

## Genomic Advances in Cucurbitaceae: Implications for Crop Improvement and Breeding

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**Abstract** The Cucurbitaceae family, encompassing a wide array of economically and nutritionally significant crops, has been the focus of extensive genomic research aimed at enhancing breeding and crop improvement. Recent advancements in sequencing technologies and bioinformatics have led to the sequencing of genomes from various Cucurbitaceae species, providing valuable insights into gene identification, genome evolution, and genetic variation. This has opened new avenues for molecular breeding, leveraging genetic transformation and gene editing technologies, including CRISPR/Cas9, to overcome the limitations of conventional breeding methods. The integration of next-generation sequencing (NGS) and omics approaches has furthered our understanding of complex traits, such as disease resistance and fruit quality, and has facilitated the development of high-density genetic maps and the identification of functional genes. Additionally, the construction of genetic and cytogenetic maps has been instrumental in revealing the genomic structure of cucurbit crops, aiding in the alignment of linkage groups with chromosomes and enhancing marker-assisted selection. The exploration of genetic diversity through the analysis of wild Cucurbitaceae species using cytogenetic mapping has also contributed to the phylogenetic understanding and breeding resource development. With the accumulation of genomic resources and the advent of high-throughput genotyping methods, new strategies such as genome-wide association studies (GWAS) and the use of multi-parent populations have emerged, leading to the discovery of quantitative trait loci (QTL) for key agronomic traits. The synergy of these genomic tools and their implications for breeding is poised to revolutionize the improvement of Cucurbitaceae crops, ensuring food security and meeting the demands of a growing population.

**Keywords** Cucurbitaceae; Genomic sequencing; Genetic transformation; Gene editing; Genetic linkage map; Cytogenetic mapping; Marker-assisted selection; Genome-wide association studies; Quantitative trait loci

The Cucurbitaceae family, encompassing a wide array of economically significant and diverse crops, is integral to global agriculture and human nutrition. This family includes species such as cucumbers, melons, squashes, and pumpkins, which are not only staples in diets worldwide but also hold cultural and medicinal value (Grumet et al., 2017). The genetic diversity within this family is remarkable, with genome sequences available for multiple species across different tribes, providing a rich resource for understanding evolutionary relationships and agronomic traits (Ma et al., 2022).

In the realm of agriculture, genomic research has become a cornerstone for crop improvement and breeding programs. The advent of next-generation sequencing (NGS) and omics technologies has revolutionized our approach to plant breeding, allowing for a more profound understanding of the genotype-phenotype relationship, particularly for complex traits (Pawełkiewicz et al., 2016). These advancements have facilitated the identification of functional genes, the development of molecular markers, and the construction of high-density genetic linkage maps, which are crucial for marker-assisted selection (MAS) in breeding (Ren et al., 2009; Fukino and Kawazu, 2016). Moreover, the recent progress in genetic transformation and gene editing technologies, such as CRISPR/Cas9, has opened new avenues for enhancing cucurbit crops' genetic diversity and overcoming the limitations of conventional breeding methods (Feng et al., 2023).

The purpose of this systematic review is to examine the impact of these recent genomic advances on the breeding and crop improvement of Cucurbitaceae species. By integrating the wealth of genomic tools and resources now available, including draft genome sequences and high-throughput genotyping methods, we aim to provide a comprehensive overview of how these technologies are shaping the future of cucurbit breeding. We will explore the implications of genomic tools for dissecting complex traits, enhancing selection efficiency, and ultimately contributing to the development of superior cucurbit cultivars with improved yield, quality, and stress tolerance (Phan and Sim, 2017). The scope of this review will encompass the latest developments in genomic research and their practical applications in cucurbit breeding, offering insights into the potential of these technologies to meet the challenges of food security and agricultural sustainability in the 21<sup>st</sup> century.

## 1 Genomic Tools and Technologies in Cucurbitaceae Research

### 1.1 Description of genomic tools (e.g., high-throughput sequencing, CRISPR/Cas9 gene editing)

The advent of genomic tools has revolutionized the field of plant breeding and genetics, particularly within the Cucurbitaceae family. High-throughput sequencing (HTS) technologies, such as next-generation sequencing (NGS), have enabled the rapid and cost-effective generation of large volumes of genomic data. These technologies facilitate the study of genotype-phenotype relationships, especially for complex traits, by allowing the discovery of new genes, regulatory sequences, and the development of extensive collections of molecular markers (Pawelkowicz et al., 2016). CRISPR/Cas9 gene editing has emerged as a powerful tool for creating targeted mutations, enabling the study of gene function and the development of crops with desirable traits. In cucurbits, CRISPR/Cas9 has been used to create mutants with compact plant architecture, which is beneficial for high-density planting and mechanical harvesting (Xin et al., 2022).

