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Case Study Open Access

Case Study: Application of CRISPR Design Algorithms in Tomato Genome Editing

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Abstract CRISPR-Cas systems have revolutionized plant genome editing by enabling precise, efficient, and targeted modifications, with tomato (*Solanum lycopersicum*) serving as a key model for translational applications in crop improvement. This study explored the application of CRISPR design algorithms in the selection and validation of guide RNAs (gRNAs) for optimizing tomato genome editing. The mechanisms of action of the CRISPR-Cas9 and Cas12a systems were analyzed, and major design tools such as CHOPCHOP, CRISPOR, and CRISPR-P were compared. The performance of the algorithms was evaluated in terms of targeting efficiency and specificity. Using the *SlMLO1* gene as a functional target, this study conducted a case study using CRISPR-P and CRISPR-GE platforms to design and evaluate gRNAs, achieving effective gene editing and enhancing tomato resistance to powdery mildew. This study highlights the challenges faced by algorithms in predicting off-target effects and adapting to the complex tomato genome, while also emphasizing the potential of integrating machine learning and tomato-specific databases to improve design accuracy. This research underscores the increasing synergy between computational biology and experimental biology, paving the way for the development of next-generation AI-assisted genome editing tools suitable for crops.

Keywords CRISPR-Cas9; Guide RNA design; Tomato genome editing; Algorithm optimization; *SlMLO1* gene

1 Introduction

In the field of plant genome editing, the CRISPR-Cas system is now almost universally mentioned, but its true impact often cannot be simply summed up as "precise and efficient". Although traditional breeding and mutagenesis methods have also played a role, they often seem inadequate when dealing with complex traits. In contrast, CRISPR's multiple editing, base modification, and even guided advanced editing methods enable researchers to deal with the issues of gene function and trait improvement more directly (Huang and Lin, 2024). Its simple operation and clear goals have provided new entry points for many old problems in plant biotechnology.

In fact, CRISPR/Cas9 initially existed in bacteria merely as an "immune tool" to recognize invading exogenous DNA. It was only later that people gradually realized that customizable Sgrnas could guide Cas proteins to specific gene loci, thereby achieving knockout, insertion or minor base modifications. Due to its relatively simple design and considerable efficiency, it has rapidly become one of the mainstream technologies in plant genetic engineering (Hu et al., 2025). In addition, some improved versions of Cas9 and strategies that support simultaneous editing of multiple genes have further expanded the application scope of this tool.

When it comes to specific examples, tomatoes are an unavoidable object. It is not only an important economic crop but also a commonly used model material for studying fruit development due to its relatively complete genomic data and mature transformation system (Reem and Van Eck, 2019). However, the genetic diversity of tomatoes is relatively limited, which instead prompts researchers to use CRISPR to improve fruit quality and enhance traits such as disease resistance and stress tolerance (Saikia et al., 2024). By rapidly manufacturing targeted mutants, the breeding cycle can be significantly shortened, and the related functional gene analysis can also be advanced more quickly.

This study does not focus on the editing principle of CRISPR itself, but rather on a more easily overlooked yet extremely crucial step, the design of gRNA. Good gRNA can enhance the success rate of editing, reduce

unnecessary off-targets, and also affect the feasibility of multi-target editing. With the continuous update of various computing tools and large-scale CRISPR libraries, the accuracy and scalability of tomato genome editing have also improved (Satheesh et al., 2021). This study will focus on discussing these technological advancements, combining their practical applications in tomato breeding and functional genomics. It will also touch upon the existing problems and possible future development directions.

2 Fundamentals of CRISPR Technology in Plants

In the field of plant genome editing, CRISPR technology has now almost become a "standard tool". Whether it is Cas9 or Cas12a, essentially they both rely on RNA to lead the way, allowing the enzyme to find the designated DNA position for cleavage (Bandyopadhyay et al., 2020). These operations may sound complicated, but their purpose is actually very direct: to modify genes more quickly and accurately, thereby promoting breeding and gene function research. Especially for crops like tomatoes, to enhance the editing effect, it is inevitable to understand the working mode of CRISPR, the role of guide RNA, and how the editing system enters cells.

