International Journal of Horticulture, 2024, Vol.14, No.5, 319-332
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Systematic Review Open Access

A Comprehensive Analysis of Genomic Advances and CRISPR/Cas9 Applications in Kiwifruit (*Actinidia chinensis* Planch.)

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International Journal of Horticulture, 2024, Vol.14, No.5 doi: 10.5376/ijh.2024.14.0033
Received: 10 Aug., 2024
Accepted: 19 Sep., 2024
Published: 23 Oct., 2024

Preferred citation for this article:
Huang B.F., 2024, A Comprehensive analysis of genomic advances and CRISPR/Cas9 applications in kiwifruit (*Actinidia chinensis* Planch.), International Journal of Horticulture, 14(5): 319-332 (doi: 10.5376/ijh.2024.14.0033)

Abstract Kiwifruit (*Actinidia chinensis* Planch.) is an important fruit crop, widely appreciated for its rich nutritional content and unique flavor. In recent years, significant advances have been made in kiwifruit genomics, providing valuable resources for biological discoveries and crop improvement. This study comprehensively analyzes the latest developments in kiwifruit genomics, with a particular focus on the application of CRISPR/Cas9 technology in this species. By synthesizing recent research findings, the study explores how genomics and gene-editing technologies can be utilized to accelerate the development of superior kiwifruit varieties. The research reveals that the kiwifruit genome has undergone multiple whole-genome duplication events, which have contributed to the diversification and specialization of genes involved in key metabolic pathways. CRISPR/Cas9 technology has been successfully applied to target specific genes, enhancing disease resistance, flavor, and nutritional content in kiwifruit. Through the use of CRISPR/Cas9, researchers are able to precisely manipulate key genes in kiwifruit, achieving objectives such as rapid flowering, enhanced disease resistance, and improved fruit quality. This provides a powerful tool for the genetic improvement of kiwifruit and guides future breeding projects. The study results offer scientific and practical guidance for promoting the sustainable development of the kiwifruit industry and enhancing its global competitiveness.

Keywords Kiwifruit (*Actinidia chinensis* Planch.); Genomics; CRISPR/Cas9 technology; Gene editing; Genetic improvement

1 Introduction

Kiwifruit (*Actinidia chinensis* Planch.) is a highly valued fruit crop known for its rich nutritional profile, particularly its high vitamin C content. It is widely cultivated and consumed globally, contributing significantly to the agricultural economies of several countries. The fruit's unique flavor, which is determined by a complex interplay of soluble sugars, organic acids, and volatile compounds, makes it a favorite among consumers (Wang et al., 2021; Shu et al., 2023). The economic importance of kiwifruit is further underscored by its role in international trade and its potential for value-added products.

The genomic study of kiwifruit has made significant strides over the past decade. The draft genome sequence of *Actinidia chinensis* was a landmark achievement, providing a comprehensive resource for biological discovery and crop improvement (Huang et al., 2013; Wu et al., 2019). This genome sequencing revealed that kiwifruit has undergone multiple whole-genome duplication events, which have contributed to the diversification and specialization of genes involved in key metabolic pathways, including those regulating vitamin C, flavonoid, and carotenoid metabolism (Huang et al., 2013). These genomic insights provide a theoretical foundation for in-depth research into the genetic and molecular mechanisms underlying the development and quality traits of kiwifruit.

CRISPR/Cas9 technology has revolutionized the field of genetic engineering, offering a precise and efficient method for genome editing. This technology allows for targeted modifications in the DNA sequence, enabling researchers to investigate gene function and develop crops with improved traits (Van Eck, 2020). In the context of kiwifruit, CRISPR/Cas9 holds immense potential for enhancing desirable characteristics such as flavor, nutritional content, and disease resistance (Zhou et al., 2020). The ability to manipulate specific genes involved in metabolic

pathways can lead to significant improvements in fruit quality and yield, addressing both consumer preferences and agricultural challenges.

This study provides a comprehensive analysis of the latest advancements in kiwifruit genomics, with a particular focus on the application of CRISPR/Cas9 technology in this species. By synthesizing the most recent research findings, it highlights the progress made in understanding the kiwifruit genome and the potential of CRISPR/Cas9 technology to accelerate the development of superior kiwifruit varieties. The study is expected to offer guidance for future research and breeding programs, contributing to the sustainability and competitiveness of the global kiwifruit industry. By integrating genomic advancements with cutting-edge gene-editing technology, the study aims to provide insights that drive innovation in kiwifruit cultivation and enhance its economic and nutritional value.

2 Advances in Kiwifruit Genomics

2.1 Key milestones in kiwifruit genome sequencing

The initial draft genome of the kiwifruit (*Actinidia chinensis*) was a significant milestone, providing a comprehensive resource for biological discovery and crop improvement (Wu et al., 2019). This draft genome was assembled from approximately 140-fold next-generation sequencing data, resulting in a total genome length of 616.1 Mb and containing 39,040 genes. This assembly revealed that the kiwifruit genome has undergone an ancient hexaploidization event shared by core eudicots and two more recent whole-genome duplication events, which have contributed to the neofunctionalization of genes involved in important kiwifruit characteristics such as vitamin C, flavonoid, and carotenoid metabolism (Huang et al., 2013).

Subsequent advancements in sequencing technologies led to the development of a high-quality, chromosome-level reference genome of *A. chinensis* (v3.0). This improved genome assembly, based on PacBio long reads and Hi-C data, spans 653 Mb with 0.76% heterozygosity and includes 40,464 annotated protein-coding genes. This version significantly improved contiguity, accuracy, and gene annotation over previous versions, providing a more reliable resource for molecular elucidation of diverse traits and breeding efforts (Wu et al., 2019).