### 1.2 Advances in genotyping and sequencing technologies applied to Cucurbitaceae

The Cucurbitaceae family has seen significant advances in genotyping and sequencing technologies. The sequencing of 18 different cucurbit species genomes has provided insights into gene identification, genome evolution, and genetic variation (Ma et al., 2022). The development of highly polymorphic simple sequence repeat (SSR) markers from whole genome shotgun sequencing has led to the construction of high-density genetic linkage maps, facilitating whole genome sequencing and molecular breeding in cucurbits like cucumber (Ren et al., 2009). Additionally, the sequencing of the mitochondrial genomes of species such as *Citrullus lanatus* (watermelon) and *Cucurbita pepo* (zucchini) has provided insights into the evolution of genome size and the content of RNA editing (Alverson et al., 2010).

### 1.3 Role of bioinformatics in genomic research: from data collection to analysis

Bioinformatics plays a crucial role in genomic research by managing and analyzing the vast amounts of data generated by HTS and other genomic technologies. The Cucurbit Genomics Database (CuGenDB) serves as a central portal for the storage, mining, analysis, integration, and dissemination of large-scale genomic and genetic datasets for cucurbits (Zheng et al., 2018). This database includes genome sequences, ESTs, genetic maps, transcriptome profiles, and sequence annotations, as well as tools for comparative genomic analysis such as synteny blocks and homologous gene pairs between different cucurbit species. The development of tools like 'SyntenyViewer' and the 'RNA-Seq' module within CuGenDB has greatly facilitated the visualization and analysis of genomic data, aiding researchers in the cucurbit breeding community (Zheng et al., 2018).

In conclusion, the integration of genomic tools and technologies, such as HTS, CRISPR/Cas9, and bioinformatics platforms, has significantly advanced the research and breeding of Cucurbitaceae crops. These advancements have not only enhanced our understanding of the genetic basis of important agronomic traits but have also provided the means for targeted crop improvement (Ren et al., 2009; Alverson et al., 2010; Pawelkowicz et al., 2016; Zheng et al., 2018; Ma et al., 2022; Xin et al., 2022).

## 2 Genomic Discoveries in Cucurbitaceae

### 2.1 Key genomic features and architectures discovered in different Cucurbitaceae species

Recent studies have shed light on the genetic architecture of fruit size and shape variation in cucurbits, revealing a complex network of quantitative trait loci (QTL) and candidate genes. In cucumbers, melons, and watermelons, over 150 consensus QTLs for fruit size, shape, and weight have been identified, and a genome-wide survey has pinpointed 253 homologs of key fruit size/weight-related genes (Pan et al., 2019). These discoveries underscore the structural and functional conservation of fruit size/shape gene homologs across cucurbits, exemplified by genes such as *CsSUN25-26-27a* and *CsTRM5* in cucumber, and *CmOFP1a* in melon (Pan et al., 2019). Additionally, the identification of 142 metal-tolerance proteins (MTPs) across eight Cucurbitaceae species highlights the importance of these transporters in plant metal tolerance and ion homeostasis (Jiang et al., 2021). The evolutionary analysis of these MTPs, which are under purifying selection, provides a basis for understanding ion transport functions and mechanisms in Cucurbitaceae (Jiang et al., 2021).

### 2.2 Comparative genomics: insights from comparing Cucurbitaceae genomes with other plant families

Comparative genomics has been instrumental in understanding the evolution of mitochondrial genome size within the Cucurbitaceae family. The sequencing of the mitochondrial genomes of *Citrullus lanatus* and *Cucurbita pepo* revealed significant size variation, which is attributed to the accumulation of chloroplast sequences and short repeated sequences (Alverson et al., 2010). This variation is decoupled from mutation rate and RNA editing frequency, suggesting independent evolutionary pathways for these genomic features (Alverson et al., 2010). Furthermore, a multi-locus chloroplast phylogeny has provided insights into the character evolution and classification within the family, correlating well with flower characters such as the number of free styles and fusion of filaments and/or anthers (Kocyan et al., 2007).

