Genome Editing in Potato Using CRISPR-Cas: Advances, Challenges and Future Perspectives

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ABSTRACT

Potato (*Solanum tuberosum* L. is a staple crop with a complex tetraploid genome, making conventional breeding for desirable traits challenging. Genome editing using CRISPR-Cas technology has emerged as a powerful tool for precise genome editing in potatoes, enabling targeted modifications for disease resistance, stress tolerance, and quality improvement. CRISPR and its related tools have really zoomed into helping to remove genes that make something vulnerable and to tweak metabolic pathways, and to generally beef up traits important to farming without throwing in DNA from other places. Compared to traditional methods of change, CRISPR and Cas are a lot finer, more efficient and considerably reduce regulatory worries too. This review explores recent advancements in CRISPR-Cas-based genome editing in potatoes, detailing its applications, challenges, and future prospects. Key aspects such as delivery methods, target genes, and regulatory considerations are discussed. While CRISPR-Cas revolutionizes potato improvement, challenges such as off-target effects, low editing efficiency in polyploids, and regulatory frameworks need further refinement. Future innovations will enhance its applicability in sustainable potato breeding.

INTRODUCTION

Potatoes—Solanum tuberosum L. as they're often called—are a huge staple crop that contributes a lot to the food supply around the globe. They're essential for millions of people across the world. The genome editing for it is hard mainly for reasons of its complex tetravalent genome, high heterozygosity and susceptibility to all kinds of diseases and environmental stresses. Traditional breeding takes a long time and a lot of hard work, while direct editing gene style techniques can be too haphazard and there are rigid requirements and problems to handle. CRISPR-Cas is really taking plant tech really big times by allowing super precise, careful changes that work splendidly. This technology allows researchers to enhance disease resistance, improve tuber quality, and increase stress tolerance in potatoes without introducing foreign DNA. CRISPR combined with newer types like base editing and prime editing (Barman *et al* 2019) is opening up huge possibilities for tuning up very important traits very well that's what we're also talking about more. CRISPR, combined with this kind of new genetic editing technology, is really promising us sweeping new ways of fine-tuning essential qualities in nature.

Mechanism of CRISPR- Cas genome editing in potato

Genome editing of potato via the CRISPR-Cas system is a new and breakthrough method to improve resistance to disease, yield, and nutritional elements in those important crop plants. CRISPR-Cas9 is a more specific gene-editing tool for potato: it introduces very targeted modifications in potato genome by incisions of double-strand breaks on those fixed DNA sequences of interest. The plant uses its own repair processes, allowing the formation of mutations through RNP delivery (ribonucleoprotein) or via NHEJ or HDR approaches. In potatoes, expensive mutated NHEJ has been knocked out for genes responsible for diseases, such as late blight due to Phytophthora infestans, responsible for potato disease processes. Moreover, reducing the accumulation of harmful compounds (acrylamide, for instance), involved with fried potatoes via starch metabolism-related gene editing, is also interesting. That is basically, enhancing pest resistance, increasing drought resistance, and improving tuber quality (Koonin *et al*., 2019).

Applications of CRISPR-Cas in Potato Genome Editing

CRISPR-Cas genome editing has transformed potato improvement by allowing for precise and targeted modifications. Its applications include enhancing disease resistance, improving tolerance to abiotic stress, boosting tuber quality, increasing yield, and advancing molecular breeding techniques.

1. **Disease Resistance:** Potatoes are vulnerable to a range of pathogens, such as fungi, bacteria, and viruses. CRISPR-Cas9 has been utilized to engineer resistance by either knocking out susceptibility (S) genes or introducing resistance (R) genes. For instance, in the case of late blight, caused by Phytophthora infestans, CRISPR-Cas9 has been used to disrupt the StDND1 and StCHL1 genes, which are linked to susceptibility. In terms of viral resistance, CRISPR has effectively targeted potato viruses like Potato virus Y (PVY) and Potato leafroll virus (PLRV) by altering genes crucial for viral replication (Makhotenko *et al*., 2019). Additionally, knocking out StSWEET genes has decreased susceptibility to Ralstonia solanacearum, the bacterium responsible for bacterial wilt.

