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Review

From Sequencing to Genome Editing in Cucurbitaceae: Application of Modern Genomic Techniques to Enhance Plant Traits

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Abstract: The availability of genome-sequencing and genome-editing techniques has increased the applicability of innovative solutions, opening up revolutionary prospects for developments in horticultural plant breeding. The Cucurbitaceae family is a group of plants of great importance in horticulture due to their high nutritional and economic value. These plants serve as important models for elucidating the principles of plant development and refining yield improvement strategies. While traditional breeding approaches have made significant contributions to the production of cucurbits, they have also been limited by the reduced genetic diversity and lower rates of variation inherent in these species. This comprehensive review summarises the latest developments in genome editing in cucurbits. It covers various aspects of enhancing plant traits to resist biotic stresses such as pathogenic fungi and viruses, as well as abiotic stresses such as adverse climate change, especially stresses caused by drought and salinity. This study focused on improvements in plant quality and on the optimisation of plant architecture, sex determination of flowers and fruit features. This review provides insights that may hold great promise for the future of horticultural crop improvement and serves as an important reference for the advancement of genome-sequencing and gene-editing technologies in cucurbits.

Keywords: genome editing; CRISPR/Cas9; sequencing; Cucurbitaceae; cucurbit crop; genetic transformation; cucumber

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1. Introduction

Growing human populations combined with the challenges posed by climate change highlight the need for resilient and high-yielding crops. Genome-editing technologies such as CRISPR/Cas9 systems have the potential to contribute to groundbreaking advances in plant breeding. However, in order to use these techniques effectively, an in-depth knowledge of plant genomics, physiology and ecology is essential [1,2]. Through genome sequencing and functional analyses, researchers have gained insights into the genes and proteins that play pivotal roles in key aspects of crop development, offering a multitude of possibilities for enhancing the traits of selected plants [3]. Trait association studies provide a wide range of knowledge that can be used to plan new traits in crop development, including basic information on phenotypic traits, genomic and transcriptomic data, and knowledge from epigenetic studies through comparative analysis across a wide range of plants (Figure 1).

Next-generation sequencing technologies have significantly improved this cognitive process, offering a cost-effective and efficient analysis of DNA or RNA at high throughput rates [4]. Sequencing offers insight into the genetics of organisms, including specific gene identification, and provides an understanding of gene function and interactions [5]. This includes the ability to interpret complex genetic information, decipher regulatory elements and identify the genetic basis of traits, including complex traits [6].

Using this specific information, researchers and breeders have acquired efficient and powerful tools for precise manipulation of genetic material through precise genome-editing techniques. CRISPR/Cas9-based genome-editing techniques have triggered a revolution in genetic modification, allowing the targeted modification of specific genes [7]. Genome editing offers unprecedented opportunities to apply an understanding of genomics to improve crop traits in many plant species, including in Cucurbits.

Cucurbitaceae is a plant family belonging to the order Cucurbitales, consisting of over 965 species grouped into 95 genera [8]. Members of the Cucurbitaceae family are vulnerable to frost and are primarily found in warmer regions of the world. Those that extend into temperate zones either survive winters underground below the frost line as tubers or exist as annuals, enduring winter in the form of seeds. The Cucurbitaceae family is most prevalent in tropical regions, particularly in tropical Africa and the neotropics [9]. Cucurbits have been integral to human nutrition and culture for over 12,000 years in both the Old and New Worlds. Consequently, alongside the Brassicaceae and Asteraceae families, the Cucurbitaceae family has exceptional significance for humans, trailing only cereals and legumes in economic importance, which makes cucurbits significant candidates for genetic enhancement [10]. Notably, the genera *Cucumis*, *Cucurbita* and *Citrullus* are deemed highly economically important [11]. Apart from these globally cultivated genera, there are other noteworthy cucurbit genera of local or regional economic importance, including *Benincasa*, *Lagenaria*, *Luffa*, *Momordica* and *Sechium* [10].

As many cucurbit genomes are known, it has been possible to use the CRISPR/Cas9 technique to target the enhancement of crucial agricultural traits [12], such as improvements in crop features, plant quality and architecture, influence on plant sex determination, resistance to biotic or abiotic stresses, including the very important aspects of greater tolerance to adverse climate change to cope with drought and salinity. Climate change constitutes a barrier to the cultivation of cucurbit crops. The increasing pressure from environmental stresses, along with extreme natural events, presents a challenge to the development of new varieties that are resilient to environmental conditions [1]. Climate change affects plants in two main ways: abiotic stress (non-living factors) and biotic stress (living factors). Rising carbon dioxide (CO_2) levels help some plants grow, but other factors such as higher temperatures and radiation can harm them, leading to desertification and more severe droughts [13]. Some regions experience droughts followed by heavy rains, causing soil salinisation [14,15]. Drought is a major problem for crops, particularly water-demanding ones such as cucumbers. It not only reduces crop quantity, but quality too. In the last decade, droughts have cost about USD 30 billion in crop losses worldwide [16]. Plants are also sensitive to soil salinity, which comes in two forms: osmotic stress (from sodium ions) and ionic stress (from toxic ions). Climate change also affects plants by exposing them to new pests and diseases due to changing temperatures. This can lead to the emergence of new strains of pathogens [17,18]. Understanding the molecular basis of a plant's reaction to changing environmental conditions and discerning the determinants contributing to multi-stress tolerance are fundamental goals in crop breeding and research on stresses in plants [19].

New and rapid technologies are therefore needed to produce varieties with the desired characteristics. One such technology is the ability to edit the genome precisely. CRISPR/Cas9 genome editing has ushered in a new era of significance in breeding and agriculture thanks to its precision and versatility. This revolutionary technology allows scientists to modify the DNA of plants precisely, offering several crucial advantages. Traditional breeding methods can be time-consuming and imprecise, whereas CRISPR/Cas9

enables specific gene edits with unprecedented accuracy. Speed is essential in addressing food security challenges caused by a growing global population.

This paper presents the accomplishments of genome editing within the Cucurbitaceae family, discussing its potential to revolutionise crop improvement, as well as the challenges and applications of this technique. By analysing new research and technological advancements, this paper aims to examine the potential opportunities and intricate methodologies to enhance the genetic capacity of cucurbit crops. One of its primary objectives is to elucidate how modern genomic techniques, with CRISPR as a central player, enable researchers to unlock the genetic potential of cucurbit plants. By sequencing their genomes and utilising CRISPR technology, scientists can precisely edit specific genes responsible for desirable traits such as disease resistance, improved yield and enhanced nutritional content. The article aims to show how CRISPR technology has become an invaluable tool for enhancing crucial plant traits and enables the development of cucurbit varieties that are more resilient to climate change, environmental stressors, pests and diseases. Furthermore, CRISPR allows for the improvement of traits relevant to human nutrition, thus contributing to food security and health. By harnessing the power of CRISPR, the article emphasises how the process of crop breeding in Cucurbitaceae can be significantly accelerated. This acceleration is vital in order to address the ever-growing global demand for food production and ensure the sustainability of agriculture.

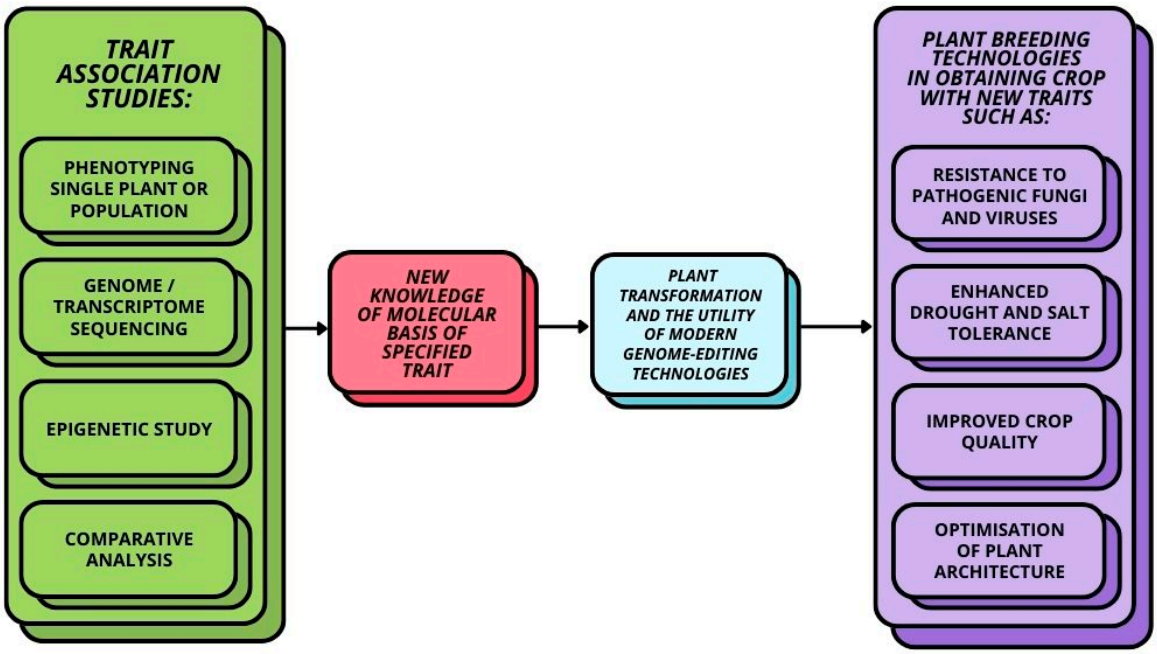

Figure 1. Overall scheme of modern technology in plant breeding.

2. Genome Sequencing

2.1. Tracing Advances in Sequencing Technologies

The overall history of genome sequencing began with the publication of an article by Frederick Sanger and his colleagues [20] in 1977 featuring the first-ever genome sequence. In detail, it was the DNA sequence of bacteriophage φX174 with only 5386 bp. Sanger was awarded his second Nobel Prize in Chemistry in 1980, shared with Walter Gilbert and Paul Berg, for developing and improving sequencing methods. It was the era of first-generation sequencing methods. Mankind had to wait 23 years, until 2000, to learn the DNA sequence of the first plant: *Arabidopsis thaliana*, a popular model organism in plant biology and genetics [21]. The human desire to understand the basic building blocks of life has driven the creation and development of sequencing technologies. Since the completion of the Human Genome Discovery Project [22], genomics has advanced rapidly around the

world. It launched the development of new sequencing technologies based on the shotgun technique to learn about the human genome. These technologies are now known as second-generation sequencing technologies. The evolution of next-generation sequencing (NGS) techniques represents a significant advancement in the field of genomics and molecular biology and has been widely described [23]. NGS technologies have revolutionised the way genetic information is studied and understood.

First-generation sequencing involved the use of chemicals to cleave bases within a DNA molecule or of chain-terminating nucleotides, followed by manual separation of fragments generated via electrophoresis [23]. Second-generation sequencing technology emerged in 2005 and marked the arrival of a new generation of sequencers to address the limitations of the first generation. The basic characteristics of second-generation sequencing technology are the generation of many millions of short reads in parallel and an acceleration of the sequencing of the process compared with the first generation. Third-generation sequencing, also known as long-read sequencing, is a class of DNA sequencing that has the capability to produce substantially longer reads than second-generation sequencing. Third-generation sequencing technologies allow direct sequencing of single DNA molecules, but they have much higher error rates than previous technologies, which can complicate downstream genome assembly and analysis of the resulting data. Third-generation sequencing technologies are undergoing active development, and it is expected that there will be further improvements in future [24,25]

Second- and third-generation sequencing technologies have greatly expanded knowledge not only of the code stored in DNA, but also of its function, interactions, modifications and evolutionary context [26]. Second-generation technologies have emerged as a faster, more accurate and more cost-effective alternative to Sanger sequencing pioneered by platforms such as Illumina's Solexa, Roche's 454 and ABI's SOLiD, and allow the sequencing process to be carried out on multiple samples at the same time, enabling millions of fragments to be sequenced simultaneously [4]. This enormous throughput has been achieved by bridging amplification or emulsion PCR, followed by cyclic synthesis or pyrosequencing. The development of NGS technology has made whole-genome sequencing possible, revealing previously undetected variants, mutations and structural changes. Whole-genome sequencing of RNA transcriptomes (RNA-Seq) has also become achievable, providing a snapshot of gene expression at any point in time, enabling transcriptome mapping and the detection of alternative splicing events [27]. An important step in understanding the complexity of genomes has been the development of third-generation sequencing technologies, often referred to as long-read sequencing, which have moved away from amplification-based NGS methods. Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) are pioneers in this field [4,26]. While PacBio's single-molecule real-time sequencing (SMRT) uses zero-mode waveguides to monitor the incorporation of individual nucleotides, ONT's technology uses nanopores to detect changes in electrical currents as DNA strands move through them [28]. By obtaining longer and longer reads of sequence fragments, scientists have been able to detect large structural changes, inversions and translocations in genomes with greater accuracy. It has also allowed for a more complete analysis of repetitive regions, which had been a major challenge for second-generation sequencing read assembly algorithms. In addition, SMRT sequencing has made it possible to directly detect DNA modifications such as methylation without the need for bisulfite, revealing complex layers of epigenetic regulation [29].