### 2.3 Functional genomics: gene discoveries related to traits such as disease resistance, fruit quality, and stress tolerance

The functional genomics landscape of Cucurbitaceae has expanded with the identification of calcium-dependent protein kinases (CDPKs) and CDPK-related kinases (CRKs) in six species. These kinases play crucial roles in plant growth, development, and stress response (Wei et al., 2019). Expression studies in watermelon have revealed genes that are induced by salinity and maintain high expression levels in male flowers, suggesting their potential roles in stress tolerance and reproductive development (Wei et al., 2019). Additionally, the complete chloroplast genome sequences of ten Cucurbitaceae species have been described, identifying genes under selection that are involved in chloroplast protein synthesis, gene transcription, energy transformation, and plant development (Zhang et al., 2018). These findings, along with the discovery of a large number of SSRs and SNPs in *Cucurbita pepo*, provide valuable resources for breeding programs aimed at improving disease resistance, fruit quality, and stress tolerance in cucurbits (Guo et al., 2020).

## 3 Applications of Genomic Research in Breeding

### 3.1 Marker-assisted selection (MAS): how genomic markers are used to accelerate breeding processes

Marker-assisted selection (MAS) is a process where molecular markers are used to assist in the selection of desirable traits in crop improvement. In the context of Cucurbitaceae breeding, MAS has been significantly advanced by the development of high-throughput genotyping technologies and the identification of single nucleotide polymorphisms (SNPs). These advancements have facilitated the discovery of key genes and molecular markers linked to important traits in vegetables, including those in the Cucurbitaceae family (Mohan et al., 1997; He et al., 2014; Hao et al., 2019). Genotyping-by-sequencing (GBS) is one such technique that combines molecular marker discovery and genotyping, proving to be a cost-effective MAS tool in plant breeding (Figure 1) (He et al., 2014).

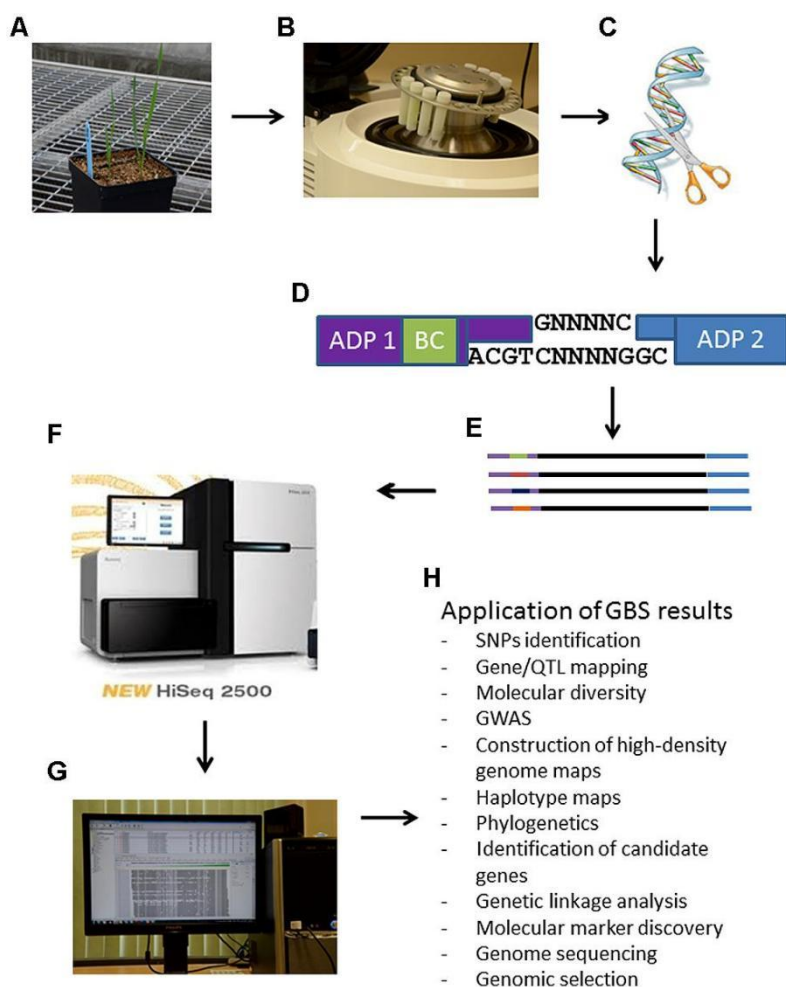


Figure 1 Schematic steps of the genotyping-by-sequencing (GBS) protocol for plant breeding (Adopted from He et al., 2014)