2.1 Mechanism of CRISPR-Cas9 and CRISPR-Cas12a systems

Both Cas9 and Cas12a belong to Class II CRISPR systems and are widely used in plant research, but their cutting methods are not exactly the same. Cas9 relies on sgRNA to identify the target, and the cut surface is generally flat. Cas12a uses crRNA and will form a sticky misaligned incision (Tang et al., 2019). In addition, Cas12a can handle crRNA arrays by itself, which makes multiple editing more convenient. It recognizes PAM rich in T, while Cas9 prefers sequences rich in G, and the two complement each other perfectly. Some experiments on corn and rice have shown that Cas9 is usually more stable in terms of efficiency and specificity, but Cas12a has its own advantages in multifunctionality and operational flexibility, although it still needs further refinement (Lee et al., 2018).

2.2 Role of guide RNA (gRNA) in target specificity and editing efficiency

In the entire editing system, the importance of gRNA is often underestimated. It determines whether nucleases can accurately locate the target site and even affects the final editing efficiency. A well-designed system will have a lower off-target rate and a more stable performance. In recent years, many computational tools have begun to optimize the prediction of gRNA. Coupled with some expression strategies (such as the single transcript unit system), the co-expression of Cas proteins and gRNA has become more reliable (Tan et al., 2024). Especially for crops with relatively complex genomes like tomatoes, the design of grnas is even more demanding; otherwise, it is difficult to achieve sufficient precision in trait improvement.

2.3 Delivery methods of CRISPR components in plant cells

No matter how advanced CRISPR is, it must first enter cells to function. There are three commonly used methods in plants: Agrobacterium-mediated, PEG protoplast transfection, and gene gun (Saini et al., 2023). The most common bacterium in tomatoes is Agrobacterium because it is stable, highly efficient and does not insert a large number of copies. PEG and gene guns, on the other hand, are more suitable for editing without exogenous DNA, which can reduce some regulatory concerns. Different delivery methods can affect editing efficiency and tissue specificity, and also determine whether transgenic final plants can be obtained.

3 Overview of CRISPR Design Algorithms

When choosing appropriate target sites, researchers now generally rely on various CRISPR design algorithms. Such tools are not designed to make the process overly complicated, but to ensure more accurate editing and fewer off-target editing. They usually identify potential targets based on PAM sequences, and then make further judgments by combining factors such as activity scores and sequence mismatches (Xie et al., 2014). By taking into account both the genomic background and sequence features, these algorithms can help identify more suitable grnas, especially in plants with relatively complex genomes like tomatoes, where this step is particularly crucial.

3.1 Key features of CRISPR design tools

Most CRISPR design tools share some common features, such as target screening, off-target prediction, and an interface where users can freely adjust parameters. When screening targets, they would first scan PAM and then

make predictions on the efficiency and specificity of possible grnas (Park et al., 2015). As for off-target prediction, algorithms usually take into account situations such as mismatch, insertion and deletion one by one. Some even use machine learning or deep learning to improve judgment (Cao et al., 2025; Du et al., 2025). Many tools also come with additional features such as primer design and multi-grNA design, facilitating further verification during the experimental stage.

3.2 Comparative analysis of commonly used tools

Among the numerous available tools, CHOPCHOP, CRISPOR and CRISPR-P are the most frequently used ones. Although they all do gRNA design, their respective focuses are not quite the same. CHOPCHOP is more like an integrated platform, with a friendly interface, flexible parameters and relatively intuitive output. CRISPOR is characterized by the integration of multiple scoring systems for targeting efficiency and off-target risk, and relies on extensive genomic databases for support (Manghwar et al., 2020). CRISPR-P has been specifically optimized for plant genomes, and its off-target prediction for crops such as tomatoes is more in line with reality (Naeem and Alkhnbashi, 2023). Recently, some new tools based on deep learning, such as DeepCRISPR and CCLMoff, have also begun to be used. They utilize larger datasets to improve prediction accuracy (Chuai et al., 2018).

3.3 Evaluation metrics: on-target efficiency, specificity score, and usability

When evaluating whether a design algorithm is user-friendly or not, people usually first look at the targeting efficiency and specificity score. Simply put, it is about whether it can accurately cut to the target and whether it will "accidentally hurt" elsewhere (Listgarten et al., 2018). In addition to these technical indicators, researchers also attach great importance to whether the interface of the tool is intuitive, whether it runs fast, and whether it can handle large genomic data (Stemmer et al., 2015). For crop editors, these factors often directly affect the pace of experiments. Tools that can balance prediction accuracy and user experience usually help researchers design reliable grnas more smoothly, reduce unnecessary trial repetitions, and accelerate the editing process of crops such as tomatoes.