2.2 Structural and functional genomics of *Actinidia chinensis*

The kiwifruit genome has been extensively studied to understand its chromosomal structure and gene organization. High-quality genome assembly reveals that at least 43% of the genome consists of repetitive sequences, with long terminal repeats accounting for 23.38%. By combining PacBio long reads and Hi-C data, the research team successfully assembled a more complete and accurate version of the genome (v3.0) (Wu et al., 2019). This study also uncovered whole-genome duplication events in *Actinidia chinensis* during its species evolution, one occurring near the K-T extinction event and another more recent, genus-specific duplication (Figure 1). Further research identified significant translocation events following the whole-genome duplications, highlighting the dynamic nature of the kiwifruit genome (Pilkington et al., 2018).

Functional genomics studies have provided insights into the regulatory networks governing important traits in kiwifruit. Integrative analyses of metabolome and genome-wide transcriptome data have identified key structural genes and transcription factors that regulate the metabolism of soluble sugars, organic acids, and volatiles, which are crucial for fruit flavor and quality (Wang et al., 2021; Shu et al., 2023). Additionally, transcriptome sequencing has revealed long noncoding RNAs and alternative splicing events that play roles in the synthesis of nutritional metabolites and the ethylene signaling pathway, which is essential for fruit ripening (Tang et al., 2016).

2.3 Insights from comparative genomics studies

Comparative genomic analyses have shown that the kiwifruit genome shares an ancient hexaploidization event with core eudicots and has undergone additional whole-genome duplications after diverging from tomato and potato. These events have contributed to the diversification and specialization of genes involved in key metabolic pathways (Huang et al., 2013). The integration of RAD-based linkage maps has further improved the genome

assembly and facilitated the identification of genomic regions associated with important traits, such as gender determination, which is valuable for marker-assisted breeding (Scaglione et al., 2015).

The kiwifruit genome provides valuable evolutionary insights, particularly within the Asterid lineage. The genome sequence of *A. chinensis*, as the first sequenced species in the Ericales, offers a unique perspective on the evolutionary history and genomic adaptations of this group. Comparative studies have highlighted the role of whole-genome duplications in the evolution of kiwifruit and related taxa, shedding light on the genetic basis of important agronomic traits (Huang et al., 2013; Wu et al., 2019).

The advances in kiwifruit genomics, from initial genome assembly to high-quality reference genomes and functional genomics studies, have significantly enhanced our understanding of the structural and functional aspects of the kiwifruit genome. Comparative genomics has provided further insights into the evolutionary history and genetic diversity of this economically important fruit crop.

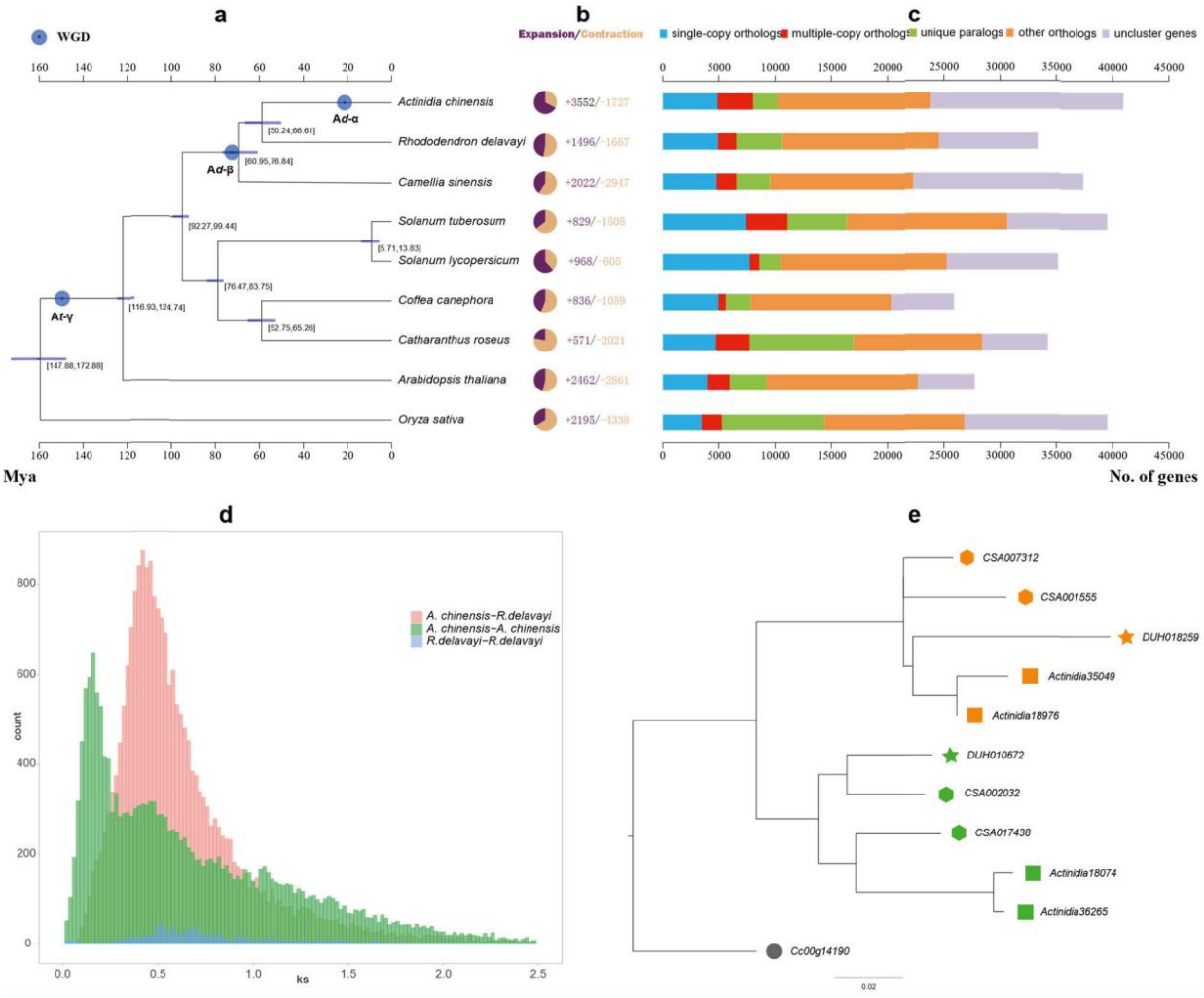

Figure 1 Phylogenetic and evolutionary analyses of the *A. chinensis* genome v3.0 (Adopted from Wu et al., 2019)