Image Caption: Panel (A): tissue is obtained from any plant species as depicted here a young triticale plant; Panel (B): ground leaf tissues for DNA isolation, quantification and normalization. At this step it is important to prevent any cross-contamination among samples; Panel (C): DNA digestion with restriction enzymes; Panel (D): ligations of adaptors (ADP) including a bar coding (BC) region in adapter 1 in random PstI-MseI restricted DNA fragments; Panel (E): representation of different amplified DNA fragments with different bar codes from different biological samples/lines. These fragments represent the GBS library; Panel (F): analysis of sequences from library on a NGS sequencer; Panel (G): bioinformatic analysis of NGS sequencing data; Panel (H): possible application of GBS results (Adopted from He et al., 2014)

### 3.2 Genomic selection: principles and case studies in Cucurbitaceae

Genomic selection (GS) is a breeding method that uses genome-wide marker data to predict the breeding values of individuals in a population. This approach is particularly useful for traits that are influenced by many genes, each with a small effect. GS has been shown to increase genetic gain per unit time and cost, and its early empirical and simulation results are promising for Cucurbitaceae crops (Heffner et al., 2009; Jannink et al., 2010; Heslot et al., 2015). The success of GS relies on the use of all available marker information to predict phenotypes, which allows for the capture of more variation due to small-effect quantitative trait loci (QTL) (Goddard and Hayes, 2007; Heffner et al., 2009). However, careful consideration of resource allocation and the cost-benefit balance of using markers is necessary for the effective implementation of GS (Heslot et al., 2015).

### 3.3 Genetic engineering and editing: targeted modifications to improve crop traits

Genetic engineering and gene editing technologies, such as CRISPR/Cas9, have opened new avenues for targeted modifications of crop genomes to improve traits. In Cucurbitaceae, these technologies have been used to overcome the limitations of conventional breeding methods, such as the narrow genetic bases and low variation

rates of these crops (Feng et al., 2023). Recent progress in genetic transformation and gene editing in cucurbits has led to improvements in genetic transformation efficiency and the application of gene editing for trait enhancement (Figure 2) (Feng et al., 2023). The integration of genomic and variomic information has the potential to rapidly increase breeding efficiency in cucurbit crops (Hao et al., 2019).

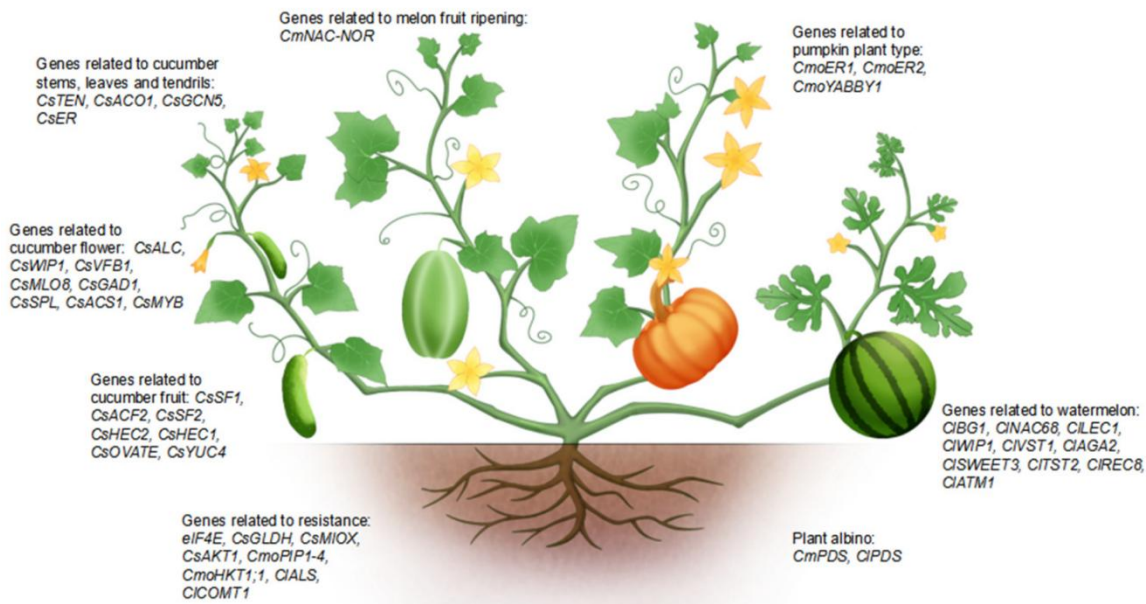


Figure 2 Illustration of editing genes in cucurbit crops (Adopted from Feng et al., 2023)