4 Criteria for Algorithm Selection in Tomato Genome Editing

4.1 Tomato genome characteristics and challenges for algorithm optimization

In the genome of tomatoes, there are many repetitive sequences and complex gene families. These characteristics often make CRISPR design less troublesome because off-target problems are prone to occur. To deal with this situation, high-quality reference genomes become very important. Chromosome-level genomes of tomato genotypes such as M82 and Sweet-100 (Figure 1) (Alonge et al., 2022) provide more details for identifying similar sequences. Algorithms often need to make more detailed adjustments based on these features to avoid mistaking similar but not target sequences for targets, so as to ensure that the final editing result is accurate enough.

4.2 Integration of genome annotation data and functional gene targets

When designing CRISPR targets for tomatoes, relying solely on the sequence itself is often insufficient; gene annotation information must also be taken into account. Information such as gene structure, regulatory regions, and functional domains can help determine which target sites are more "important" and more likely to affect traits such as yield and stress resistance (Chandrasekaran et al., 2021). This approach can reduce the situation where irrelevant areas are mistakenly edited and is more in line with the goals of breeding and biological research. Therefore, it is very practical when designing grnas.

4.3 Need for customization based on gene family, promoter regions, or non-coding RNAs

The tomato genome contains many elements that regulate gene expression, including gene families, promoters, and various non-coding RNAs. In the face of this situation, the design of gRNA cannot be one-size-fits-all and often requires additional customization. The reason is also quite simple: these areas may be similar to each other, but their functions are different. If the design is not fine enough, it is very easy to cause off-target and even affect other gene members, thereby resulting in unwanted trait changes (Hashimoto et al., 2018). Appropriate customization can enable editors to focus more on truly relevant regulatory points and enhance the effectiveness of the entire editing project.

