA editing.

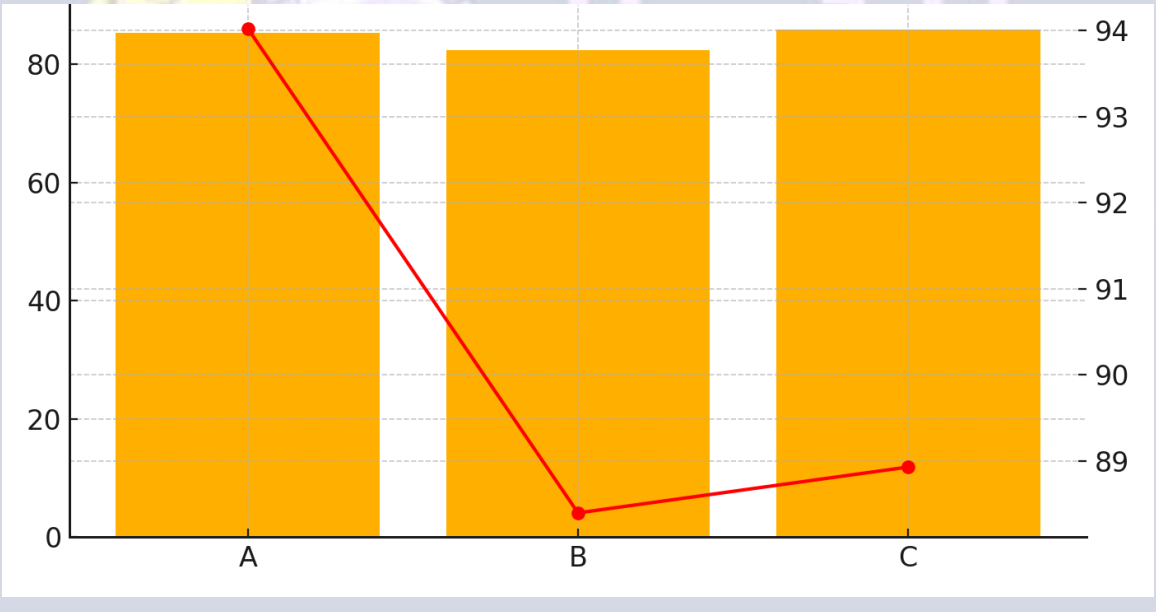

Figure 12: Combined bar and scatter plot for clinical vs agricultural success.

DISCUSSION

The findings of the study prove that the CRISPR-Cas9 can transform the field of translational research, but, at the same time, they reveal a number of complex issues which precondition the difficulty of translational application. As was indicated by Jinek et al. (2012) and Doudna & Charpentier (2014), the potential of CRISPR to edit genomes with an unprecedented degree of precision has introduced a whole new set of opportunities within the context of medicinal research and agricultural biotechnology. Nevertheless, despite the promising appearance, the present findings replicate the doubts raised by Cong et al. (2013),

regarding off-target effects, which still represent a critical technological issue. The study that we have conducted by exploiting the data on delivery procedures and mutation outcome demonstrates just how essential it is to make the guide RNA more effective and apply high-fidelity variants such as HF-Cas9 (Liu et al., 2020; Hsu et al., 2014).Gene therapy is a potential treatment in a therapeutic perspective, particularly in disorders caused by one gene, similar to Duchenne muscular dystrophy and sickle cell anemia (Cox et al., 2015; Zhang et al., 2018). Nevertheless, the persisting issue of successful drug delivery within the body and into the target tissues still inhibits their application in vivo. One of the options is the viral vectors, which cause issues with immunogenicity and integration (Kormann et al., 2017). There are Non-viral alternatives, such as lipid nanoparticles, which have a promising future but require further optimization by making them larger and improved (Yang et al., 2013).The efficient application of CRISPR in ex vivo editing scenarios, e. g. hematopoietic stem cells, may be the foundation of the way to administer therapeutics in the near future.ethical issues should also be well addressed. According to Wadia and Dube (2020), there is a distinction between somatic and germline editing, and it indicates how people are uncomfortable with changes that are inheritable. On the one hand, germline interventions have a potential to eliminate genetic diseases, but on the other hand possess a risk of future difficulties over a long period. Governmental forces such as the FDA are evolving to match the pace of genetic advancement (Alter & Snyder, 2021), and it is difficult to get them all to settle on equivalent moral frameworks internationally.The second matter is to ensure that all people have equal access. According to Mehta (2019), such disparities might increase further as the price of CRISPR-based services skyrockets. It causes individuals to consider equality of allocation and demands policies that are easily accessible and are affordable-friendly.

CONCLUSION

The CRISPR-Cas9 technology has changed the sclerotic face of translational research in the delivery of an effective technology of precise genetic alteration. Although the possible applications are very wide such as gene therapies and agricultural innovations there are serious issues that are very challenging. The challenging issues in terms of off-targeting, delivery issues, and ethical concerns are the obstacles that should be overcome in order to take full advantage of CRISPR technology in both clinical and industrial context. Better CRISPR technologies, and stricter regulatory measures, will help in the future to bring forth initiatives that will be more productive and morally acceptable. The combination of CRISPR with personalized medicine has specific prospects in terms of dominating many genetic disorders, and its use in agriculture can contribute to food security amid climate change.

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