Image Caption: This diagram illustrates the CRISPR/Cas9 gene editing process adopted for cucurbit crops. The system introduces engineered endonucleases into the plant via genetic transformation, enabling precise and efficient modification of specific nucleotide sequences within the genome. Key steps include the design of the CRISPR system, introduction into the target plant, and the eventual elimination of carrier fragments through hybridization. Successful applications in various cucurbit species demonstrate the technology's adaptability and effectiveness in enhancing desirable crop traits (Adapted from Feng et al., 2023)

The implementation of CRISPR/Cas9 technology in cucurbit crops marks a significant advancement in agricultural biotechnology, offering a powerful tool for precise genomic manipulation. This approach not only accelerates the process of crop improvement but also allows for the targeted editing of genes with high specificity and minimal off-target effects. By enabling specific genetic modifications, CRISPR technology facilitates the study of gene functions and the development of cucurbit varieties with improved traits, such as increased resistance to pathogens or enhanced nutritional profiles.

In conclusion, the application of genomic research in Cucurbitaceae breeding, through MAS, GS, and genetic engineering and editing, is revolutionizing the way breeders approach crop improvement. These genomic tools enable more precise and efficient selection, ultimately accelerating the development of improved crop varieties to meet the demands of a growing global population.

## 4 Case Studies

### 4.1 Successful implementation of genomic advances in Cucurbitaceae crop improvement

The Cucurbitaceae family, encompassing crops such as cucumber, melon, watermelon, squash, and pumpkin, has seen significant advancements in genomic research that have facilitated crop improvement and breeding efforts. The development of high-density genetic linkage maps, as reported in the cucumber genome, has been instrumental in enhancing the resolution of recombination breakpoints and has enabled the integration of gene and trait knowledge across cucurbits (Ren et al., 2009). The advent of next-generation sequencing (NGS) and omics technologies has further revolutionized Cucurbitaceae breeding by allowing the discovery of new genes and

regulatory sequences, thereby aiding in the understanding of complex traits such as disease resistance, cold tolerance, and fruit quality (Pawelkowicz et al., 2016).

#### **4.2 Example 1: Enhancing disease resistance in cucumbers through genomic selection**

Cucurbit viruses, including *Potyvirus*es and *Cucumovirus*es, pose a significant threat to cucurbit crops worldwide. The identification of natural resistances within the Cucurbitaceae family has been a cornerstone in breeding for disease resistance. Genetic studies have mapped dominant and recessive resistance genes, and markers for marker-assisted selection (MAS) are now available, although higher mapping resolutions are needed for gene identification (Martín-Hernández and Picó, 2020). The development of DNA markers in Cucurbitaceae breeding has been pivotal in constructing genetic linkage maps and consensus maps with phenotypic traits and quantitative trait loci (QTLs), which are essential for MAS in cucurbit breeding programs (Fukino and Kawazu, 2016).

#### **4.3 Example 2: Genetic modification of watermelon for improved drought tolerance**

The identification of salt-tolerant genotypes within the Cucurbitaceae family is a research priority, especially in the context of climate change and intensive agricultural practices. A greenhouse study assessing the response to salinity in seedlings of various cucurbit genotypes has identified potential candidates for salt-resistant rootstocks, which could be introduced in grafting programs to secure yield stability in salt-affected areas (Modarelli et al., 2020). This approach exemplifies the use of genomic information to select and improve traits such as drought tolerance in watermelon and other cucurbits.

#### **4.4 Example 3: Breeding of bitter melon with improved fruit quality using marker-assisted selection**

The transferability of SSR markers from cucumber to other cucurbit crops has been evaluated, demonstrating that cucumber-derived SSR markers can cross-amplify in other species, including bitter melon. This transferability facilitates the molecular characterization of less studied cucurbit crops and supports genetic diversity analysis, which is crucial for breeding programs aimed at improving fruit quality and other desirable traits (Natenuch et al., 2020). The use of SSR markers in cucurbit breeding has been a breakthrough, allowing for the development of varieties with resistance to multiple diseases and the incorporation of specific horticultural characteristics (Sitterly, 1972).