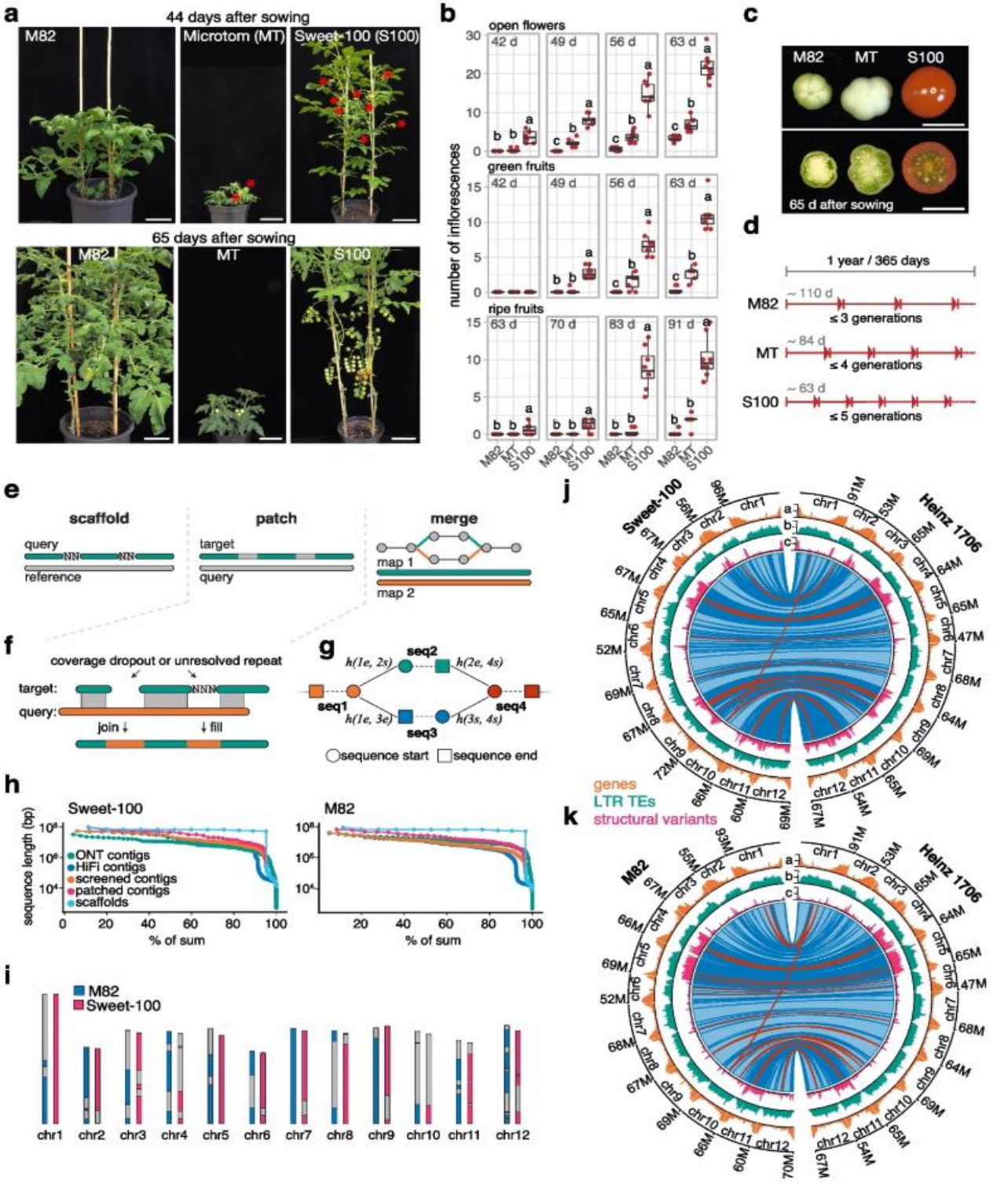

Figure 1 RagTag enables rapid generation of new reference genomes for the tomato genotypes Sweet-100 and M82 (Adopted from Alonge et al., 2022)

5 Case Study in Place: CRISPR Editing in Tomato

5.1 Objective: functional disruption of *SlMLO1* gene using algorithm-assisted CRISPR design

In the research on powdery mildew in tomatoes, the *SlMLO1* gene has always been regarded as a typical "susceptibility gene". As long as it is deprived of function, tomatoes can acquire natural resistance to the powdery mildew pathogen *Oidium neolycopersici*. It is not intended to introduce exogenous DNA here. Instead, it is hoped to directly create damage similar to natural mutations on this gene through CRISPR/Cas9, such as the 19 bp

deletion that has occurred in some wild tomatoes (Figure 2) (Berman et al., 2025). Therefore, the main objective of this case is to design a set of grnas that can precisely induce this functional deficiency, enabling tomatoes to acquire lasting resistance without sacrificing breeding efficiency.