Image caption: The figure illustrates the phylogenetic analysis and whole-genome duplication (WGD) events of the *Actinidia chinensis* genome v3.0. Figure a reveals the phylogenetic relationships between *Actinidia chinensis* and other plant species, indicating its closest relationship with the Rhododendron genus. Figure b shows the expansion and contraction of gene families, indicating that the *Actinidia chinensis* genome has undergone significant gene expansion. Figure c presents the clustering analysis of gene families, while Figure d displays the distribution of synonymous substitution rates (Ks), revealing three WGD events, particularly the one shared with the Rhododendron genus. Figure e supports the existence of these WGD events, confirming the association between the evolutionary history of the *Actinidia chinensis* genome and WGDs (Adapted from Wu et al., 2019)

3 Identification of Key Genes in Kiwifruit

3.1 Genes involved in fruit development and ripening

Ripening in kiwifruit is a complex process regulated by various transcription factors. Ethylene plays a crucial role in this process, and its effects can be modulated by inhibitors such as 1-methylcyclopropene (1-MCP). Transcriptomic analysis has identified several key transcription factors, including AcEIL, which is responsive to both ethylene and 1-MCP treatments, indicating its significant role in the ethylene-mediated ripening process (Choi et al., 2023). Additionally, MADS-box transcription factors such as SEP4/RIN have been shown to be involved in the regulation of ethylene production and response, further highlighting their importance in the ripening stages of kiwifruit (McAtee et al., 2015).

Cell wall modification is a critical aspect of fruit softening during ripening. Polygalacturonase (PG) enzymes, which degrade pectin, are key players in this process. In kiwifruit, 51 *AcPG* genes have been identified, with specific genes such as *AcPG4* and *AcPG18* showing strong correlations with pectin content and fruit firmness during softening (Huang et al., 2020). The activities of other cell wall-modifying enzymes like pectinesterase (PE) and pectate lyase (PL) also contribute to the changes in cell wall composition, further influencing the texture of the ripening fruit.

3.2 Disease resistance genes in *Actinidia chinensis*

The identification of resistance gene analogs (RGAs) is essential for understanding the disease resistance mechanisms in kiwifruit. High-quality genomic data, such as the improved chromosome-level reference genome of *A. chinensis*, provides a robust platform for identifying these genes. This genome contains 40,464 annotated protein-coding genes, many of which are involved in disease resistance pathways (Wu et al., 2019). The comprehensive annotation and improved contiguity of this genome facilitate the identification and functional analysis of RGAs.

Functional characterization of disease resistance pathways involves understanding the interactions between various genes and their regulatory networks. Transcriptomic and metabolomic analyses have revealed that certain transcription factors and signaling pathways are crucial for disease resistance. For instance, the MAPK signaling pathway, which is enriched in differentially expressed genes in kiwifruit, plays a significant role in plant defense mechanisms (Wu et al., 2020). These insights are vital for developing strategies to enhance disease resistance in kiwifruit through genetic manipulation.

3.3 Genes linked to flavor and nutritional content

Flavor in kiwifruit is determined by the biosynthesis of volatile compounds, soluble sugars, and organic acids. Integrative analyses of metabolome and transcriptome data have identified key structural genes and transcription factors that regulate the metabolism of these flavor-related compounds. For example, specific transcription factors have been shown to modulate the biosynthesis of important volatiles, contributing to the unique flavor profile of kiwifruit (Wang et al., 2021; Shu et al., 2023).

Nutrient content in kiwifruit, including vitamins and minerals, is regulated by various biosynthetic pathways. Transcriptomic studies have identified genes involved in the synthesis of essential nutrients such as vitamin C, carotenoids, and anthocyanins. These genes undergo alternative splicing and differential expression during fruit development and ripening, indicating their dynamic regulation (Tang et al., 2016). Additionally, the application of growth regulators like CPPU has been shown to enhance the biosynthesis of soluble sugars and vitamin C, further improving the nutritional quality of kiwifruit (Wu et al., 2020).

4 Applications of CRISPR/Cas9 in Kiwifruit

4.1 Genome editing for disease resistance

CRISPR/Cas9 technology has been instrumental in targeting specific resistance genes in kiwifruit to enhance defense against pathogens. For instance, the identification and manipulation of Multiple Organellar RNA Editing Factor (MORF) genes, which play a crucial role in the plant's response to pathogen stress, can be a potential target

for CRISPR/Cas9 editing to improve disease resistance in kiwifruit (Xiong et al., 2022). Additionally, quantitative trait loci (QTLs) associated with resistance to bacterial canker caused by *Pseudomonas syringa*e pv. *actinidiae* (Psa) have been mapped, providing specific genetic targets for CRISPR/Cas9 to enhance resistance (Tahir et al., 2019).

Several studies have demonstrated the potential of CRISPR/Cas9 in improving disease resistance in fruit crops, including kiwifruit. For example, the application of CRISPR/Cas9 in other fruit crops has shown significant reductions in disease susceptibility, which can be translated to kiwifruit (Zhou et al., 2020). The successful identification of QTLs for Psa resistance in kiwifruit provides a roadmap for using CRISPR/Cas9 to edit these loci and develop resistant varieties (Tahir et al., 2019).