In conclusion, the case studies presented here illustrate the successful implementation of genomic advances in the breeding and improvement of Cucurbitaceae crops. The integration of genetic and genomic resources, such as high-density linkage maps, omics technologies, and DNA markers, has significantly contributed to the development of disease-resistant, drought-tolerant, and high-quality cucurbit varieties.

## **5 Challenges and Limitations**

Despite the significant strides in genomic research within the Cucurbitaceae family, several challenges and limitations persist that hinder the full potential of crop improvement and breeding programs. One of the primary challenges is the narrow genetic bases and low variation rates in cucurbit crops, which have led to a breeding bottleneck. This limitation has been a significant obstacle in conventional breeding methods, which, while having contributed to cucurbit production, are now facing diminishing returns (Feng et al., 2023).

The development of genetic transformation and gene editing techniques for cucurbit crops has lagged behind other major crops. Although recent progress has been made, the efficiency of these techniques in cucurbits still requires improvement. The screening of germplasm, application of physical treatments, morphogenic genes, and selection markers are areas that need further refinement to enhance genetic transformation efficiency in cucurbit crops (Feng et al., 2023).

Another limitation is the availability of genetic and genomic resources for plant improvement. For instance, the construction of saturated molecular linkage maps has been impeded by the narrow genetic base of some cucurbit species, such as cucumber. While the development of highly polymorphic simple sequence repeat (SSR) markers and high-density genetic linkage maps has been reported, these resources are still relatively scarce compared to other crops (Ren et al., 2009).

Genomic innovation is crucial for increasing crop production and reducing its impact on ecosystems. However, accessing the large and complex genomes of crops and their wild relatives remains a daunting task. The association of genetic diversity with agronomic phenotypes requires detailed characterization of plant genomes, which is a resource-intensive process. Moreover, the integration of improved phenotyping assays and functional genomic studies into crop-breeding systems is still in its infancy (Bevan et al., 2017).

The rapid development of sequencing technologies has facilitated the generation of genome sequences for numerous Cucurbitaceae species. However, the translation of this genomic information into practical applications for molecular breeding is a complex process that involves understanding evolutionary relationships and functional genes associated with important agronomic traits. The pace at which this genomic information can be applied to molecular breeding programs is still slow, and there is a need for more research to bridge the gap between genomic data and breeding applications (Ma et al., 2022).

Lastly, while novel molecular and transgenic approaches have been employed in cucumber breeding, the development of genomic tools and their application in breeding programs is a time-consuming process. The success of these approaches in rapidly developing new cultivars to meet the demands of a growing population is yet to be fully realized. The improvement of various traits such as sex expression, disease resistance, and quality through these novel approaches is still an ongoing effort (Kaur and Sharma, 2021).

In conclusion, while genomic advances hold great promise for the improvement of Cucurbitaceae crops, significant challenges and limitations must be addressed to fully harness these technologies for crop improvement and breeding.

## **6 Future Directions**

### **6.1 Emerging technologies and their potential impact on Cucurbitaceae genomics and breeding**

The advent of next-generation sequencing (NGS) and omics technologies has ushered in a new era for Cucurbitaceae genomics and breeding. The analysis of NGS data facilitates the discovery of new genes, regulatory sequences, and provides a wealth of molecular markers, which are invaluable for plant breeding programs (Pawełkiewicz et al., 2016). The integration of these technologies has the potential to significantly accelerate the understanding of genotype-phenotype relationships, especially for complex traits such as disease resistance, fruit quality, and tolerance to abiotic stresses (Pawełkiewicz et al., 2016; Feng et al., 2019). The future of Cucurbitaceae breeding is poised to be transformed by these emerging technologies, enabling breeders to create high-density genetic maps and apply genomic selection and modification methods more effectively (Ren et al., 2009; Pawełkiewicz et al., 2016).

### **6.2 The role of genome editing in future crop improvement strategies**

Genome editing, particularly CRISPR/Cas9-mediated gene knockout and base editing, represents a revolutionary tool in the field of crop improvement (Feng et al., 2023). For Cucurbitaceae, the application of gene editing technologies is still in its infancy but has shown great promise in recent years (Feng et al., 2023). The ability to precisely edit the genome allows for the targeted modification of key agronomic traits, potentially overcoming the limitations of conventional breeding and genetic transformation methods (Ezura, 2016; Feng et al., 2023). As these techniques become more refined, they will likely play a central role in developing new Cucurbitaceae cultivars with enhanced yield, quality, and stress resilience (Feng et al., 2019; Feng et al., 2023).