NGS methods have become more prevalent, allowing for the rapid and cost-effective sequencing of large portions of genomes or even entire genomes [30]. This has led to the possibility of reference genomes being created. However, the resequencing of genomes is also worthy of mention. The primary purpose of resequencing is not to determine the sequence itself but to identify variations or mutations in the DNA of a specific individual or species compared with the reference [30]. While sequencing provides fundamental insights into the genetic makeup of an organism, resequencing offers insights into genetic

diversity and evolution, and can help identify mutations associated with diseases or specific traits [31].

Also noteworthy is epigenetics research, the study of heritable changes in gene function that are not accompanied by changes in the underlying DNA sequence. They play a key role in plant development, stress response and adaptation. One of the most powerful tools in modern epigenetic research is NGS, which provides a high-resolution, genome-wide view of epigenetic changes. In plants, NGS has revolutionised the understanding of epigenomics and its impact on phenotype. DNA methylation, the addition of a methyl group to cytosine or adenine DNA nucleotides, is the major epigenetic change in plants that leads to inhibition of gene expression. Whole-genome bisulfite sequencing (WGBS), an NGS-based method, is widely used to generate comprehensive methylation maps in various plant genomes [32]. Chromatin immunoprecipitation sequencing (ChIP-Seq) is a technique for studying protein–DNA interactions, such as transcription factor binding sites or the locations of histone modifications throughout the genome. In plants, ChIP-Seq has played a key role in identifying the distribution of various histone modifications and elucidating their role in processes such as flowering time regulation, seed development and response to biotic and abiotic stresses [33]. Small RNAs (sRNAs), typically 20–24 nucleotides in length, play a key role in gene silencing and endogenous gene regulation in plants. NGS-based sRNA sequencing enables the identification and quantification of these small RNA molecules, leading to insights into their biogenesis, modification and function [34]. Another level of epigenetic regulation is chromatin accessibility, which often correlates with active gene expression. The assay for transposase-accessible chromatin with sequencing (ATAC-Seq) is an NGS-based method that examines open regions of chromatin. In plants, ATAC-Seq has been used to study dynamic changes in chromatin accessibility during processes such as fruit ripening and seed germination [35]. Next-generation sequencing has had a groundbreaking impact on unravelling complex epigenetic networks in plants. As the technology continues to evolve and computational tools improve, knowledge of plant epigenetics will only increase, paving the way for innovative applications in agriculture and plant biology.

In addition, genome-wide association studies (GWAS) use sequenced genomes to correlate specific genetic changes with observable traits [36]. These methods have allowed the detection of genomic variants associated with either traditional agronomic phenotypes or biochemical and molecular phenotypes. These associations, in turn, enable applications in gene cloning and accelerated crop breeding through marker-assisted selection or genetic engineering. Furthermore, the integration of genome sequencing with transcriptomics has enabled the identification of regulatory elements such as enhancers and promoters, providing insight into the diverse regulatory networks that control gene expression [37].

When comparing second- and third-generation sequencing technologies, it is worth noting that while second-generation sequencing offers high sequencing throughput at a reduced cost per base, it is unable to read longer DNA fragments, making de novo assembly and detection of structural variants difficult. However, it offers much longer reads, often exceeding 10 kb or even 100 kb, at the cost of slightly lower accuracy. Combining the strengths of both technologies has become a common strategy in modern genomics to ensure comprehensive genome analyses.

However, the development of NGS technologies has revolutionised the view of the genetic code and its functions. With NGS democratising access to genomic data and providing unprecedented transparency of complex genomic structures, the understanding of how genes interact in complex organisms has improved immeasurably, paving the way for innovations in medicine, agriculture and environmental sciences.

2.2. Cucurbits Genomes, Complexity and Characterisation

Plant genomes are inherently complex, often characterised by polyploidy, extensive repetitive sequences and epigenetic modifications [38–40]. Comprehensive knowledge of these complexities is essential for the design of effective genome-editing strategies. A well-described genome sequence is the foundation of genome editing. However, an understanding of gene function, regulatory elements and synteny is equally important [41]. Genome complexity is influenced by factors such as repeated DNA sequences, polyploid events and transposable elements (TEs). TEs can change their position in the genome and contribute significantly to the size and complexity of the plant genome, especially retrotransposons with long terminal repeats [42]. TEs not only enlarge the genome but also induce genome reorganisation and affect gene regulation, providing an evolutionary playground for adaptation and diversification [43]. In addition, complex regulatory networks modulate gene expression in response to environmental cues. The presence of cis-regulatory elements, transcription factor binding sites and epigenetic markers adds another layer of complexity to the genome, facilitating adaptation and phenotypic plasticity [44]. Understanding the complexity of the plant genome is of great importance in the era of precision agriculture. Complex genome annotation, facilitated by advanced sequencing technologies, is paving the way for targeted crop improvement. By unravelling the complex network of genes, regulatory elements and structural changes, breeders can improve yields, resistance and nutritional profiles [45]. Focusing on the genomes of plants in the Cucurbitaceae family, the year 2009 represented a significant milestone as it provided the first genome within this family and, additionally, the genome of the first vegetable crop, i.e., cucumber (*Cucumis sativus*) [46]. Since then, scientists around the world have described the genomes of 87 different plant varieties and cultivars within the Cucurbitaceae family. Summarised data on sequenced genomes and the annual distribution of papers on this topic are presented in Figure 2. Moreover, detailed information on the cultivars, sequencing technologies, chromosome number, genome size and coverage, as well as the number of scaffolds, contigs and protein-coding genes, is presented in Table 1. Information concerning whether a given genome is a reference genome comes from the National Center for Biotechnology Information (NCBI) [47] and the updated database CuGenDBv2 (Cucurbit Genomics Database) [48].

As mentioned above, the cucumber species was the first in this family to be sequenced. *Cucumis sativus*, commonly known as cucumber, is characterised by well-established reference lines, such as 9930 (a Chinese line) and Gy14 (a North American line) and the longest B10v3 (North European) [49–51]. Recently, a system for integrating experimental data with high-throughput genomic data was used by Turek et al. [52], where a model of genome comparison and a merging of experimental data were proposed [52]. As more and more genomes are sequenced and assembled, comparative genomics methods and adaptive genome drafts are increasingly needed to standardise gene location and annotation information. Research is also being carried out on so-called pangenomes, an example of which is the research on *Cucumis sativus*, in which a pangenome was created using 12 sequences of cucumber lines [53]. This analysis revealed structural, functional and sequence differences related to agronomic and domestication traits. The resulting pangenome contains information from cucumber genomes assembled at the chromosome level, highlighting the need for a comprehensive reference that captures structural details at the gene level as well as larger chromosomal structures [53].

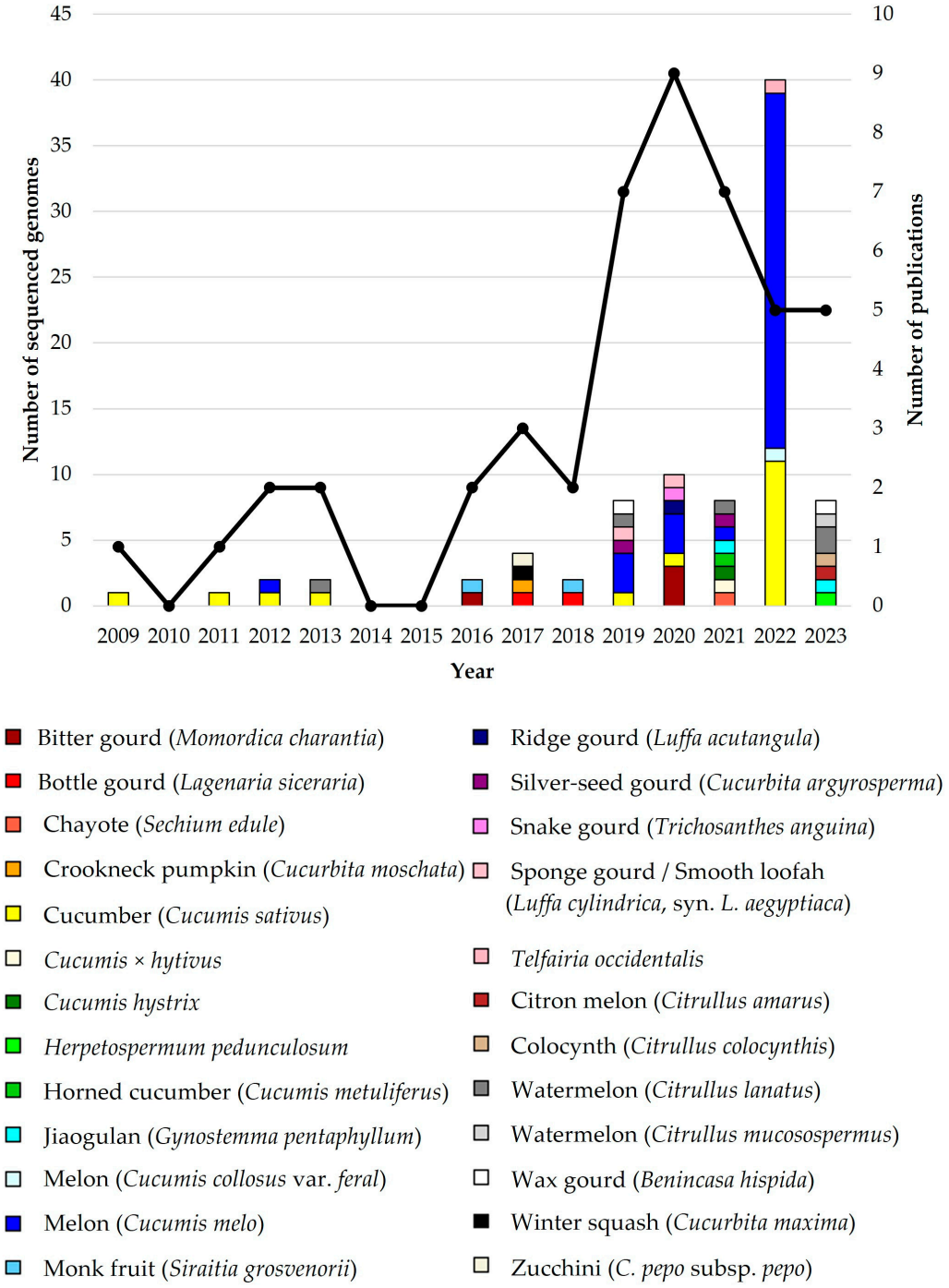

Figure 2. Overview of sequenced genomes (n = 88) and the annual distribution of publications (n = 46, black solid line) that refer to genome sequencing in the Cucurbitaceae family.

Table 1. Summary of the sequenced genomes in the Cucurbitaceae family.