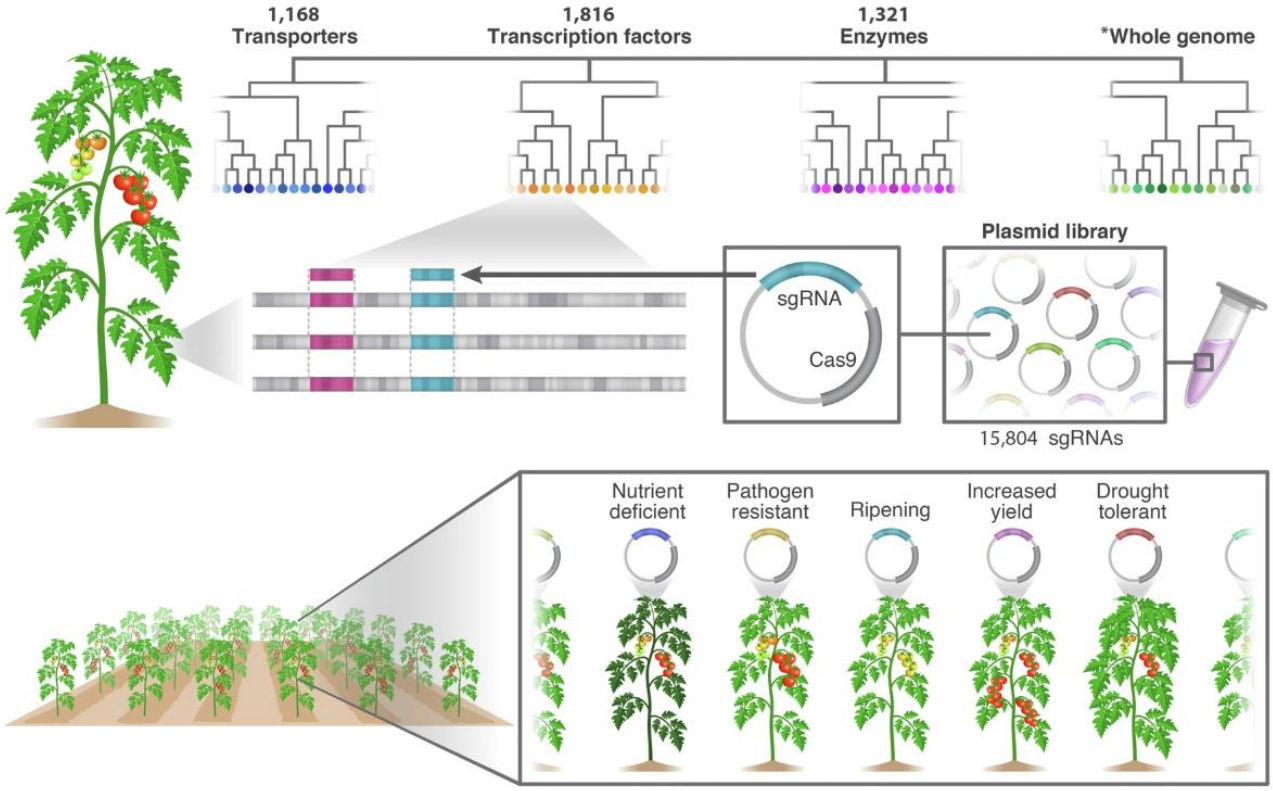

Figure 2 Schematic overview illustrating the library workflow, from design to screening (Adopted from Berman et al., 2025)

5.2 Step-by-step use of CRISPR-P and CRISPR-GE tools for gRNA design and validation

In practical operation, instead of directly starting from one or two candidate sequences, we first generated a large number of possible gRNAs through CRISPR-P and CRISPR-GE. Subsequently, screening was carried out step by step based on conditions such as whether PAM matched, potential off-target situations, MIT-specific scores, distances from natural ol-2 sites, and stability of sequence secondary structures (Prajapati and Nain, 2021). The remaining gRNA needs further verification of cutting efficiency and specificity. Only those with stable performance will enter the transformation stage. With the help of these tools, the process of selecting highly specific grnas has become clearer, and the additional costs caused by off-target during the experimental stage have also been reduced.

5.3 Outcomes: editing efficiency, off-target analysis, and phenotypic evaluation of powdery mildew resistance

CRISPR editing on *SlMLO1* performed quite well, and biallelic mutations were observed in the first-generation plants (Brooks et al., 2014). Subsequent off-target detection also revealed that non-target sequences were hardly affected, indicating that the algorithm-assisted design indeed improved the accuracy of editing. More direct evidence comes from phenotypic results: these edited strains demonstrated complete resistance in powdery mildew inoculation trials, confirming the inactivation of *SlMLO1* and also demonstrating the value of CRISPR design algorithms in disease-resistant breeding. This case demonstrates that the combination of computational tools and genome editing can more efficiently promote the improvement of crop traits.

6 Challenges in Applying Design Algorithms to Tomato

6.1 Inaccuracies in off-target prediction for the complex tomato genome

In the tomato genome, there are many repetitive sequences and genes from the same family often look very similar, which often makes off-target prediction less reliable. Existing algorithms often struggle to distinguish

these highly similar sequences, and if not careful, they may target similar family members as "targets", leading to some unwanted editing changes. This not only may affect the final phenotype, but also make subsequent analysis more troublesome. For this reason, models that are closer to the structure of the tomato genome are more urgent. They need to be able to handle this complexity more accurately and make the editing results more controllable.

6.2 Limited algorithm adaptability to different CRISPR systems (e.g., Cas12a vs Cas9)

At present, most design tools seem to be more inclined towards Cas9, while systems like Cas12a are often not fully considered due to different PAM requirements and cutting methods (Tiwari et al., 2023). This leads to a practical problem: some tomato targets are actually more suitable for Cas12a, but the algorithm cannot provide ideal design suggestions. This "incompatibility" among systems also limits researchers from making full use of various CRISPR variants. To truly expand the scope of editing and ensure that different tools perform their respective duties, the adaptability of algorithms will eventually have to be improved.