4.2 Enhancing fruit quality and yield using CRISPR/Cas9

CRISPR/Cas9 has been employed to edit genes that regulate key metabolites affecting fruit quality, such as sugars, flavonoids, and vitamins. In kiwifruit, uncovering the comprehensive metabolic pathways that regulate major quality traits can guide the editing of specific genes to improve fruit texture and flavor. For example, research has shown that specific transcription factors, such as *AcMYB123-2* and *AcERF192*, strictly regulate the accumulation of soluble sugars, proanthocyanidins, and vitamin C (Shu et al., 2023). These factors influence key metabolic pathways, including starch degradation, flavonoid biosynthesis, and the ascorbate cycle, which are crucial for enhancing the desirable qualities of kiwifruit (Figure 2).

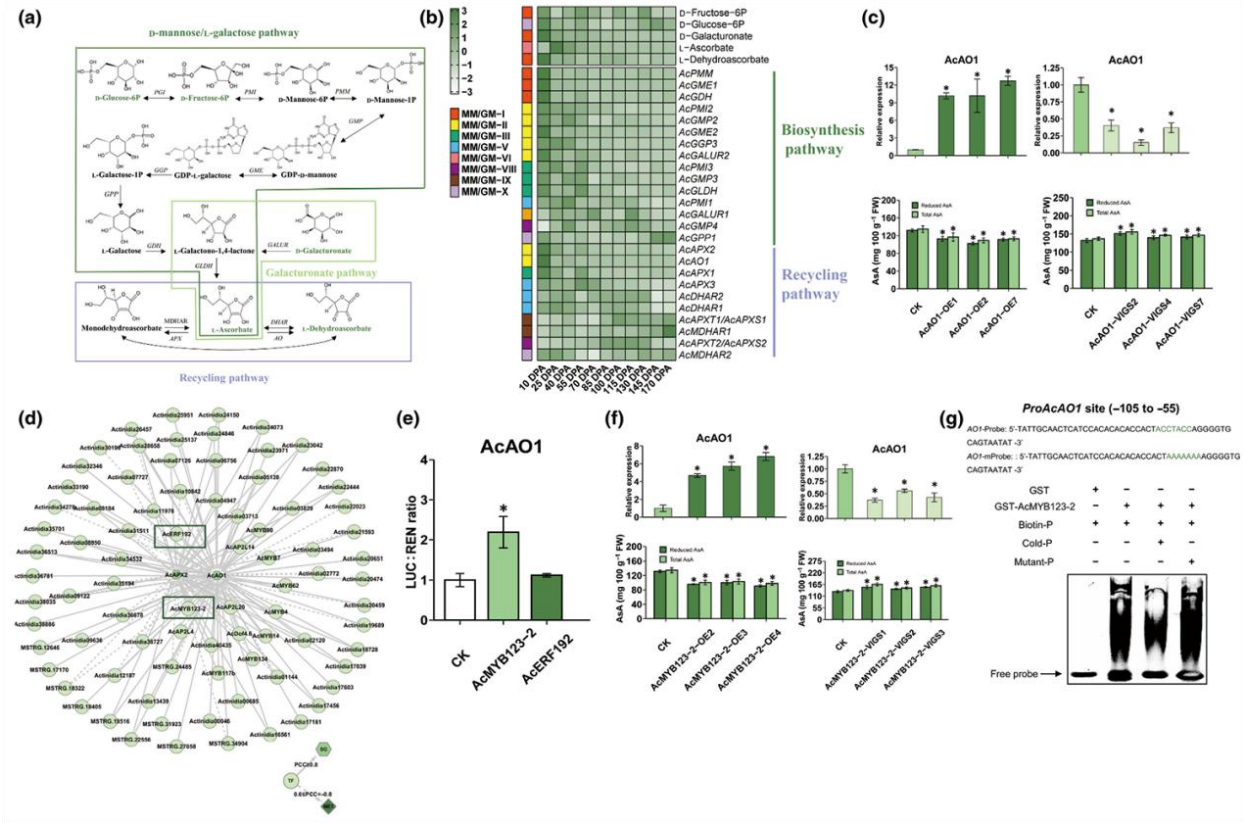

Figure 2 Transcription factor (TF) AcMYB123-2 modulates vitamin C (L-ascrobate, AsA) degradation by regulation of *AcAO1* in kiwifruit (*Actinidia chinensis* cv Hongyang) (Adopted from Shu et al., 2023)

Image caption: The figure analyzes the AsA metabolism and recycling pathways, revealing that the transcription factor *AcMYB123-2* influences AsA degradation by regulating the expression of the *AcAO1* gene. Experiments demonstrated that *AcMYB123-2* directly binds to the *AcAO1* promoter, promoting its expression, which leads to a reduction in AsA content. This result reveals that the accumulation of AsA in kiwifruit is regulated by the recycling pathway and confirms the critical role of *AcMYB123-2* in AsA degradation, providing a potential molecular breeding target to enhance the nutritional quality of kiwifruit (Adapted from Shu et al., 2023)

The application of CRISPR/Cas9 in fruit crops has also focused on increasing yield by modifying genes that control plant architecture and fruit development. In kiwifruit, the identification of genes involved in stress responses, such as the R1R2R3-MYB transcription factor *AcMYB3R*, which enhances tolerance to environmental stresses, can be edited to improve overall plant health and yield (Zhang et al., 2019). Additionally, the integration of CRISPR/Cas9 in breeding programs can accelerate the development of high-yielding kiwifruit varieties (Zhou et al., 2020).

4.3 Potential for developing new kiwifruit varieties

Breeding programs that integrate CRISPR/Cas9 technology can significantly enhance the development of new kiwifruit varieties. The high-quality reference genome of *Actinidia chinensis* provides a robust foundation for identifying and editing genes associated with desirable traits (Wu et al., 2019). By combining traditional breeding methods with CRISPR/Cas9, it is possible to introduce specific genetic modifications more efficiently and accurately (Zhou et al., 2020).