Species	Cultivar	Year	Sequencing Technologies	Chromosome Number	Genome Size (Mb)	Percentage Assembly	Genome Coverage	Number of Scaffolds	Number of Contigs	Protein-Coding Genes	Reference Genome	Reference
Cucumber (*C. sativus* var. *sativus*)	"Chinese long" inbred line 9930	2019	PacBio RSII, PacBio Sequel, 10x Genomics, and Hi-C technologies	7	226.2	93.3	50.0x	85	174	24,317	YES	[49]
	"Chinese long" inbred line 9930	2009	Sanger and Illumina GA	7	243.5	72.8	72.2x	47,837	62,412	26,682	No	[46]
	Borszczagowski	2011	454 Sequencing and Sanger—Celera/Arachne	7	323.0	N/A *	12.0x	13,129	16,547	26,587	No	[54]
	Gy14	2012	No data	7	173.1	86.0	4.3x	244	N/A	N/A	No	[50]
	B10v3 (Borszczagowski)	2020	PacBio RS II and Illumina HiSeq 2000	7	342.3	93.9	69.8x	N/A	8035	27,271	No	[51]
	MSC19	2020	Illumina HiSeq 2000	7	342.3 **	N/A	32.5x	N/A	8035	27,271	No	[55]
	320	2020	Illumina HiSeq 2000	7	342.3 **	N/A	36.8x	N/A	8035	27,271	No	
	y-gc	2020	Illumina HiSeq 2000	7	342.3 **	N/A	34.8x	N/A	8035	27,271	No	
	212	2021	Illumina HiSeq 2000	7	342.3 **	N/A	34.6x	N/A	8035	27,271	No	
	224	2021	Illumina HiSeq 2000	7	342.3 **	N/A	34.6x	N/A	8035	27,271	No	[56]
	225	2021	Illumina HiSeq 2000	7	342.3 **	N/A	34.5x	N/A	8035	27,271	No	
	XTMC (East Asian line)	2022	PacBio RSII and PacBio Sequel	7	240.1	N/A	53.0x	N/A	926	25,167	No	
	Cu2 (East Asian line)	2022	PacBio RSII and PacBio Sequel	7	247.1	N/A	64.0x	N/A	851	25,382	No	
	Cuc37 (Eurasian line)	2022	PacBio RSII and PacBio Sequel, 10x Genomics, and Hi-C	7	238.4	N/A	54.0x	865	967	24,490	No	
	Gy14 (Eurasian line)	2022	PacBio RSII and PacBio Sequel	7	239.4	N/A	47.0x	N/A	926	25,042	No	[53]
	9110gt (Eurasian line)	2022	PacBio RSII and PacBio Sequel	7	242.9	N/A	58.0x	N/A	830	24,992	No	
	Cuc80 (Xishuangbanna line)	2022	PacBio RSII and PacBio Sequel, 10x Genomics, and Hi-C	7	237.4	N/A	47.0x	887	923	24,578	No	
	Hx14 (Indian line)	2022	PacBio RSII and PacBio Sequel	7	234.6	N/A	52.0x	N/A	865	24,914	No	
	Hx117 (Indian line)	2022	PacBio RSII and PacBio Sequel	7	243.7	N/A	49.0x	N/A	1015	26,033	No	
Cucumber (*C. sativus* var. *hardwickii*)	PI183967 (CG0002)	2013	Illumina GA IIx and Illumina HiSeq 2000	7	204.8	95.3	20.9x	187	6113	23,836	No	[56]
	Cuc64 (Indian line)	2022	PacBio RSII and PacBio Sequel, 10x Genomics, and Hi-C	7	232.5	N/A	46.0x	796	842	24,583	No	
	W4 (Indian line)	2022	PacBio RSII and PacBio Sequel	7	251.1	N/A	58.0x	N/A	894	25,703	No	[53]
	W8 (Indian line)	2022	PacBio RSII and PacBio Sequel	7	241.9	N/A	56.0x	N/A	907	25,531	No	

Table 1. *Cont.*

Species	Cultivar	Year	Sequencing Technologies	Chromosome Number	Genome Size (Mb)	Percentage Assembly	Genome Coverage	Number of Scaffolds	Number of Contigs	Protein-Coding Genes	Reference Genome	Reference
Cucumis hystrix	-	2021	PacBio, Illumina HiSeq X-Ten, Illumina HiSeq 2500 and 10x Genomics	12	289.9	90.4	360.0x	2284	6072	23,864	YES	[57]
Cucumis × *hytivus*	-	2021	Illumina HiSeq 2000, Illumina X-Ten, PacBio SMRT, BioNano, and Hi-C	19	540.7	97.2	104.0x	562	771	45,687	No	[58]
Bitter gourd (*Momordica charantia*)	OHB3-1	2016	Illumina MiSeq and Illumina HiSeq 2500	11	285.6	84.0	110.0x	1052	20,427	45,859	YES	[59]
	OHB3-1	2020	PacBio Sequel and Illumina HiSeq 2500	11	303.0	96.3	84.0x	193	221	N/A	No	[60]
	Dali-11	2020	Illumina HiSeq 2000	11	296.3	97.9	251.0x	297	8600	264,27	No	[61]
	TR	2020	Illumina HiSeq 2000	11	296.3	98.7	185.0x	1643	23,789	28,827	No	
Bottle gourd (*Lagenaria siceraria*)	Hangzhou gourd	2018	PacBio SMRT and Illumina HiSeq 4000	11	297.9	N/A	77.0x	27	71	23,541	YES	[62]
	USVL1VR-Ls	2017	Illumina HiSeq 2500	11	313.4	93.8	395.0x	444	18,083	22,472	No	[63]
Chayote (*Sechium edule*)	-	2021	Nanopore and Hi-C	14	608.2	99.7	151.0x	103	356	28,237	No	[64]
Crookneck pumpkin (*Cucurbita moschata*)	Rifu	2017	Illumina HiSeq 2500	20	269.9	72.6	215.5x	3500	17,340	32,205	YES	[65]
Herpetospermum pedunculosum	-	2023	PacBio Sequel IIe and HiC	10	804.1	90.45	27.3x	189	250	23,924	YES	[66]
Horned cucumber (*Cucumis metuliferus*)	PI 482,460 (CM27)	2021	PacBio SMART and Hi-C	12	329.1	98.0	93.0x	N/A	432	29,214	No	[67]
Jiaogulan (*Gynostemma pentaphyllum*)	-	2023	DNBSEQ™ and PromethION	11	609.0	99.99	275.0x	18	158	26,588	YES	[68]
	-	2021	Illumina, PacBio Sequel II, and Hi-C	11	582.9	91.65	403.0x	578	1232	25,285	No	[69]
Melon (*Cucumis melo* subsp. *melo*)	AY	2022	PacBio Sequel II	12	438.3	N/A	44.0x	1309	1548	28,628	YES	[70]
	Double-haploid line DHL92	2020	PacBio SMRT and Illumina	12	357.6	96.2	50.0x	13	1178	29,980	No	[71]
	MR1	2022	PacBio Sequel II	12	438.3	N/A	53.0x	1030	1374	N/A	No	[70]

Table 1. *Cont.*

Species	Cultivar	Year	Sequencing Technologies	Chromosome Number	Genome Size (Mb)	Percentage Assembly	Genome Coverage	Number of Scaffolds	Number of Contigs	Protein-Coding Genes	Reference Genome	Reference
Melon (*C. melo*)	Double-haploid line DHL92	2012	454 Sequencing and Sanger	12	374.8	83.3	13.5x	1594	60,752	27,427	No	[72]
Melon (*C. melo* subsp. *agrestis* var. *chinensis*)	BAHC	2022	Oxford Nanopore MinION	12	361.9	N/A	60.0x	170	247	36,981	No	[73]
	PI161375	2022	Oxford Nanopore MinION	12	360.1	N/A	44.0x	242	458	36,593	No	
Melon (*C. melo* subsp. *agrestis* var. *conomon*)	TOG	2022	Oxford Nanopore MinION	12	361.2	N/A	46.0x	161	273	36,802	No	[73]
Melon (*C. melo* subsp. *agrestis* var. *makuwa*)	SW3	2019	Illumina HiSeq 2500	12	354.0	94.9	258.0x	7202	29,154	38,173	No	[74]
	Chang Bougi	2019	Illumina HiSeq 2500	12	344.0	96.9	258.0x	11,309	43,251	36,235	No	
	ESL	2022	Oxford Nanopore MinION	12	358.6	N/A	39.0x	163	560	36,345	No	[73]
	OHG	2022	Oxford Nanopore MinION	12	360.7	N/A	54.0x	113	174	36,883	No	
	SAS	2022	Oxford Nanopore MinION	12	361.0	N/A	41.0x	309	432	36,725	No	
Melon (*C. melo* subsp. *agrestis* var. *momordica*)	PI414723	2022	Oxford Nanopore MinION	12	363.4	N/A	101.0x	157	230	36,458	No	
Melon (*C. melo* subsp. *agrestis*)	-	2020	Illumina HiSeq, PacBio SMRT, PacBio Sequel, and Hi-C	12	366.2	98.2	100.0x	101	298	28,898	No	[75]
	IVF77	2021	PacBio SMART and Hi-C	12	364.3	96.3	84.0x	N/A	1698	27,073	No	[67]
Melon (*C. melo* subsp. *melo* var. *adzhur*)	PI164323	2022	Oxford Nanopore MinION	12	367.9	N/A	53.0x	750	1029	36,394	No	[73]
Melon (*C. melo* subsp. *melo* var. *ameri*)	AY	2022	Oxford Nanopore MinION	12	367.5	N/A	53.0x	176	280	37,183	No	

Table 1. *Cont.*

Species	Cultivar	Year	Sequencing Technologies	Chromosome Number	Genome Size (Mb)	Percentage Assembly	Genome Coverage	Number of Scaffolds	Number of Contigs	Protein-Coding Genes	Reference Genome	Reference
Melon (*C. melo* subsp. *melo* var. *cantalupensis*)	BEL	2022	Oxford Nanopore MinION	12	373.8	N/A	54.0x	142	226	37,193	No	[73]
	NDD1	2022	Oxford Nanopore MinION	12	365.1	N/A	59.0x	208	282	37,122	No	
	NY	2022	Oxford Nanopore MinION	12	365.7	N/A	39.0x	148	221	36,919	No	
	VEP	2022	Oxford Nanopore MinION	12	365.4	N/A	48.0x	392	528	36,984	No	
	Charmono	2022	PacBio RSII, 10x Genomics, and Hi-C	12	366.8	99.5	100.0x	43	236	31,348	No	[76]
Melon (*C. melo* subsp. *melo* var. *duda'im*)	DUD	2022	Oxford Nanopore MinION	12	362.9	N/A	49.0x	214	357	36,602	No	[73]
Melon (*C. melo* subsp. *melo* var. *flexuosus*)	DOYA	2022	Oxford Nanopore MinION	12	366.7	N/A	49.0x	277	473	36,513	No	[73]
Melon (*C. melo* subsp. *melo* var. *inodorus*)	Payzawat	2019	PacBio RSII and Illumina X-Ten	12	386.5	94.1	81.0x	623	882	22,924	No	[77]
	BDR	2022	Oxford Nanopore MinION	12	366.0	N/A	57.0x	346	462	37,136	No	[73]
	NA	2022	Oxford Nanopore MinION	12	367.3	N/A	80.0x	155	239	37,259	No	
	PSR	2022	Oxford Nanopore MinION	12	368.7	N/A	44.0x	165	258	37,232	No	
	TAD	2022	Oxford Nanopore MinION	12	364.9	N/A	72.0x	98	173	37,120	No	
	TVT	2022	Oxford Nanopore MinION	12	364.8	N/A	55.0x	142	245	36,970	No	
Melon (*C. melo* subsp. *melo* var. *khandalak*)	ARJ	2022	Oxford Nanopore MinION	12	365.0	N/A	72.0x	327	525	36,773	No	
	INB	2022	Oxford Nanopore MinION	12	363.6	N/A	45.0x	117	231	36,626	No	
Melon (*C. melo* subsp. *melo* var. *reticulatus*)	Harukei-3	2020	PacBio RSII, Illumina HiSeq 2000, and Oxford Nanopore MinION	12	368.5	N/A	73.0x	80	112	33,829	No	[78]
	DUL	2022	Oxford Nanopore MinION	12	365.5	N/A	57.0x	63	124	36,175	No	[73]
	KRY	2022	Oxford Nanopore MinION	12	369.4	N/A	54.0x	295	441	37,158	No	

Table 1. *Cont.*

Species	Cultivar	Year	Sequencing Technologies	Chromosome Number	Genome Size (Mb)	Percentage Assembly	Genome Coverage	Number of Scaffolds	Number of Contigs	Protein-Coding Genes	Reference Genome	Reference
Melon (*C. collosus* var. *feral*)	QME	2022	Oxford Nanopore MinION	12	363.6	N/A	70.0x	184	269	36,578	No	[73]
Monk fruit (*Siraitia grosvenorii*)	-	2016	Illumina TSLR	14	420.1	N/A	36.9x	12,772	25,166	N/A	No	[79]
	"Qingpiguo" variety	2018	Illumina HiSeq X-Ten and PacBio SMRT	14	469.5	N/A	73.8x	N/A	4128	30,565	No	[80]
Ridge gourd (*Luffa acutangula*)	AG-4	2020	PacBio SMRT, Chicago, and HiC	13	735.6	92.2	47.5x	7871	17,812	42,211	YES	[81]
Silver-seed gourd (*Cucurbita argyrosperma* subsp. *sororia*)	-	2021	Illumina HiSeq 4000 and PacBio Sequel	20	255.2	92.8	288.4x	72	959	30,592	YES	[82]
Silver-seed gourd (*C. argyrosperma* subsp. *argyrosperma*)	-	2019	Illumina HiSeq 2000, Illumina MiSeq, and PacBio RS II	20	228.8	95.6	151.0x	920	1481	28,298	No	[83]
Snake gourd (*Trichosanthes anguina*)	-	2020	PromethION and Hi-C	11	919.8	99.9	108.5x	69	202	22,874	No	[84]
Sponge gourd/smooth loofah (*Luffa cylindrica*, syn. *L. aegyptiaca*)	P93075	2020	Illumina HiSeq X-Ten, 10x Genomics, PacBio Sequel, and Hi-C	13	656.2	96.9	100.0x	332	480	25,508	YES	[85]
	-	2020	Illumina Hiseq X-Ten, PacBio Sequel, and Hi-C	13	669.7	99.5	101.0x	798	1156	31,661	No	[86]
Telfairia occidentalis	-	2022	Illumina HiSeq	N/A	745.3	N/A	105.0x	852,383	874,487	N/A	YES	[87]
Watermelon (*Citrullus lanatus*)	242-1	2023	Oxford Nanopore MinION and Illumina HiSeq 2500	11	361.7	95.9	22.0x	N/A	43	23,921	YES	[88]
	Charleston Gray	2019	Illumina GAIIx, Illumina HiSeq 2500, and Illumina MiSeq	11	396.4	94.6	228.0x	2034	21,498	22,546	No	[89]
	159-1	2023	Oxford Nanopore MinION and Illumina	11	362.1	95.8	25.0x	N/A	103	24,451	No	[88]