6.3 Need for integration with transcriptome and epigenomic datasets to enhance target selection

Most of the existing CRISPR design algorithms are based on the DNA sequence itself, which is of course useful. However, in the tomato crop, factors such as gene expression and chromatin openness can also affect whether the editing is smooth (Cardi et al., 2023). Some regions, although the sequence looks appropriate, have unsatisfactory editing effects due to tight chromatin or special expression features. Therefore, to select more functional targets, it would be more reliable to incorporate transcriptome and epigenomic data into the design process. After incorporating this information, the selection of gRNA can also be more in line with the actual breeding and research needs.

7 Future Directions in CRISPR Design Tool Development

7.1 Incorporation of machine learning and AI to improve guide RNA design

In the genome editing design of tomatoes, future tools are likely to increasingly rely on machine learning and artificial intelligence. The main reason is not the technological trend, but that rule-based algorithms in the past often appeared inaccurate when facing complex sequences (Naeem et al., 2024). AI methods can learn from a large amount of data from edited experiments which grnas perform better and which are more likely to go off-target, and provide more detailed predictions based on this. Sometimes, it can even automatically adjust the design parameters based on the genomic characteristics of tomatoes themselves to avoid unnecessary mutations. From an overall trend perspective, the design of gRNA is likely to become more dynamic rather than relying on fixed scoring rules.

7.2 Development of tomato-specific databases and genome editing platforms

Although many general-purpose CRISPR tools are already available, there is still a lack of truly "tailor-made" databases and platforms for tomato research. If genomic, transcriptomic and even phenotypic information could be integrated into a unified system, target selection would be more based and it would be easier to design multiple editing strategies (Vu et al., 2020). Some tools like CRISPR-GuideMap have demonstrated the possibility of this direction. They can track large-scale sgRNA libraries and are quite helpful in addressing the issues of gene redundancy and functional overlap in tomatoes. If these platforms can be more systematic in the future, tomato breeding and gene function research will proceed more smoothly.

7.3 Real-time, high-throughput screening integration for rapid validation of gRNAs

In the verification of gRNA, if it is completely dependent on step-by-step experiments, the efficiency is often not high enough. Combining real-time high-throughput screening with CRISPR design tools can detect a large number of candidate sequences at one time, saving a lot of time (Hu et al., 2019). For instance, through the barcode gRNA tracking system, the performance of different editing sites can be evaluated synchronously without having to test each one individually. In this way, the cycle of design, verification, and re-optimization will be completed more quickly, and grnas with stable performance and fewer off-targets can be found earlier. For scientific research and breeding, this way of enhancing speed and accuracy is undoubtedly more practical.

8 Concluding Remarks

In tomato research, the role of the CRISPR design algorithm is now almost self-evident, but the changes it has brought about have actually accumulated gradually. Whether it is fruit quality, disease resistance, stress tolerance, or various metabolism-related traits, these tools have been able to make stable targeted modifications within a relatively short period of time. Even in many cases, mutation effects can be observed in the first generation of plants. The use of multiple grnas has also made trait overlay and metabolic engineering easier to expand, making CRISPR increasingly "irreplaceable" in both basic research and breeding projects.

However, the genomic structure of tomatoes is not simple, with numerous repetitive sequences, complex regulatory networks, and even epigenetic particularities. These factors often make editing and design less straightforward. Therefore, although the existing tools are already available, algorithms that better suit the characteristics of tomatoes are still needed to further enhance specificity and efficiency. Integrating multi-omics data such as transcriptomics and epigenomes can also help reduce off-targets and further enhance the functional performance after editing. Establishing a dedicated database and design platform for tomatoes is also a necessary step in the long run.

The future direction is likely to make the boundary between computational design and experimental verification increasingly blurred, forming a rapid round-trip iterative process. The participation of machine learning, the development of high-throughput screening, and the continuous maturation of multiple editing technologies can all make the selection of gRNA more reliable and the editing results more controllable. With the increasingly close integration of computational biology and experimental biology, the efficiency and accuracy of tomato breeding have the opportunity to reach a new level, respond more quickly to the demands of agricultural production, and better meet consumers' expectations for crop quality.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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