There have been successful case studies in other fruit crops where CRISPR/Cas9 has been used to develop new varieties with improved traits. For example, the use of CRISPR/Cas9 to edit genes related to fruit quality and yield in crops like tomatoes and strawberries has shown promising results, which can be applied to kiwifruit (Zhou et al., 2020). The development of new kiwifruit varieties through CRISPR/Cas9 can also benefit from the detailed genetic and metabolic maps available, which provide insights into the regulatory networks governing key traits (Shu et al., 2023).

5 Genetic Mapping and QTL Analysis

5.1 SNP Genotyping Array

The development of SNP arrays for kiwifruit has been a significant advancement in genetic studies and breeding applications. A high-density SNP genotyping array was developed by performing genome-wide DNA sequencing of 40 kiwifruit genotypes. This process identified 134,729 unique SNPs, which were stringently filtered for sequence quality, predicted conversion performance, and distribution over the available *Actinidia chinensis* genome. The array was evaluated by genotyping 400 kiwifruit individuals, demonstrating its effectiveness in distinguishing kiwifruit accessions and facilitating genetic studies (Wang et al., 2022).

The SNP genotyping array has been utilized in various genetic studies, including the construction of an integrated linkage map and QTL analysis. Research indicates that SNP arrays and resequencing technologies are complementary in detecting quantitative trait loci (QTL). By combining different SNP distributions and densities, the study found that the integration of these two technologies can enhance the ability to detect QTLs and potentially more precisely pinpoint causal polymorphisms (Negro et al., 2019). For instance, using a tetraploid F1 population, researchers constructed a linkage map covering 3060.9 cM across 29 linkage groups. This map was instrumental in performing QTL analysis for the sex locus identified on Linkage Group 3 (LG3) in *Actinidia arguta*. The array's comprehensive design makes it a valuable tool for genetic studies and breeding applications in kiwifruit (Wang et al., 2022).

5.2 QTL Analysis

Quantitative Trait Loci (QTL) analysis has been pivotal in identifying loci linked to important traits in kiwifruit. The high-density SNP genotyping array facilitated the identification of QTLs, such as the sex locus on LG3 in *Actinidia arguta*. This identification is crucial for understanding the genetic basis of key traits and for the development of marker-assisted selection strategies (Wang et al., 2022). Another study on kiwifruit constructed a high-density linkage map of hexaploid kiwifruit through genotyping-by-sequencing (GBS) and used this map for QTL analysis of sex loci and fruit traits. This is the first time that QTLs have been discovered and reported in hexaploid kiwifruit with complex traits, providing a foundation for molecular marker-assisted selection in kiwifruit (Popowski et al., 2021).

The identified QTLs are utilized in marker-assisted selection (MAS) to enhance breeding efficiency. By integrating QTL information into breeding programs, breeders can select individuals with desirable traits more accurately and efficiently. This approach accelerates the development of new kiwifruit cultivars with improved characteristics, such as disease resistance, fruit quality, and yield (Wang et al., 2022).

5.3 Genetic diversity

Assessing genetic diversity within kiwifruit populations is essential for effective breeding and conservation strategies. The SNP genotyping array has been used to perform multidimensional scaling analysis, which revealed the diversity of kiwifruit germplasm. This analysis is crucial for understanding the genetic variation within and between kiwifruit populations, aiding in the management of genetic resources (Wang et al., 2022).

The genetic diversity within kiwifruit populations has significant implications for breeding programs. High genetic diversity provides a broader genetic base for selecting desirable traits and developing new cultivars. It also enhances the adaptability and resilience of kiwifruit to environmental changes and biotic stresses. Therefore, maintaining and utilizing genetic diversity is vital for the long-term success of kiwifruit breeding programs (Wu et al., 2019; Wang et al., 2022). By leveraging these genomic advances and CRISPR/Cas9 applications, researchers and breeders can make significant strides in improving kiwifruit cultivars, ensuring their sustainability and economic viability.

6 Case Studies

6.1 Successful induction of early flowering and normal reproductive development in kiwifruit mediated by CRISPR/Cas9

CRISPR/Cas9 technology has been successfully applied in *Actinidia chinensis* to manipulate specific genes and achieve desired phenotypic changes. A notable example is the mutation of the kiwifruit CENTRORADIALIS-like genes (*AcCEN4* and *AcCEN*). By editing the *AcCEN4* and *AcCEN* genes, researchers successfully transformed this woody vine, known for its long juvenile phase and axillary flowering, into a plant with a compact growth habit and rapid terminal flowering (Figure 3) (Varkonyi-Gasic et al., 2018). The study demonstrated that a double allelic mutation could lead to early flowering, while a double gene mutation further accelerated the flowering process and enhanced the compact growth form.

This research highlights the potential of CRISPR/Cas9-mediated targeted mutations to accelerate kiwifruit breeding, particularly for indoor cultivation and the planting of annual crops. The findings provide new insights into the rapid domestication and improvement of long-lived woody plants like kiwifruit and contribute to enhancing the sustainability of fruit production and food security (Varkonyi-Gasic et al., 2018).

6.2 Study on the sustained growth phenotype of kiwifruit mediated by CRISPR-Cas9 targeted editing of the *AcBFT2* Gene

Herath et al. (2022) used CRISPR-Cas9 gene editing technology to create targeted mutations in the *AcBFT2* gene of kiwifruit (*Actinidia chinensis*), revealing its critical role in regulating plant dormancy and growth cessation. The results showed that mutations in the *AcBFT2* gene led to a "sustained growth" phenotype, characterized by delayed growth cessation and dormancy, as well as early bud break, without negatively impacting flowering or reproductive maturity (Figure 4). This indicates that the *AcBFT2* gene plays a key role in controlling growth cessation and dormancy in kiwifruit.

This discovery is significant because it offers the potential to cultivate kiwifruit varieties that are better suited to warmer climates with lower winter chilling requirements, reducing the dependence on chemical dormancy-breaking agents. The study provides valuable insights into the genetic regulation of dormancy and growth in temperate fruit crops and holds important application prospects for enhancing crops' ability to cope with climate change.