2. **Abiotic Stress Tolerance:** The productivity of potatoes can be significantly impacted by drought, heat,

salinity, and cold stress. CRISPR-Cas has been employed to enhance resilience to these stresses by modifying stress-responsive genes. For example, knocking out ABA receptors (PYR/PYL) has improved drought tolerance by regulating stomatal responses. Furthermore, CRISPR-Cas9 editing of CBF genes has resulted in increased cold tolerance without negatively affecting yield (Tiwari *et al*., 2022).

3. **Tuber Quality Improvement:** Enhancing nutritional content, extending shelf life, and reducing harmful metabolites are essential objectives in potato breeding. Acrylamide, a carcinogenic compound that forms in fried potatoes, can be reduced through CRISPR-Cas9 knockout of StAsn1 (asparagine synthetase). Additionally, editing GBSS genes has facilitated the development of potatoes with altered starch composition for industrial uses. Targeting StGA20ox1, a gene involved in gibberellin biosynthesis, has also contributed to extending the shelf life of potatoes (Johansen *et al*., 2019).

4. **Enhanced Yield and Biomass:** Potato productivity is essential for food security at the global level. CRISPR-Cas9 has been utilized to edit StSP6A (a tuberization regulator), leading to enhanced tuber number and size. Editing of regulatory genes implicated in chloroplast function has boosted biomass production.

Challenges in CRISPR-Cas-Mediated Potato Genetic Transformation

i. **Polyploidy and Genetic Redundancy-** Potato is a tetraploid with four chromosome sets, so genetic modification is complicated. Four alleles of a target gene must be mutated for a knockout, and it takes more than one editing event. Multiplex CRISPR editing and base editing can enhance efficiency.

ii. **Low Efficiency of Homology-** Directed Repair (HDR)-Precise gene insertion through HDR is low in plants, such as potatoes, restricting its applications. Increasing HDR efficiency by donor template optimization, CRISPR-Cas modifications such as prime editing, and modulation of DNA repair pathways.

iii. **Off-Target Mutations-** Unsolicited DNA modifications might take place, with unforeseen phenotypic consequences. Employing high-fidelity Cas9 variants (e.g., Cas9-HF1, eCas9), gRNA design optimization, and utilization of base editing to prevent double-strand breaks.

CRISPR-Cas Future View of Potato Improvement

In order to make a complete use of CRISPR's potential, future research is needed to optimize efficiency, develop new applications, and solve the regulatory issues.

1. Development of CRISPR Technologies:

Prime Editing: This new-generation CRISPR strategy enables precise DNA editing without inducing double-strand breaks, and as such is very efficient for polyploid crops such as potatoes. Base Editing: Facilitates targeted point mutations without the induction of DNA breaks, minimizing off-target modifications. CRISPR-Cas12 and Cas13: These Cas orthologues present novel editing strategies, e.g., RNA editing for transient modifications.

2. Improved Delivery Method

Nanoparticle-Based Delivery: May permit non-transgenic genome editing in potatoes.

Virus-Induced Gene Editing (VIGE): Employing plant viruses to target CRISPR components without fixed DNA integration to minimize regulatory challenges.

3. Climate-Resilient and Sustainable Agriculture of Potatoes

CRISPR can enhance the breeding of climate-resilient potato types with enhanced tolerance for droughts and heat stress. It will also facilitate breeding for low-input agriculture, eliminating or minimizing pesticides and fertilizers.

CONCLUSION

CRISPR-Cas has transformed potato genome editing providing precise, efficient, and targeted alterations to improve disease resistance, stress tolerance, and tuber quality. Even with the obstacles of polyploidy, delivery efficiency, and regulatory barriers, ongoing advancements in CRISPR technologies, delivery strategies, and regulatory environments will augment its utility further. The combination of CRISPR with prime editing, synthetic biology, and AI-based breeding strategies has tremendous potential for the future of sustainable potato cultivation. With the right technological development and policy support, CRISPR-edited potatoes can become a key player in global food security and climate-resilient agriculture

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