Table 1. *Cont.*

Species	Cultivar	Year	Sequencing Technologies	Chromosome Number	Genome Size (Mb)	Percentage Assembly	Genome Coverage	Number of Scaffolds	Number of Contigs	Protein-Coding Genes	Reference Genome	Reference
Watermelon (*C. lanatus* subsp. *vulgaris*)	97103	2013	Illumina GAII and Illumina HiSeq 2000	11	353.5	83.2	108.6x	1793	41,945	23,440	No	[90]
Watermelon (*C. lanatus* subsp. *cordophanus*)	-	2021	PacBio Sequel SMRT, Illumina, and Hi-C technologies	11	367.9	84.1	388.8x	33	86	23,043	No	[91]
Citron melon (*C. amarus*)	USVL246-FR2	2023	PacBio and Illumina	11	356.8	93.6	284.2x	1422	38,258	22,028	YES	[92]
Colocynth (*C. colocynthis*)	PI 537277	2023	PacBio and Illumina	11	360.2	99.7	370.1x	1536	15,928	22,723	YES	
Watermelon (*C. mucosospermus*)	USVL531-MDR	2023	PacBio and Illumina	11	365.3	99.4	84.8x	N/A	77	22,377	YES	
Wax gourd (*Benincasa hispida*)	B227	2019	PacBio RSII and Illumina HiSeq 4000	12	913.0	94.1	50.0x	2197	26,315	27,467	YES	[93]
	pf3	2023	PacBio Sequel II, Illumina NovaSeq 6000, and Hi-C	12	975.6	94.9	86.0x	1862	1897	31,562	No	[94]
Winter squash (*Cucurbita maxima*)	Rimu	2017	Illumina HiSeq 2500	20	271.4	70.2	282.7x	8299	25,524	32,076	YES	[65]
Zucchini (*C. pepo* subsp. *pepo*)	MU-CU-16	2017	Illumina HiSeq 2000	20	263.5	93.0	198.0x	26,025	32,754	27,870	YES	[95]

* Not applicable. ** Resequencing performed.

Between 2009 and today, scientists have sequenced a total of 13 cucumber (*C. sativus* var. *sativus*) genomes, with their results described in six papers. Two varieties of cucumber have been sequenced: "sativus" and "hardwickii". The latter is a wild form of cucumber considered to be the progenitor of *C. sativus* var. *sativus*. Qi et al. [56] performed de novo sequencing and assembly of this wild cucumber of PI183967 accession. Through the application of Illumina GA IIx and Illumina HiSeq 2000 with 20.9x coverage, a total length of the assembly of 204.8 Mb was obtained, and the authors predicted a total of 23,836 genes [56]. In the study of Li et al. [53], three "hardwickii" Indian line cultivars, W4, W8 and Cuc64, were also sequenced, and the assembled genome size ranged from 232.5 to 251.5 Mb, with the number of protein-coding genes ranging from 24,583 to 25,703. Qin et al. [57] reported the genome assembly of another *Cucumis* representative, i.e., *C. hystrix*, a monoecious climbing vine. A genome size of 289.9 Mb was revealed, where 90.4% of the sequences were anchored onto 12 chromosomes and 23,846 genes were annotated. Moreover, a comparative genomic analysis conducted by the authors stated that *C. hystrix* is phylogenetically closer to cucumber than to melon [57].

Yu et al. [58] undertook sequencing of the *Cucumis* × *hytivus* genome, which is a synthetic allotetraploid species consisting of 2n = 4x = 38 chromosomes containing both genomes of two different species described above (*C. sativus* and *C. hystrix*). The use of different sequencing approaches (Illumina, PacBio, BioNano, and Hi-C) allowed a total assembly size of 540.7 Mb and 45,687 predicted genes to be obtained, and to date it is the first fully sequenced synthetic allopolyploid [58].

Subsequently, four genomes of bitter gourd (*Momordica charantia*) have been revealed. The first from the OHB3-1 cultivar, officially recognised by the NCBI as the reference genome, was sequenced in 2017 by Urasaki et al. [59]. *M. charantia* is characterised by 11 chromosomes, and the authors described a genome size of 285.6 Mb and 45,859 predicted genes. Matsumura et al. [60] again sequenced the genome of this cultivar and using PacBio Sequel and Illumina HiSeq 2500 obtained a size of 303.0 Mb. Cui et al. [61] also contributed to the research on bitter gourds, and two other cultivars, i.e., Dali-11 and TR, were tested. The authors achieved similar results on the genome size, in detail about 296.3 Mb for both samples. In contrast to the reference genome, the number of protein-coding genes was significantly lower (26,427 and 28,827 vs. 45,859) [61].

Among other gourds, *Lagenaria siceraria* (bottle gourd) was another species under consideration in the study of the genomes of Cucurbitaceae. Both Hangzhou gourd [62] and USVL1VR-Ls [63] varieties were analysed. In the case of the former, a genome size of 297.9 Mb was provided, while for the latter it was 313.4 Mb. Bottle gourds have 11 chromosomes, and in both studies about 23,000 genes were predicted.

Another example of a gourd is chayote (*Sechium edule*). In 2021, Fu et al. [64] applied Nanopore and Hi-C technologies to obtain a dataset of 151.0x coverage, and then based on the 103 scaffolds and 356 contigs, the authors described the 14 chromosomes of gourd with a relatively large genome size of 608.2 Mb [64].

Rifu and Rimu are the names of two cultivars, *Cucurbita moschata* (crookneck pumpkin) and *C. maxima* (winter squash), respectively, for whom Sun et al. [65] reported genome sequences. In both cases, Illumina HiSeq 2500 was used and high-quality reads with the coverage of 215.5x and 282.7x for Rifu and Rimu, respectively, were obtained. Moreover, de novo assemblies resulted in draft genomes of 269.9 and 271.4 Mb, and in comparison with cucumber, a higher number of protein-coding genes were predicted: 32,205 for *C. moschata* and 32,076 for *C. maxima* [65].

Moreover, *Herpetospermum pedunculosum* [66], horned cucumber (*Cucumis metuliferus*) [67] and jiaogulan (*Gynostemma pentaphyllum*, n = 2) [68,69] are subsequent species within the Cucurbitaceae family whose genomes have been reported in the last three years.

Interestingly, melon (*Cucumis melo*) was the most frequently studied species within this plant family in terms of genome sequencing. Over the years, ten papers [67,70–78] have described the sequences of different melon subspecies, varieties and cultivars. The first genome sequence of *C. melo* was revealed in 2012, as the second species after the

cucumber genome in the Cucurbitaceae family. The genome of double-haploid line DHL92 was obtained via 454 and Sanger sequencing methods. Low genome coverage by current standards of 13.5x allowed a genome size to be obtained of 374.8 Mb with 83.3% percentage assembly and prediction of 27,427 protein-coding genes [72]. In comparison, sequencing and assembly of *C. melo* subsp. *melo* AY, the NCBI reference genome, was accomplished in 2022 [70]. The authors applied PacBio Sequel II with a coverage of 44.0x, and ultimately a genome size of 438.3 Mb was obtained.

An important work contributing greatly to the discovery of melon genomes was published in 2022 by the team of Oren et al. [73]. The authors sequenced the genomes of 25 different melons, i.e., they provided 17 accessions of "*melo*" subspecies (in detail: 5x *inodorus*, 4x *cantalupensis*, 2x *reticulatus*, 2x *khandalak*, 1x *duda'im*, 1x *flexuosus*, 1x *ameri* and 1x *adzhur* cultivars), 7 for "*agrestis*" subspecies (2x *chinensis*, 1x *conomon*, 3x *makuwa* and 1x *momordica*), and 1 for *C. collosus* var. *feral*, an under-exploited wild melon fruit traditionally used in folk remedies [73]. Among all the sequenced melon genomes, their size ranged from 357.6 to 438.3 Mb, and predicted genes from 22,924 to 38,173 (Table 1).

The development of genome cognition techniques and the interest of different scientific groups in this issue allowed the discovery of the genomes of subsequent cucurbits: monk fruit (*Siraitia grosvenorii*) [79,80], ridge gourd (*Luffa acutangula*) [81] and silver-seed gourds, i.e., *Cucurbita argyrosperma* subsp. *sororia* [82] or *C. argyrosperma* subsp. *argyrosperma* [83]. Moreover, the study by Ma et al. [84] revealed that the genome of the snake gourd (*Trichosanthes anguina*) is one the largest genomes ever within the Cucurbitaceae family. The use of PromethION and Hi-C technologies with 108.5x coverage led to the genome size of 919.8 Mb being obtained and compared with the large genome the authors described 22,874 protein-coding genes.

More recently, i.e., between 2019 and 2023, the genomes have been identified of well-known tropical vines grown in Asia, i.e., sponge gourd known also as smooth loofah (*Luffa cylindrica*, syn. *L. aegyptiaca*) and *Telfairia occidentalis*. The referenced genome for loofah is organised in 13 chromosomes with a size of 656.2 Mb [85], and the second paper for this species showed a size of 669.7 Mb [86]. In the case of the African fluted pumpkin (*T. occidentalis*), Pirro et al. [87] applied the Illumina HiSeq sequencing system to reveal its genome (745.3 Mb), where the genome assembly includes 852,383 scaffolds but no assembled chromosomes.

Another important species in relation to a high cultivation rate worldwide is watermelon (*Citrullus lanatus*). The referenced genome of 242-1 isolate was published in 2023 [88], where the authors utilised Oxford Nanopore MinION and Illumina HiSeq 2500 systems and described the genome size of 361.7 Mb with 22.0x data coverage and 23,921 protein-coding genes. Due to the existence of a huge number of watermelon varieties, the genome sequencing of other subspecies and species has also been attempted several times by other researchers. DNA sequences for the Korean cultivar 159-1 [88], as well as for Charleston Gray [89], a dessert watermelon cultivar, have also been obtained. Moreover, in 2013 the genome of *C. lanatus* subsp. *vulgaris* 97,103 was accomplished [90], and in 2021 the sequencing for *C. lanatus* subsp. *cordophanus* was performed [91]. In addition, Wu et al. [92] sequenced the genomes of *C. amarus*, *C. colocynthis*, and *C. mucosospermus*, i.e., other wild and cultivated watermelons, for a better understanding of the evolution and domestication of watermelons. All watermelons tested so far have been characterised by a similar genome size, ranging from 353.5 to 396.4 Mb (Table 1).

Finally, the genomes of the two edible cucurbits, i.e., wax gourd (*Benincasa hispida*) and zucchini (*C. pepo* subsp. *pepo*) MU-CU-16, have been sequenced. The genome sequence of the former and its two cultivars, i.e., B227 and pf3, were discovered in 2019 [93] and 2023 [94]. The first had a size of 913.0 Mb, making it one of the largest genomes in the Cucurbitaceae family, and pf3 had the largest described sequence amounting to 975.6 Mb. Furthermore, a genome sequence of the zucchini was accomplished using the Illumina HiSeq 2000 technique, which had a size of 263.5 Mb [95].