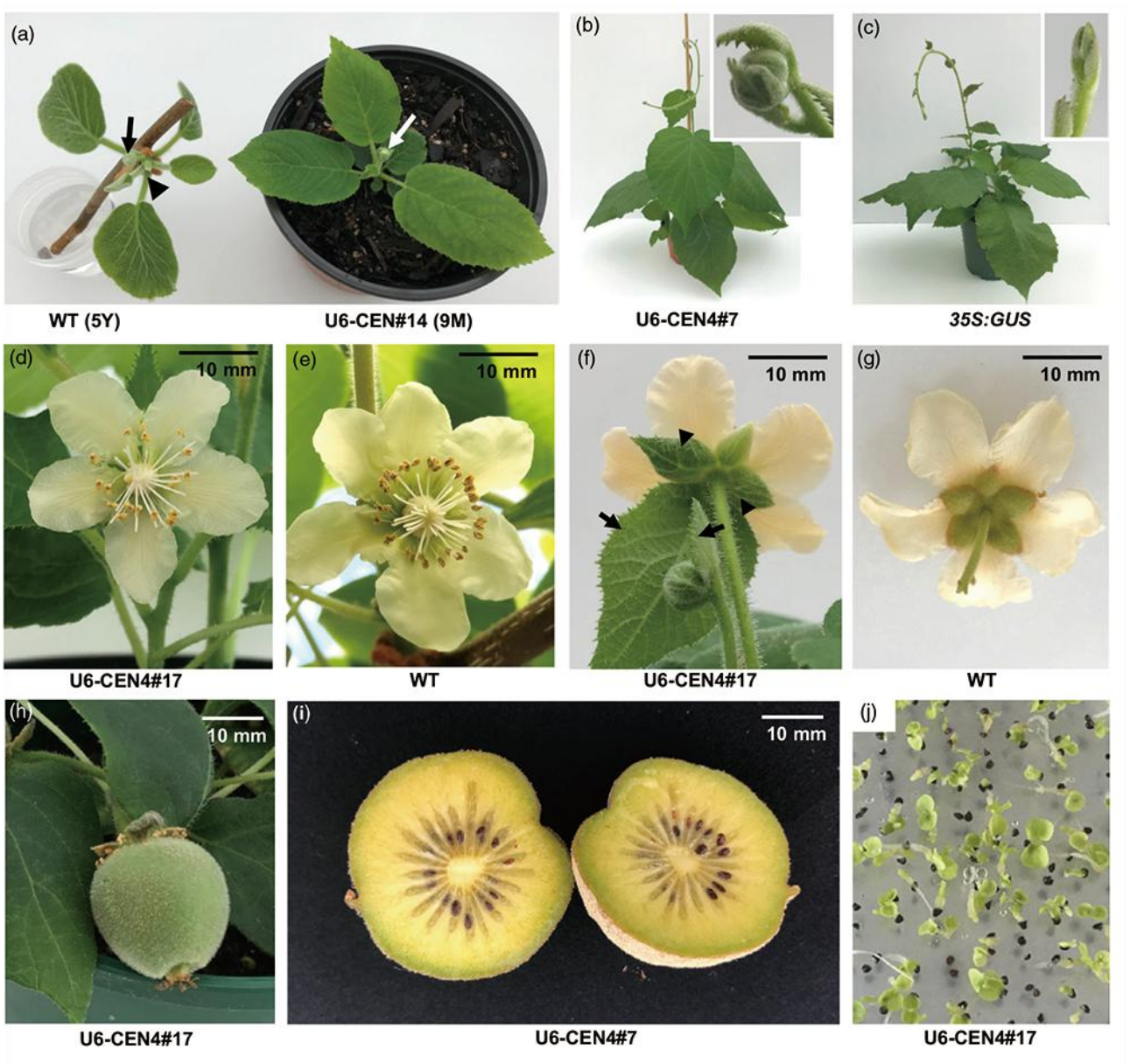

Figure 3 Accelerated flowering and normal reproductive development in *Actinidia chinensis* transgenic lines (Adopted from Varkonyi-Gasic et al., 2018)

Image caption: (a) The apical flower bud (white arrow) of U6-CEN4 line 14 (right) compared to the apical vegetative bud (black arrow) and axillary flower bud (black arrow) on the extending shoot from a wild-type (WT) kiwifruit plant (left). (b, c) The appearance of U6-CEN4 line 7 and the control 35S transgenic kiwifruit. (d, e) The normal appearance of the ovary. (f, g) The accessory leaves (arrows) and leafy sepals (arrows) in the terminal flowers. (h) Developing fruit at 30 days after pollination. (i) Mature fruit at 120 days after pollination. (j) Seed germination. The results shown in the figure indicate that the edited plants were able to form terminal flower buds at an early stage, leading to earlier flowering compared to the wild-type plants. The images also demonstrate that these terminal flower buds were capable of normal development and fruiting, confirming that gene editing successfully altered the growth habit of kiwifruit, transforming it from the traditional axillary flowering to terminal flowering and significantly shortening the juvenile phase. The findings support the crucial regulatory role of the *CEN4* and *CEN* genes in kiwifruit flowering time and plant morphology (Adapted from Varkonyi-Gasic et al., 2018)