### **6.3 Integration of genomics with other omics technologies for a systems biology approach**

The integration of genomics with other omics technologies, such as proteomics and metabolomics, is essential for adopting a systems biology approach in Cucurbitaceae research and breeding (Bevan et al., 2017; Pawełkiewicz et al., 2016). This holistic view allows for a more comprehensive understanding of the molecular networks that govern plant growth, development, and response to environmental stimuli (Bevan et al., 2017). By combining genomic information with protein and metabolite profiles, researchers can unravel the complex interactions that define important agronomic traits (Bevan et al., 2017; Pawełkiewicz et al., 2016). This integrated approach is

expected to lead to more efficient breeding strategies and the development of Cucurbitaceae crops with optimized performance and quality (Ezura, 2016; Bevan et al., 2017; Pawelkiewicz et al., 2016).

In conclusion, the future of Cucurbitaceae genomics and breeding is bright, with emerging technologies providing powerful tools for crop improvement. The integration of genome editing and omics technologies will enable breeders to tailor crops to meet the demands of a growing population and changing climate. As these technologies continue to evolve, they will undoubtedly unlock new possibilities for innovation in Cucurbitaceae breeding.

## **7 Concluding Remarks**

### **7.1 Summary of the impacts of genomic advances on Cucurbitaceae crop improvement and breeding**

The genomic advances in the Cucurbitaceae family have significantly impacted crop improvement and breeding strategies. The development of molecular techniques, such as genetic transformation and gene editing technologies, has led to breakthroughs in horticultural crop breeding, overcoming the limitations of conventional breeding methods and the narrow genetic bases of these crops (Feng et al., 2023). The integration of next-generation sequencing (NGS) and omics technologies has facilitated the study of genotype-phenotype relationships, particularly for complex traits, and has enabled the discovery of new genes, regulatory sequences, and molecular markers (Pawelkiewicz et al., 2016). The availability of genome sequences for numerous Cucurbitaceae species has accelerated research on gene identification, genome evolution, genetic variation, and molecular breeding, providing valuable insights into important agronomic traits (Ma et al., 2022). Additionally, the construction of high-density genetic linkage maps and the use of fluorescent in situ hybridization (FISH) have facilitated whole genome sequencing and the integration of gene-trait knowledge in cucurbits (Ren et al., 2009).

### **7.2 The potential of genomics to meet future challenges in agriculture**

Genomics has the potential to address future challenges in agriculture by enabling more precise and efficient crop improvement. Detailed characterization of plant genomes and genetic diversity is crucial for increasing crop production while reducing its impact on ecosystems (Bevan et al., 2017). Advances in genomics are providing new foundations for crop-breeding systems, allowing breeders to design genotypes *in silico* and practice whole genome selection (Varshney et al., 2005). Genome-wide association studies (GWAS) and genomic selection (GS) are powerful approaches that accelerate the rate of genetic gain in crops and reduce the breeding cycle in a cost-effective manner (Bentley et al., 2022). These genomic tools are essential for developing high-yielding varieties capable of responding to global agricultural challenges, including climate change and food security.

### **7.3 Final thoughts on the evolution of breeding techniques driven by genomic research**

The evolution of breeding techniques driven by genomic research has transformed the landscape of Cucurbitaceae crop improvement. The integration of genomic information into breeding programs, known as genomics-assisted breeding, is gradually evolving from marker-assisted breeding and selection (Varshney et al., 2005). The use of high-throughput phenotyping and functional genomic studies, along with the elucidation of fundamental mechanisms such as heterosis and epigenetics, is enhancing the efficiency and precision of crop improvement (Bevan et al., 2017). The future of cucurbit breeding lies in leveraging the genetic diversity and genomic innovations to develop varieties that are resilient to biotic and abiotic stresses, nutritionally enhanced, and adapted to diverse environmental conditions (Bentley et al., 2022). As we look to the future, the continued application of genomic advances will undoubtedly lead to more innovative and sustainable approaches to meet the ever-growing demands of agriculture (Grumet et al., 2017).

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## Conflict of Interest Disclosure

The authors affirm 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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