3. Genome Editing

3.1. The Evolution of Genome Editing Technologies

Deciphering the vast expanse of genomic sequences is just the tip of the iceberg; the profound value lies in interpreting the role of individual genes and their organised functions in organisms. Genome sequencing is a fundamental tool of functional genomics, which aims to understand the relationship between a genome sequence and the resulting physiological traits. A well-described genome sequence is the basis for genome editing. However, understanding gene function, regulatory elements and synteny is equally important [41]. High-resolution genomic maps obtained by sequencing are the foundation of genomic editing as they provide the basis for designing precise genome-editing systems to manipulate target genes, thus influencing their phenotypic consequences [96]. Gene-editing techniques have significantly evolved, directly impacting phenotypic outcomes in targeted genes. The progression of these methods can be charted from the 1970s, with the use of restriction enzymes to manipulate DNA; in the 1980s, zinc finger nucleases (ZFNs) were introduced, which are artificially engineered restriction enzymes for custom site-specific genome editing; in 2011, transcription activator-like effector nucleases (TALENs) were developed, which are similar to ZFNs, but use a different DNA-binding domain; in 2013, the CRISPR/Cas9 system was discovered, which is a simple and efficient genome-editing tool; in 2017, base editing was introduced, which allows for precise single-base changes; and in 2019, prime editing was developed, which enables all types of base conversion, small deletions and insertions [97–99]. ZFNs, TALENs and CRISPR/Cas9 are three widely used gene-editing techniques that have revolutionised genome engineering. ZFNs are composed of a zinc finger domain and a Fok1 endonuclease domain [97,99,100]. Each zinc finger can recognise three to six nucleotide bases and requires the endonuclease domain to dimerise before creating a double-strand break in the DNA. The ZFNs technique is less flexible and more expensive than TALENs. The TALENs technique is cheaper and produces faster results than ZFNs [100]. It is more flexible and easier to design due to well-defined target specificities. CRISPR/Cas9 is an RNA-based bacterial defence mechanism composed of two types of RNA (trans-activating crRNA and a single guide RNA) and Cas9 endonuclease. It is simpler, cheaper and more efficient than ZFNs and TALENs [97–99]. In summary, each technique has its advantages and disadvantages, and the choice of which one to use depends on the specific requirements of the research. CRISPR/Cas9 is currently the most popular choice due to its simplicity, cost-effectiveness and efficiency.

The widespread use of gene-editing techniques in many plant species has been made possible by major advances in transformation methodology. In gene editing, an endonuclease recognises specific DNA or guides RNA sequences through its DNA binding domain (DBD), facilitating precise and efficient incisions in the target DNA sequence, resulting in changes in specific regions of DNA [101]. Modern research has led to a number of gene-editing techniques using nucleases that recognise their loci through protein–DNA interactions, examples of which include meganucleases (MNs), zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [102,103]. A separate category of nucleases includes CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated) and CRISPR/Cpf1 (class 2/type V CRISPR RNA-guided endonuclease), which identify their loci by RNA–DNA base complementarity [104].

It is important to consider performance, specificity, versatility and cost when comparing these genome-editing tools. CRISPR/Cas9 generally shows a superior performance compared with ZFNs and TALENs. This is primarily due to the ease of designing targeting RNAs for CRISPR compared with custom proteins for ZFNs and TALENs [105]. All systems may have off-target effects when considering the specificity of the techniques. However, modifications in the design of the guide RNA and Cas9 protein may improve the specificity [105]. In terms of versatility, although ZFNs and TALENs are limited by the protein engineering requirements for each target, CRISPR/Cas9's reliance on guide RNAs makes it easily programmable and versatile for many targets [105]. CRISPR technology is

considered the optimal method for generating modified genomes due to its low cost, high flexibility and reliability [106–108].

In recent times, numerous plant viral vectors have been effectively designed to transport CRISPR/Cas reagents for genome editing in both model and non-model plants. Virus-induced gene editing (VIGE) has emerged as a powerful technique, providing substantial advantages over alternative methods, including enhanced efficiency, precision and ease of use [109]. Notably, VIGE facilitates genome editing without the need for tissue culture, as it directly delivers transgenes to the meristem. In the past decade, VIGE systems have been developed and applied across various host plants, demonstrating successful outcomes in genome editing [110]. In cucurbits, there is a potential application of cucumber green mottle mosaic virus (CGMMV)) as a VIGE vector, which has been successfully employed in virus-induced gene silencing [111].

3.2. Genome Editing in Cucurbitaceae

Recently, scientists have been using gene-editing technologies, particularly CRISPR, on cucurbits (Figure 3) such as cucumber, melon, watermelon and squash. Analysing the data presented in Figure 3, there have been a total of 63 scientific papers on CRISPR-based genome editing in the Cucurbitaceae family registered in the Scopus database between 2016 and 2023. Of these, 73% were research articles, 21% were review papers and the remainder (6%) were conference papers, book chapters and letters. In recent years a dynamic increase in the number of publications in this subject has been observed. In particular, in 2022 the number of papers reached 19 and the same in 2023, but it should be noted that at the time of publication of this review article, more articles may still appear by the end of 2023. The growing interest in the application of CRISPR-based technologies may also be linked to the number of genomes sequenced so far within this plant family, as well as to the possibility of different gene functions by deleting or editing them, leading to the improvement of plant traits in terms of their resistance to many biotic and abiotic stresses, climate change and global warming.

Figure 3. Number of publications connected with genome editing in Cucurbitaceae (Source: Scopus; *n* = 63, accessed on 16 October 2023).

Figure 4 presents a network visualisation of keywords based on their co-occurrence, where the size of each frame is proportional to the number of occurrences. The given keywords are divided into five clusters, but all of them are strictly related to "CRISPR/Cas9". The grouping into these clusters results from the common appearance of individual words

in articles from the Scopus database, and the detailed connections will be described below. One of the clusters refers to virus resistance and genome editing within this subject, and the eIF4E (eukaryotic translation initiation factor 4E) gene appears here, whose disruption in cucumber led to the development of virus-resistant plants [112]. Furthermore, the green and blue boxes show an interest in CRISPR-based genome editing in the case of physiological aspects and climate correlation, respectively, with the following keywords: "auxin", "ethylene", "salinity", etc.

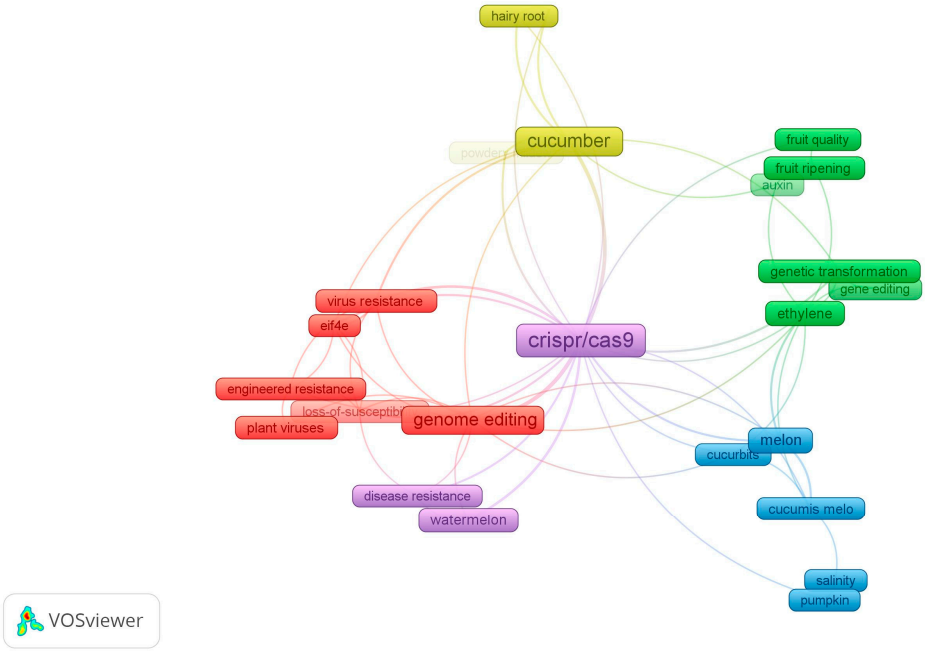

Figure 4. Network visualisation of keywords based on their co-occurrence, obtained by VOSviewer.

Plants have developed a range of physiological, biochemical and anatomical mechanisms to withstand abiotic stresses such as drought, flooding and salinity, which are becoming more prevalent due to climate change. Knowledge of the genomic sequences of cucurbit plants combined with novel genome-editing techniques have enabled the elucidation of the functions of numerous key genes in the physiology and stress response of Cucurbitaceae. These insights, in conjunction with the ongoing development of genome-editing methodologies, constitute an impressive tool for breeders, facilitating the rapid generation of new plant cultivars. New cultivars should be characterised not only by improved yield quantity and quality but also by their enhanced adaptability to the challenges posed by a changing environment [1]. In Table 2, examples of genome editing in Cucurbitaceae plants are presented, which enhances understanding of their physiology and the application of this knowledge in breeding. The sections below present the potential utilisation of genome editing in obtaining cucurbit plants that will confront the challenges of a dynamic environment (drought, salinity, limited arable land and new biotic stressors). It is noteworthy that induced modifications aimed at various objectives (e.g., improving crop quality and understanding of sex determination) may also be beneficial in breeding plants resistant to multistress conditions posed by climate change.

Table 2. Examples of genome editing found in the Cucurbitaceae family.

Species	Gene	Direct Effect of Genome Editing (CRISPR/Cas9)	Revealed Physiological Function of Gene	Functional Trait	References
Cucumber	*SF2* (*Csa2G337260*, HDC1 homolog)	A knockout of *SF2* resulted in inhibition of shoot growth	SF2 controls cell proliferation by histone deacetylation of genes involved in multiple pathways related to cytokinin and polyamine biosynthesis and transduction	Fruit quality/ stress response	[113]
Cucumber	*CsMYB* (*CsaV3_6G044410*, MYB transcription factor); *CsACS1* (*CsaV3_6G044400*, 1-aminocyclopropane-1-carboxylate synthase)	*Wip1* mutants produce female flowers with some bisexual at lower nodes genes	Upregulation of *ACS1G* in cucumber induces the development of female flowers and leads to the overproduction of ethylene	Sex determination/ stress response	[114]
Cucumber	*SF1* (*Csa2G174140*, cucurbit-specific RING-type E3 ligase); *ACS2* (*Csa1G580750*, rate-limiting enzyme for ethylene biosynthesis)	A knockout of *ACS2* mutants produces only male flowers	Regulation of female flower generation due to ethylene biosynthesis	Sex determination/ stress response	[115]
Cucumber	*eIF4E* (*XM_004147349*, eukaryotic translation initiation factor 4E)	CRISPR/Cas9 mediated mutations in *eIF4E* resulted in virus resistance	Mimicking natural mutation in *eIF4E* genes, which results in the potyvirus resistance	Biotic stress response	[112]
Cucumber	*CsALC* (*Csa2G356640.1*, bHLH transcription factor)	CRISPR/Cas9 *Csalc* mutant maintains normal vegetative growth and fruit length but produces very few seeds.	The bHLH transcription factor CsALC, expressed in the ovaries, plays a role in cucumber pollen tube emergence	Sex determination	[116]
Cucumber	*eIF4E* (*XM_004147349*, eukaryotic translation initiation factor 4E)	Utilising CRISPR/Cas9 mutants in breeding for virus resistance	Mass production of virus-resistant cultivars	Biotic stress response	[117]
Cucumber	*CsERF39* (ethylene response factor); *CsGLDH* (*Csa4M236360.1*, L-galactono-1,4-lactone dehydrogenase)	A knockout *CsERF39* and *CsGLDH* led to a decreased ascorbate level in leaves	*CsGLDH* is a direct target for CsERF39 in ascorbate biosynthesis	Stress response	[118]
Cucumber	*CsHEC1* (*Csa4G639900*, HECATE 1)	Mutation in *CsHEC1* resulted in shortened fruit neck	CsHEC1 stimulates the expression of *CsYuc4*, leading to increased auxin biosynthesis	Fruit quality/ stress response	[119]
Cucumber	*NS* (*Csa2G264590*, auxin transporter-like protein 3)	A knockout of *NS* by CRISPR/Cas9 resulted in spine-rich fruits	Expression pattern of auxin transporter of the AUX1/LAX type in cucumber plant	Fruit quality/ stress response	[120]