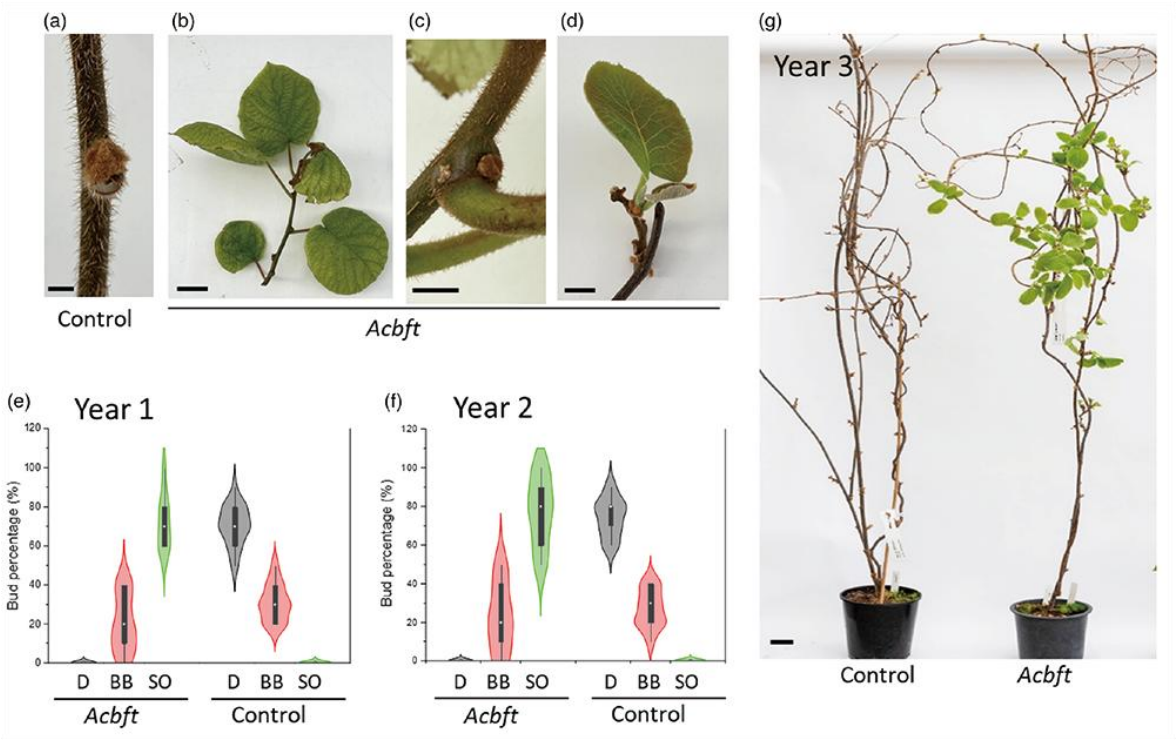

Figure 4 Delayed dormancy and early budbreak in *Acbft* kiwifruit lines (Adopted from Herath et al., 2022)

Image caption: Figure a shows the normal dormant bud in the control group, while Figures b to d display the "evergrowing" phenotype of the *AcBFT2* double allele mutant lines, including delayed leaf senescence and continuous bud emergence. Figures e and f quantify the early budbreak observed in the edited lines after pruning and defoliation, which occurs significantly earlier than in the control group. Figure g further confirms the advanced budbreak in these mutants following winter. These results indicate the critical role of the *AcBFT2* gene in regulating dormancy and growth cessation in kiwifruit, particularly in the process of breaking winter dormancy (Adapted from Herath et al., 2022)

6.3 Comparative outcomes of CRISPR/Cas9 applications in *Actinidia chinensis*

Comparative studies of CRISPR/Cas9 applications in *Actinidia chinensis* have highlighted the versatility and effectiveness of this technology in achieving various genetic improvements. For instance, the simultaneous targeting of *CEN4* and *SyGl* genes resulted in rapid flowering hermaphrodites with restored gynoecial function and viable pollen, providing functional evidence for the role of *SyGl* in suppressing feminization (De Mori et al., 2020; Varkonyi-Gasic et al., 2021). This approach not only accelerated breeding but also facilitated the development of self-pollinating kiwifruit lines.

Furthermore, the development of a high-quality reference genome for *Actinidia chinensis* has provided a solid foundation for future CRISPR/Cas9 applications, enabling more precise and efficient gene editing (Wu et al., 2019; Yue et al., 2022). These advancements underscore the potential of CRISPR/Cas9 technology to revolutionize kiwifruit breeding and cultivation, paving the way for improved crop varieties with enhanced traits.

7 Ethical and Regulatory Considerations

7.1 Ethical concerns in CRISPR/Cas9 applications

The application of CRISPR/Cas9 technology in kiwifruit (*Actinidia chinensis*) raises several ethical concerns. One primary issue is the potential for unintended off-target effects, which could lead to unforeseen genetic changes and ecological impacts. The precision of CRISPR/Cas9, while significantly advanced, is not foolproof, and the long-term consequences of these genetic modifications are not fully understood (Wang et al., 2018; Fizikova et al., 2021).

Additionally, there is a debate over the moral implications of genetically modifying organisms, particularly when it comes to altering the genetic makeup of crops that are integral to human diets and economies (Zhou et al., 2020).

The potential for creating genetically modified organisms (GMOs) that could crossbreed with wild relatives and alter natural ecosystems is another significant concern (Herath et al., 2022).

7.2 Regulatory landscape for gene-edited crops

The regulatory landscape for gene-edited crops, including kiwifruit, varies significantly across different regions. In some countries, gene-edited crops are subject to the same stringent regulations as traditional GMOs, requiring extensive testing and approval processes before they can be commercially released. For instance, the European Union has stringent regulations that classify gene-edited crops under the same category as GMOs, necessitating rigorous safety assessments and labeling (Zhou et al., 2020).

In contrast, other countries like the United States have a more lenient approach, where gene-edited crops that do not contain foreign DNA may not be subject to the same level of regulation (Fizikova et al., 2021). This disparity in regulatory frameworks can create challenges for international trade and the global adoption of CRISPR/Cas9 technology in agriculture.