Table 2. *Cont.*

Species	Gene	Direct Effect of Genome Editing (CRISPR/Cas9)	Revealed Physiological Function of Gene	Functional Trait	References
Cucumber	*CsHEC2* (Csa2G285890, HECATE 2)	A knockout of *CsHEC2* leads to reduced wart density on fruit peel	CsHEC2, through interactions with the CKT hydroxylase-like gene promoter, promotes the expression of cytokinins	Fruit quality/ stress response	[121]
Cucumber	*CsWIP1* (*Csa4M290830*, gynoecy gene); *CsVFB1* (*Csa4M641640*, VIER F-BOX PROTEINE); *CsMLO8* (*Csa5M623470*, powdery mildew susceptibility gene); *CsGAD1* (*Csa5M348050*, glutamate decarboxylase 1 gene)	Mutation in *CsVFB*1 led to developing smaller leaves with smooth margin of leaf blade. Exhibited a gynoecious trait, where the upper nodes exclusively bore female flowers.	The successful development of gynoecious inbred lines by CRISPR/Cas9	Sex determination	[122]
Cucumber	*CsSRP43* (A candidate gene encoding a chloroplast signal recognition particle 43 protein)	Mutations in *CsSRP43* resulted in disturbed chloroplast development and yellowing of the leaves	CsSRP43 direct interact with LHCP and cpSRP54 proteins as its chaperone.	Stress response	[123]
Cucumber	*CsAKT1* (*CsaV3_1G029650*, K^+ transporter)	A knockout of *CsAKT* resulted in salt-sensitive plants	Induction of oxidative stress in plants with a *CsAKT* knockout, confirming that CsAKT plays a significant role in the response to salinity and could be a target for interventions aimed at mitigating this stress	Abiotic stress response	[124]
Cucumber	*CsGCN5* (*Csa6G527060*, General Control Nonderepressible protein 5)	Mutation of the *CsGCN5* resulted in extremely dwarf plants	A methodological article aimed at establishing homozygous mutants within the first generation (T0), without comprehensive physiological analysis	Plant architecture	[125]
Cucumber	*CsaMLO1* (*ON528941.2*, powdery mildew susceptibility gene); *CsaMLO8* (*ON528937.2*, powdery mildew susceptibility gene); *CsaMLO11* (*ON528948.2*, powdery mildew susceptibility gene)	Single, double and triple mutants in MLO genes resulted in resistance to powdery mildew	Plants exhibiting strong pre-invasion or post-invasion resistance to *Podosphaera xanthii*	Biotic stress response	[126]
Cucumber	*CsIAGLU* (*CsaV3_6G009300*, Indoleacetic acid glucosyltransferase gene)	*Csiaglu* mutants accumulated auxins and formed great leaf pedicle angle	CsIAGLU catalyses the glycosylation of free indole-3-acetic acid (IAA) to produce glucose conjugate ensuring the maintenance of suitable free IAA concentrations	Plant architecture	[127]

Table 2. *Cont.*

Species	Gene	Direct Effect of Genome Editing (CRISPR/Cas9)	Revealed Physiological Function of Gene	Functional Trait	References
Cucumber	*CsBPC2* (BASIC PENTACYSTEINE transcription factor)	Mutation in *CsBPC2* resulted in phenotype hyper-sensitive to salt stress	CsBPC2 is involved in the abscisic acid signalling pathway and is crucial for ABA-induced synthesis and transcription of genes related to ABA signalling	Abiotic stress response	[128]
Cucumber	*CsER* (*CsaV3_4G036080*—ERECTA gene homologs)	*Csre* mutants exhibit dwarf phenotype with shorter internodes	A methodological article aimed at optimising CRISPR/Cas9-mediated mutagenesis, without comprehensive physiological analysis	Plant architecture	[129]
Cucumber	*CsSEC23* (*Csa5G585430*, gene encoding the core component of COPII vesicles)	i mutants are characterised by strong glossiness of fruit peel	Deposition of cutin wax on the surface of fruit is determined by *CsSEC23* expression	Fruit quality/ stress response	[130]
Cucumber	*CsbHLH66*; *CsbHLH82* (Basic helix-loop-helix (bHLH) transcription factors)	Mutation in *CsbHLH82* led to the root hair sparse phenotype, simultaneous mutations in both *CsbHLH82* and *CsbHLH66* genes resulted in the root hair-less phenotype	A methodological article on establishing hairy root transformation system, without comprehensive physiological analysis	Plant architecture	[131]
Melon	*CmNAC-NOR* (*MELO3C016540.2*, NAC transcription factor)	Knockout of *CmNAC-NOR* results in fruits that do not emit ethylene, do not form an abscission layer, and do not undergo external colour change.	CmNAC-NOR is a critical and essential component responsible for the ripening of climacteric fruits. In the *nor-1* mutant, there is a suppressed production of ethylene, and it does not respond to exogenous ethylene.	Fruit quality/ stress response	[132]
Melon	*CmeIF4E* (eukaryotic translation initiation factor 4E)	C-to-T and C-to-G substitution in *CmeIF4E* gene	A methodological article aimed at optimising CRISPR/Cas9-mediated mutagenesis, without comprehensive physiological analysis	Biotic stress response	[133]
Melon	*CmCTR1-like* (*MELO3C024518*, serine/threonine kinase); *CmROS1* (*MELO3C024516*, homolog of DNA demethylase AtROS1)	A knockout of *CmCTR1* and *CmROS1* resulted in early ethylene production	CmROS1 plays a significant role in the demethylation of promoter regions of genes responsible for hormonal control of climacteric fruit ripening	Fruit quality/stress response	[134]

Table 2. *Cont.*

Species	Gene	Direct Effect of Genome Editing (CRISPR/Cas9)	Revealed Physiological Function of Gene	Functional Trait	References
Melon	*CmPDS* (*MELO3C017772.2*, melon phytoene desaturase gene)	CRISPR/Cas9 mediated mutations in CmPDS resulted in dwarf and albino plants	A methodological article aimed at facilitating and optimising CRISPR/Cas9 techniques in melon	Plant architecture	[135]
Melon	*CmACO1* (*MELO.jh010107.1*, 1-aminocyclopropane-1-carboxylic acid oxidase 1 gene)	A knockout of *CmACO1* resulted in strong decrease in ethylene emission in fruits	Fruits from mutant lines of *CmACO1* were characterised by low ethylene emission, no changes in pericarp colour, and firm flesh, resulting in an extended shelf life	Fruit quality/ stress response	[136]
Melon	*CmPDS* (*MELO3C017772.2*, melon phytoene desaturase gene)	A knockout of *CmPDS* results in dwarf and albino plants	A methodological article aimed at facilitating and optimising precise techniques of genome editing in melon	Plant architecture/ stress response	[137]
Melon	*eIF4E* (*MELO3C002698.2*, eukaryotic translation initiation factor 4E)	Homozygous mutant plants exhibited resistance to Moroccan watermelon mosaic virus	A mutation in the *eIF4E* gene is responsible for virus resistance, but it can also lead to the development of male sterile lines	Sex determination	[138]
elon	*CmER* (*MELO3C016916*, ERECTA gene homologs)	*Cmre* mutants exhibit dwarf phenotype with shorter internodes	A methodological article aimed at optimising CRISPR/Cas9-mediated mutagenesis, without comprehensive physiological analysis	Plant architecture	[129]
Pumpkin	*RBOHD* (*CmoCh14G010850*, respiratory burst oxidase homolog D)	rbohd-cas9 mutants were characterised by decreased H_2O_2 and K^+ content	Confirmation of the signalling role of reactive oxygen species in salt stress tolerance	Abiotic stress response	[139]
Pumpkin	*CmoER10* (*CmoCh09G003660*); *CmoER2* (*CmoCh01G017570*)—ERECTA gene homologs	*Cmoer10* and *cmoer2* mutants exhibit dwarf phenotype	A methodological article aimed at optimising CRISPR/Cas9-mediated mutagenesis, without comprehensive physiological analysis	Plant architecture	[129]
Pumpkin and Cucumber/ Pumpkin graft	*CmoHKT1;1* (High-affinity K+ transporter1); *CmoNHX4* (Sodium hydrogen exchanger4, pumpkin tonoplast Na+/H+ antiporter gene)	*CmoHKT1;1*^{CR} accumulate NaCl in the shoots	A salt stress tolerance in the cucumber/pumpkin grafting system	Abiotic stress response	[140]
Watermelon	*ClCOMT1* (*Cla97C07G144540*, caffeic acid O-methyltransferase)	The knockout of *ClCOMT1* reduces melatonin content in watermelon calli	Melatonin is an important signalling molecule involved in the response to abiotic stress also in watermelon	Abiotic stress response	[141]

Table 2. *Cont.*

Species	Gene	Direct Effect of Genome Editing (CRISPR/Cas9)	Revealed Physiological Function of Gene	Functional Trait	References
Watermelon	*ClREC8* (*Cla97C07G132920*, member of RAD21/REC8 family)	The knockout of *ClREC8* resulted in decreased pollen vitality	Understanding the function of ClREC8 in meiosis and unravelling the basis of seedless watermelon fruits	Fruit quality	[142]
Watermelon	*ClAGA2* (*Cla97C04G070460*, alkaline alpha-galactosidase); *ClSWEET3* (*Cla97C01G000640*, plasma membrane-localised hexose transporter in watermelon fruit parenchymal cells); ClTST2 (Cla97C02G036390, Tonoplast Sugar Transporter)	Mutation of the *ClAGA2* blocked raffinose oligosaccharides hydrolysis. *Cltst2* mutants were characterised by decreased sugar content and delayed fruit colouration, *clsweet3* mutants accumulate less sugars in fruits	ClAGA2, ClTST2 and ClSWEET3 are key elements in sugar transport, redistribution and unloading in watermelon	Fruit quality	[143]
Watermelon	*ClVST1* (*Cla97C02G031010*, vacuolar sugar transporter)	The knockout of *ClVST1* resulted in bearing lighter fruits with lower sugar content.	Vacuolar sugar transporter (*ClVST)* in fruit phloem cells is responsible for sucrose and glucose efflux and unloading in watermelon	Fruit quality	[144]
Watermelon	*ClGRF4* (*Cla97C02G034420*, GROWTH-REGULATING FACTOR4); *ClGIF1* (*Cla97C02G042620*, GRF-INTERACTING FACTOR1)	ClGRF4 and ClGIF1 double mutants produce seedless fruits	A primarily methodological work that confirms the involvement of *ClGRF4*, *ClGIF1* genes in melon reproduction development	Fruit quality	[145]
Watermelon	*ClNAC68* (*Cla97C03G059250*, NAC transcription factor)	The knockout *ClNAC68* led to a reduction in fruit sugar content and a delay in seed maturation	ClNAC68, a member of the NAC transcription factor family, plays a critical role in sugar accumulation in fruit and seed development by increasing the pool of free IAA	Fruit quality/ stress response	[146]
Watermelon	*ClBG1* (*Cla97C08G153160*, β-glucosidase l)	*Clbg1* mutants were characterised by a decrease in seed size and weight	CLBG1 is responsible for the hydrolysis of ABA esters with glucose, and by increasing pool of available ABA, it regulates melon seed development	Fruit quality/ stress response	[147]
Watermelon	*ClPDS* (*Cla010898*, phytoene desaturase)	Mutants in *ClPDS* gene exhibit albino phenotype	A methodological article aimed at optimising CRISPR/Cas9-mediated mutagenesis, without comprehensive physiological analysis	Stress response	[148]

Table 2. *Cont.*

Species	Gene	Direct Effect of Genome Editing (CRISPR/Cas9)	Revealed Physiological Function of Gene	Functional Trait	References
Watermelon	*ClALS* (*Cla019277*, acetolactate synthase)	Substitution of C to T in *SlALS* resulted in high resistance to tribenuron herbicide	A methodological article on optimising targeted base editing to achieve resistance to herbicides, without comprehensive physiological analysis	Herbicide resistance	[149]
Watermelon	*ClWIP1* (*Cla008537*, a putative C2H2 zinc finger transcription factor)	Mutation in ClWIP resulted in the formation of female flowers, with bisexual flowers bearing viable pollen produced only in the lower nodes	Confirmation of the role of *ClWIP* in creating gynoecious lines by inhibiting carpel primordia at the early stages of flower development	Sex determination/ stress response	[150]
Watermelon	*ClATM1* (*Cla010576*, the bHLH transcription factor Abnormal Tapetum 1 gene)	CRISPR/Cas9 edited lines exhibited typical vegetative growth but displayed male flower abnormalities, including reduced petal size and degraded anthers with nonviable pollen	The role of ClATM1 in the regulation of anther development	Sex determination/ stress response	[151]
Watermelon	*ClPDS* (Cla97C07G142100, phytoene desaturase gene)	The CRISPR/Cas9 edited line exhibited an albino phenotype	A methodological article aimed at optimising CRISPR/Cas9-mediated mutagenesis, without comprehensive physiological analysis	Stress response	[152]
Cucumber	*CsMS* (*CsaV3_1G009520*, malate synthase)	The knockout of *CsMS* synthase in hairy roots led to resistance against root-knot nematodes	CsMS, through its involvement in carbohydrate metabolism, serves as a crucial link in the transport of sucrose from the phloem to the giant cells of the nematode	Biotic stress response	[153]
Melon	*Prv* (*MELO3C022145*, nucleotide binding-leucine-rich repeat proteins)	The mutant displays a dwarf phenotype, accompanied by an increase in salicylic acid concentration and the expression of resistance genes	One of leucine-rich repeat proteins—prv is essential in melon resistance to papaya ringspot virus and *Fusarium oxysporum* f.sp. *Melonis* via hypersensitive. The corrected sentence is: "One of the leucine-rich repeat proteins, Prv, is essential for melon resistance to Papaya Ringspot Virus and Fusarium oxysporum f. sp. melonis through hypersensitive response	Biotic stress response	[154]

Table 2. *Cont.*

Species	Gene	Direct Effect of Genome Editing (CRISPR/Cas9)	Revealed Physiological Function of Gene	Functional Trait	References
Cucumber	*CsBPC2* (BASIC PENTACYSTEINE transcription factor)	*Csbpc2* mutants were characterised by root growth inhibition, reduction in surface area, volume and the number of roots, along with a transformation in root system architecture from dichotomous branching to herringbone branching	BPC2 plays a crucial role in regulating root growth and development by stimulating gibberellin synthesis	Plant architecture	[155]
Watermelon	*ClDMP4* (*Cla97C06G121370*, DOMAIN OF UNKNOWN FUNCTION 679 homolog)	*Cldmp4* mutants decreased the number of viable seeds and raised the number of aborted seeds	A methodical article that utilises the CRISPR/Cas9 technique to study the production of double haploids	Fruit quality	[156]
Pumpkin	*CmCNIH1* (*CmoCh07G013500*, cornichon homolog)	The knockout of *CmCNIH1* resulted in Na+ accumulation in shoot and roots	Confirmation that CmCNIH1 plays a key role in enhancing stress resistance in pumpkin, as well as in other cucurbits grafted onto pumpkin	Stress response	[157]
Cucumber	*CsARN6.1* (gAAA ATPase domain-containing protein)	CRISPR/Cas9 editing of *CsARN6.1* resulted in disturbed development of adventitious roots in flooding conditions	CsARN6.1 interacts with CsPrx5, a class-III peroxidase responsive to waterlogging, resulting in enhanced adventitious root growth through the signalling action of hydrogen peroxide signalling	Plant architecture	[158]
Cucumber	*CsTRM5* (*CsaV3_2G013800*, TONNEAU1 recruiting motif protein 5)	The knockout of *CsTRM5* resulted in formation of spherical fruits	CsTRM5 controls fruit shape by influencing the orientation of cell division and cell enlargement through ABA accumulation	Fruit quality/stress response	[159]
Cucumber	*CsTIC21* (component of cucumber translocon at the inner membrane of chloroplasts)	The knockout of *CsTIC21* resulted in chloroplast malformation, leading to albino phenotypes and ultimately death in cucumber plants.	Nuclear factor YCs–TIC21 is a key element of chloroplast development induced by light	Development	[160]

Table 2. *Cont.*

Species	Gene	Direct Effect of Genome Editing (CRISPR/Cas9)	Revealed Physiological Function of Gene	Functional Trait	References
Melon	*CmRDR1c1/c2* (RNA-dependent RNA polymerase 1)	*Cmrdr1c1/c2* mutant plants were more susceptible to cucumber mosaic virus while susceptibility to zucchini yellow mosaic virus was not affected	RNA-dependent RNA polymerase 1b in melon is responsible for differential susceptibility to viruses from various families	Biotic stress response	[161]
Watermelon	*Clpsk1* (*Cla97C01G016930*, phytosulfokine precursor)	The knockout of *clpsk1* resulted in increased resistance to Fusarium oxysporum f.sp. niveum in watermelon seedlings	Confirmation that phytosulfokine-associated signalling attenuates the plant's response to pathogens	Biotic stress response	[162]
Pumpkin	*CmoPIP1-4* (plasma membrane intrinsic proteins)	*Cmopip1-4* mutants exhibited extremely salt-sensitive phenotypes	Confirmation that plasma membrane intrinsic proteins are crucial factors in signalling pathways related to stress responses, particularly salt stress	Abiotic stress response	[163]
Watermelon	ClphyB (Cla97C05G088180.1, phytochrome B)	A mutation in CmphyB led to elongation of the hypocotyl, decreased leaf angle, and suppressed branch growth	Phytochrome B plays an important role in regulating the branching in watermelon plants	Plant architecture	[164]

3.3. Gene Editing of Phytohormone Metabolism to Improve Fruit Quality and Enhance Multiple Stress Resistance

Phytohormones and growth regulators play a crucial role not only in regulating plant growth, development and flower sex determination but also in responding to stress throughout a plant's life cycle. Ethylene is involved in regulating various developmental processes such as organ abscission, seed germination, transition from vegetative to generative phases, flowering, seed maturation and responses to biotic and abiotic stresses. Depending on the species and interactions with other phytohormones, ethylene can induce opposing effects in some of these processes. The biosynthesis of ethylene begins with methionine, which is converted into S-adenosylmethionine (SAM) by SAM synthetase. SAM is then transformed into 1-aminocyclopropane (ACC) by ACC synthase (ACS). Finally, ethylene is released from ACC due to the activity of ACC oxidase (ACO). A very important aspect in which ethylene plays a crucial role is the regulation of sex determination [165]. The sex determination system in the family Cucurbitaceae is conserved, which inspires a broader understanding of sex differentiation in plants. Genetic studies have revealed that sex expression in cucumber is controlled by loci connected with ethylene biosynthesis genes: *F (ACS1)*, *M* (*ACS2*) and *A (ACS11)* in combination with *G (WIP)* (the WIP family C2H2 zinc finger transcription factor gene WIP1) and the ethylene biosynthetic genes ACC oxidase 2 (*ACO2*) [165]. Selected aspects of the above reports are confirmed in some works presenting gene editing (Table 2). Using CRISPR/Cas9, the downregulation of ACO genes resulted in a significant decrease in ethylene production in generative organs and extended flower longevity. However, this reduction in ethylene production in seeds also negatively affected germination. Upregulating the expression of *ACS1G* in cucumber induces the development of female flowers, but in combination with *ACO2* activity leads to the over-production of ethylene [114]. Maize (*Zea mays* L.) mutants with reduced ACS expression (*ZmACS6*) were characterised by increased drought resistance associated with delayed leaf senescence (increased chlorophyll content, proteins, including RuBisCO) [166]. The cucumber mutant SF1 (Short-fruit1), which produces short fruits (due to arrested cell divisions), is characterised by *ACS2* accumulation. Using CRISPR/Cas9 to generate null mutants of *ACS2* results in androecy and the production of male flowers only [114]. A knockout of *ACS2* mutants produces only male flowers [115]. Beyond sex determination control, *ACS2* plays a crucial role in cucumber fruit development, potentially accompanied by changes in drought resistance [115]. Another gene related to sex expression is WIP, a transcription factor that could block the expression of ethylene biosynthesis genes and inhibit stamen development. In watermelon, a direct mutation in the *ClWIP* gene, introduced by CRISP/Cas, resulted in the formation of female flowers [150]. Other studies linked to sex determination were connected to, among other things, transcription factors due to the bHLH transcription factor *CsALC* expressed in the ovaries, which plays a role in cucumber pollen tube emergence [116]. A study of watermelon showed that the CRISPR/Cas9-edited lines (*ClATM1*) exhibited typical vegetative growth, but displayed male flower abnormalities, including reduced petal size and degraded anthers with nonviable pollen [151]. Gynoecious inbred lines of cucumber have been successfully generated using CRISPR/Cas9 by introducing mutations in *CsVFB1* [122]. In melon, a mutation in the *eIF4E* gene introduced by CRISP/Cas9 is responsible for virus resistance, but it can also lead to the development of male sterile lines [138].

Cucumber *sf2*, carrying a recessive allelic variation in the hdc1 Arabidopsis homolog, produces shorter fruits due to inhibited cell proliferation by 70%. Through the CRISPR/Cas9 technique, the function of the cucumber *sf2* gene, encoding histone deacetylase homologous to HDC1 in Arabidopsis, was elucidated (Table 2). Genomic analysis indicated that *SF2* promotes histone deacetylation of genes involved in multiple pathways related to phytohormone biosynthesis and transduction. *SF2* promotes the actions of auxins, gibberellins and cytokinins by repressing negative regulators of these phytohormones, while repression of positive regulators of abscisic acid, jasmonic acid and ethylene reduces the impact of these hormones. Furthermore, *SF2* targets and inhibits several SAM decarboxylase genes, which code enzymes involved in polyamine biosynthesis [113]. Un-

doubtedly, polyamines present a prominent metabolic feature in plants when exposed to a range of abiotic stressors, including drought, salinity, cold, heat, waterlogging, ultraviolet radiation, heavy metals and herbicides [167]. Polyamines assume a crucial role in upholding the protein balance, countering the effects of reactive oxygen species (ROS), instigating protective antioxidative mechanisms, and serving as molecular chaperones in stressful circumstances. Consequently, due to polyamines, plants acquire a versatile capacity to withstand a multitude of stress factors [167].

Certain varieties of melons produce climacteric fruits, which, as they mature, exhibit a respiratory burst and increase in ethylene emission [168]. Delaying melon post-harvesting ripening improves fruit shelf life. Understanding the mechanisms behind climacteric melon fruit physiology is of interest to researchers, but changes in ethylene metabolism may bring about alterations in plant sensitivity to stresses [136]. Mutation of the *CmNAC-NOR* transcription factor gene led to inhibited ethylene production, the absence of an abscission layer, and also any change in fruit colour (Table 2) [132]. *CmROS1* (MELO3C024516) encodes a homolog of the main DNA demethylase *AtROS1* in Arabidopsis, primarily acting on sequences of transposable elements and regulating certain genes involved in pathogen responses and epidermal cell organisation [169]. Methylome analysis of *ROS1* knockout mutants revealed changes in DNA methylation in promoter regions of key ripening genes, such as *ACS1* and *ACO1*, suggesting the importance of DNA demethylation through ROS1 in initiating fruit ripening in melon [134]. Potential utilisation of these findings in stress-resistant plant breeding should encompass functional analyses of these genes not only in fruits but also in whole plants. Knockout of *CmACO1* (which is predominantly expressed in ripe fruits) using CRISPR/Cas9 significantly reduced ethylene emission, extending the shelf life of fruits (Table 2) [136]. Understanding the precise functions of the remaining four *CmACO* genes, which are expressed in different organs, could be of great relevance in the context of breeding more stress-resistant varieties.

Auxins are among the most thoroughly characterised phytohormones, which play a significant role at all stages of plant growth and development. Currently, the attention of an expanding group of researchers is drawn to the involvement of auxins not only in growth and development processes but also in plant responses to stress [170]. Cytokinins are commonly characterised as growth-promoting phytohormones, although various compounds with cytokinin-like properties have been identified as regulators of a wide spectrum of developmental and physiological processes in plants. Numerous studies have demonstrated that cytokinins can exert both beneficial and adverse effects on stress tolerance [171]. Many reports suggest that the response to drought is accompanied by a reduction in cytokinin biosynthesis in the roots and their transport to the shoots. Increased cytokinin levels enhance stomatal apertures and stimulate transpiration. However, elevated cytokinin concentrations result in the mitigation of stress-related symptoms, such as the delay in leaf senescence [172] (Table 2). Auxins, along with other phytohormones and growth regulators, are responsible for the architecture of the whole plant [173]. Modifying plant architecture not only enhances yield by optimising resource utilisation but also potentially increases resistance to biotic and abiotic stresses [174].

The *CsHEC1* gene is responsible for fruit neck development in cucumber. Genetic editing has allowed a detailed understanding of its function. *CsHeC1* knockout results in the formation of fruits with shorter necks, attributed to reduced auxin accumulation (Table 2) [119]. *CsHEC1* directly activates the *CsYuc4* gene, a member of the YUCCA family that catalyses the conversion of indole-3-pyruvic acid to IAA, the final step in the most common natural auxin biosynthesis pathway. The function of the numerous spines (ns) gene *Csa2G264590* in cucumber was studied by Liu et al. [120]. The ns gene encodes an auxin transporter of the AUX1/LAX type. Mutants with ns-cr had inhibited auxin transport to the epidermis, resulting in numerous spines. However, the study was limited to fruit peel and spines. The authors indicated the expression of auxin transporter genes in other plant organs such as stems (*Csa4G308640* and *Csa5G201310*), roots (*Csa5G20131* and *Csa5G374730I)*, which exhibited expression in most tissues (Table 2). Regulating auxin

biosynthesis and transport within the plant and various organs could be crucial in breeding plants more adapted to changing environmental conditions [175].