7.3 Public perception and acceptance of CRISPR technology

Public perception and acceptance of CRISPR technology play a crucial role in its adoption and implementation in agriculture. There is a general lack of understanding and awareness about the differences between traditional GMOs and gene-edited crops, which can lead to public resistance and skepticism (Zhou et al., 2020). Efforts to communicate the benefits and safety of CRISPR/Cas9 technology are essential to gain public trust and acceptance. Highlighting consumer-friendly traits, such as improved nutritional content and reduced pesticide use, can help in garnering support for gene-edited crops (Zhou et al., 2020). Moreover, transparent communication about the ethical considerations, regulatory measures, and potential risks associated with CRISPR technology is vital to address public concerns and foster informed decision-making (Fizikova et al., 2021).

While CRISPR/Cas9 technology holds immense potential for the genetic improvement of kiwifruit, addressing ethical concerns, navigating the complex regulatory landscape, and fostering public acceptance are critical for its successful application and integration into modern agriculture.

8 Future Directions in Kiwifruit Genomics and Genome Editing

8.1 Emerging trends in kiwifruit genomics

The field of kiwifruit genomics is rapidly evolving, driven by advancements in sequencing technologies and bioinformatics tools. Recent studies have highlighted the importance of optimizing genome editing systems specifically for kiwifruit to enhance their efficiency and effectiveness (Yue et al., 2020). For instance, the development of a new cloning strategy for generating paired-sgRNA/Cas9 vectors has significantly improved the mutagenesis frequency in kiwifruit, demonstrating a 10-fold increase in efficiency compared to traditional methods (Wang et al., 2018). This optimization is crucial for achieving precise genetic modifications and advancing functional genomic studies in kiwifruit.

8.2 Innovations in CRISPR/Cas9 technology

CRISPR/Cas9 technology has revolutionized genome editing across various plant species, including kiwifruit. Innovations such as the polycistronic tRNA-sgRNA (PTG) cassette have been shown to enhance the efficiency of CRISPR/Cas9 systems, making them more powerful tools for genome editing in kiwifruit (Wang et al., 2018). Additionally, emerging CRISPR systems like base editing (BE), xCas9, and Cas12a (Cpf1) offer new possibilities for precise genetic modifications without the need for double-strand breaks (Manghwar et al., 2019). These advancements are expected to facilitate the development of kiwifruit varieties with improved traits, such as disease resistance and enhanced fruit quality (Zhou et al., 2020; Keul et al., 2022).

8.3 Potential for integrating multi-omics approaches

The integration of multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, with CRISPR-based genome editing holds great promise for advancing kiwifruit research. By combining these technologies, researchers can gain a comprehensive understanding of the molecular mechanisms underlying

important traits and identify key genes for targeted editing. For example, the use of omics technologies has already provided valuable insights into the functional attributes of food microbes and their impact on host health (Pan and Barrangou, 2020). Applying similar strategies to kiwifruit could lead to the identification of novel targets for genetic improvement and the development of superior kiwifruit varieties (Concordet and Haeussler, 2018; Razzaq et al., 2019).

The future of kiwifruit genomics and genome editing is bright, with emerging trends and innovations in CRISPR/Cas9 technology paving the way for significant advancements. The integration of multi-omics approaches will further enhance our understanding of kiwifruit biology and facilitate the development of improved varieties, ultimately benefiting both growers and consumers.

9 Concluding Remarks

Recent advancements in genomic research and CRISPR/Cas9 applications have significantly contributed to the understanding and improvement of kiwifruit (*Actinidia chinensis* Planch.). Key findings include the successful manipulation of kiwifruit genes to alter plant architecture and flowering time. For instance, CRISPR/Cas9-mediated mutagenesis of CENTRORADIALIS-like genes (*AcCEN4* and *AcCEN*) transformed kiwifruit from a climbing woody perennial into a compact plant with rapid terminal flowering, demonstrating the potential for accelerated breeding and indoor farming. The development of optimized paired-sgRNA/Cas9 systems has enhanced the efficiency of multiplex genome editing in kiwifruit, providing a powerful tool for genetic improvement. Furthermore, high-quality genome assemblies, such as the chromosome-level reference genome of *A. chinensis* and the telomere-to-telomere assembly of *A. chinensis* cv. 'Hongyang', have provided comprehensive genomic resources that facilitate molecular elucidation of diverse traits and breeding efforts.

The genomic advances and CRISPR applications in kiwifruit research have profound implications for both basic and applied sciences. The ability to manipulate specific genes using CRISPR/Cas9 has opened new avenues for functional genomic studies, allowing researchers to dissect the roles of key genes in plant development, flowering, and stress responses. For example, targeting the *SyGI* gene, which suppresses female development, has potential applications in modifying sex expression and improving breeding efficiency in dioecious plants like kiwifruit. Moreover, the integration of metabolome and transcriptome data has revealed regulatory networks governing flavor formation, providing insights into the metabolic pathways that can be targeted for flavor improvement. These advancements not only enhance our understanding of kiwifruit biology but also offer practical solutions for breeding programs aimed at developing superior cultivars with desirable traits such as early flowering, compact growth, and improved flavor.

The future of kiwifruit research looks promising, with continued advancements in genomic technologies and CRISPR/Cas9 applications poised to drive further innovations. The development of high-quality reference genomes and efficient genome editing tools will enable more precise and targeted breeding strategies, accelerating the development of new kiwifruit varieties with enhanced traits. Additionally, the integration of multi-omics approaches, including genomics, transcriptomics, and metabolomics, will provide a holistic understanding of the complex regulatory networks underlying key traits, facilitating the identification of novel targets for genetic improvement. As climate change poses new challenges to agriculture, the ability to engineer kiwifruit plants with improved resilience to environmental stresses will be crucial for ensuring sustainable production.

Overall, the synergistic application of genomic advances and CRISPR technologies holds great potential for transforming kiwifruit research and breeding, ultimately contributing to the development of high-value crops that meet the demands of both growers and consumers.

Acknowledgments

Author extend his sincere gratitude to the two anonymous peer reviewers for their contributions during the evaluation of this manuscript.

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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