CsHEC2 in cucumber is responsible for spine formation on the fruit skin and its function is related to cytokinin content. Knockout of *CsHEC2* reduced cytokinin levels, while overexpression increased them. *CsHEC2* regulates cytokinin levels by controlling the expression of CTK hydroxylase-like1, an enzyme involved in cytokinin biosynthesis (Table 2). Utilising CRISPR/Cas9, the *CsHEC2* function can be precisely studied to develop cucumber varieties with enhanced resistance to abiotic stresses [121].

3.4. Increasing Salt Stress Tolerance through Gene Editing

A plant's response to salinity depends on the species and its adaptation. Initial symptoms of salinity stress include inhibited root and shoot growth. Under conditions of salinity stress, leaves age prematurely, chlorophyll and protein concentrations decrease, and membrane permeability increases. Plants also experience an imbalance in ion content, with higher levels of chloride and sodium ions and lower concentrations of calcium and potassium ions. However, plants have the capacity to adapt to salinity stress by utilising biochemical pathways that maintain or promote growth through improved water or ion management [176].

Homeostasis of K^+/Na^+ is a key factor in plant resistance to salt stress. In cucumber, the K^+ transporters CsAKT control K^+ influx. Understanding the function of CsAKT can be useful for breeding cultivars more resistant to sodium toxicity and improving the efficacy of salt-mitigating treatments, such as cerium oxide nanoparticle spraying [124] (Table 2).

Salt stress induces *CsBPC2* expression, and mutated *CsBPC2* leads to reduced osmolyte content, antioxidant enzyme activity and activity of ATP-dependent ion pumps. Mutated *CsBPC2* impedes osmotic adjustments and eliminates ROS, simultaneously causing unfavourable anion balance disturbances. These changes negatively affect plant tolerance to salinity stress. *CsBPC2* is also involved in the abscisic acid signalling pathway and is essential for ABA-induced biosynthesis and transcription of ABA-related signalling genes [128].

The basic pentacysteine (BPC) transcription factor, encoded by the *BPC* gene, plays a role in plant responses to abiotic stress [177]. In Arabidopsis, the homologous transcription factor *BPC1/BPC2* enhances plant salt resistance by suppressing the expression of galactan synthase 1 [178], but other studies suggest that *BPC2* may increase sensitivity to osmotic stress by repressing the expression of the LEA protective protein [179].

Knocking out the gene encoding RBOHD (Respiratory Burst Oxidase Homolog D, NADPH-dependent enzyme catalysing •OH formation) in salt-tolerant pumpkin (*Cucurbita moschata*) cv. Chaojiquanwang reduced the H_2O_2 levels in the root, resulting in decreased K^+ concentration and increased susceptibility to drought stress [139]. These studies have contributed to a better understanding of the signalling function of reactive oxygen species in Cucurbitaceae in response to salt stress.

An essential determinant of fruit quality pertains to the abundance of simple sugars and disaccharides in fruit. Modern genome-editing techniques are employed to investigate fundamental mechanisms underlying the transport of oligosaccharides, their unloading from the phloem, and hydrolysis within watermelon plants (Table 2) [143,144,146]. Simple sugars and disaccharides, classified as osmolytes, play a crucial role in osmoregulation, consequently mitigating the adverse effects of drought and salinity. The study by Xuan et al. [180] revealed that the majority of *ClSWEET* genes (encoding plasma membrane-localised hexose transporters) exhibited higher expression levels in response to stress.

3.5. Enhancing Resistance to Biotic Stresses through Genome Editing

Climate change exacerbates the likelihood of disease outbreaks through its influence on pathogen evolution, interactions between hosts and pathogens, and the promotion of novel pathogenic strains. It can also lead to shifts in the geographic range of pathogens, thereby expanding the prevalence of plant diseases into previously unaffected regions [17]. The most environmentally friendly approach to addressing new pathogen issues is through

resistance breeding, which is greatly enhanced by genome sequencing and emerging genome-editing techniques.

Powdery mildew poses a significant threat to cucumber cultivation [181]. Resistance to the pathogens that cause this disease is polygenic. Modern gene-editing techniques, however, can elucidate the role of individual genes, which can be crucial for effective resistance breeding. Tek et al. [126] used CRISPR/Cas9 to investigate the function of genes responsible for *mlo* resistance to powdery mildew. A mutation in *CsaMLO8* corresponded to pre-invasion response resistance, while genes *CsaMLO1* and *CsaMLO11* were identified as negative regulators in a post-invasive response to *Podosphaera xanthii* (Table 2). A double mutation of *CsaMLO1* and *CsaMLO11* was suggested as a potential approach to powdery mildew resistance relying on over-sensitivity. Therefore, CRISPR/Cas9 can be used to create cucumber varieties resistant to powdery mildew, exhibiting strong pre-invasion resistance with *CsaMLO8* mutations or post-invasion resistance with *CsaMLO1*/*CsaMLO11* mutations. Zhang et al. [162], applying CRISPR/Cas9 technology, generated a knockout line of watermelon clpsk1, which exhibited resistance to *Fusarium oxysporum* f.sp. *niveum*, thus confirming that phytosulfokine-associated signalling mitigates the plant's response to pathogens.

Plant-parasitic nematodes represent a serious category of plant pathogens. Their effective control is hampered by the restricted availability of nematocides. Furthermore, these nematodes themselves may serve as vectors for viruses. By knockout of the *CsMS* gene (using CRISPR/Cas9), cucumber plants resistant to root-knot nematodes were obtained (Table 2) [153].

Another example of CRISPR/Cas9 application in cucumber genome editing, which may be useful in breeding for increased stress resistance, is introducing virus resistance. Chandrasekaran et al. [112] used CAS9/subgenomic RNA to disrupt the function of the eukaryotic translation initiation factor 4E (eIF4E) transcription factor (Table 2). The resulting mutants were resistant to Ipomovirus (cucumber vein yellowing virus) and potyviruses (zucchini yellow mosaic virus and papaya ring spot mosaic virus-W). A mutation in the gene encoding the cap-binding protein eIF4E induced resistance to the Moroccan watermelon mosaic virus in melon [133,138]. CRISPR/Cas9 mutations in eIF4E were successfully applied for breeding cucumber plants resistant to zucchini yellow mosaic virus, watermelon mosaic virus and papaya ringspot virus (Table 2) [117]. Through the induction of mutations in the gene *CmRDR1c1/c2* encoding RNA-dependent RNA polymerase 1b, it has been possible to gain a deeper understanding of the mechanisms of diversified resistance in melon against viruses from various families [161].

3.6. Modifications to Plant Architecture Resulting from Genome Editing

The world's increasing population and decreasing arable land necessitate a higher crop yield per unit area. Plant architecture, a complex agronomic trait determining crop yield under high planting density, comprises many factors, including plant height and branching. Leaf angle is a significant factor determining plant canopy architecture, which can increase photosynthetic efficiency and facilitate dense plant spacing. Indole-3-acetic acid is an essential hormone that appears to play a crucial role in leaf angle regulation. A decrease in IAA content leads to a greater leaf angle in maize, while an increase in IAA concentration results in a smaller leaf angle [182]. The petiole angle (LPA) in cucumbers can impact not only yield and planting density but also fruit quality and disease occurrence due to light capture and air circulation. The gene encoding indole-3-octenoic acid glucosyltransferase (*CsIAGLU*) negatively regulates the leaf petiole angle by modifying the active IAA pool (glycosylation of free IAA) at the petiole base and the size of adaxial cells in cucumber (Table 2) [127]. *CsIAGLU* acts as a negative regulator of leaf petiole angle development by expanding cells, mediated by auxin. This provides a valuable strategy for breeding cucumbers with smaller leaf petiole angles by increasing *CsIAGLU* expression, likely through gene editing using CRISPR/Cas9 in the promoter region [127]. Being the outermost layer on terrestrial plants, the lipophilic cuticle primarily coats aerial plant structures,

including non-woody stems, leaves, flowers and fruits. It serves as a protective barrier against various abiotic and biotic stresses [183]. Mutation using CRISPR/Cas9 has allowed the understanding of the function of the *CsSEC23* gene, which modifies the deposition of cutin wax on fruit surfaces and has a significant impact on resistance to abiotic stresses [130]. To sum this up, an overview of crop improvement through CRISPR/Cas9-mediated genome editing in plants of the Cucurbitaceae family is presented in Figure 5.

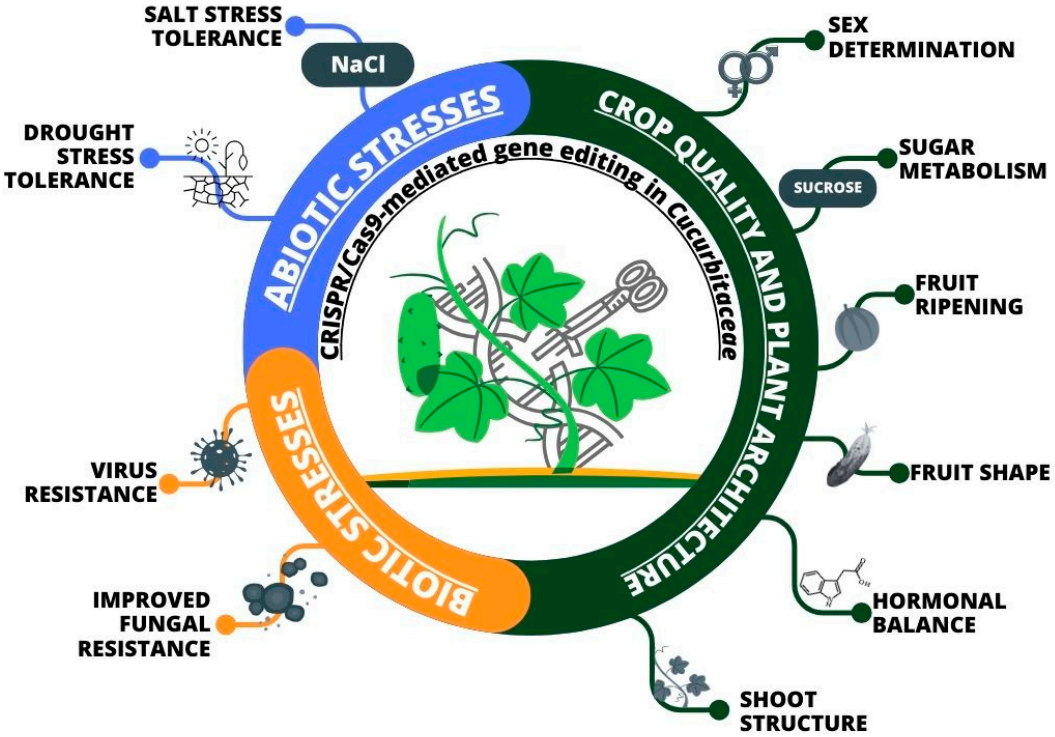

Figure 5. An overview of crop improvement through genome editing in Cucurbitaceae.

4. Legal Framework for Plant Genome Editing in Agriculture

The agricultural sector has experienced significant technological advancements leading to groundbreaking innovations. Among these innovations is plant genome editing, a technique that has attracted significant attention. As described above, this cutting-edge technique holds the promise of revolutionising agriculture by improving crop traits, increasing yields and ensuring food security. However, implementing plant genome editing in agriculture poses legal complexities and challenges. This analysis covers the crucial legal aspects and established conventions related to the use of plant genome editing in agriculture. There is a need for strict international regulatory frameworks to govern the application of this technique. The legal framework regulating plant genome editing is intricate and encompasses global agreements, regional regulations and domestic laws. The Cartagena Protocol on Biosafety [184], established under the Convention on Biological Diversity, is a significant factor at the international level. The protocol was adopted on 29 January 2000 in Cartagena, Colombia, and came into force on 11 September 2003. It has been ratified by numerous countries and plays a crucial role in the regulation of genetically modified organisms (GMOs) and biotechnology on a global scale. This protocol has the objective of guaranteeing the safe management, conveyance and utilisation of altered living organisms, comprising genetically modified plants. Additionally, the International Union for the Protection of New Varieties of Plants (UPOV) [185] offers a framework to secure the intellectual property rights of breeders who generate new plant species through genome editing. UPOV establishes guidelines to protect plant breeders' rights and distribute benefits resulting from the utilisation of new plant varieties equitably. Additionally, compliance with national legislation and regulations is necessary. At the national level,

different countries have differing approaches to regulating plant genome editing. The United States, for instance, has adopted a relatively lenient position, with the United States Department of Agriculture (USDA) asserting that certain genome-edited crops might not be subject to the same regulations as conventional genetically modified organisms (GMOs) [186]. This view is based on the belief that genome-edited crops may be identical to those created through conventional breeding methods. In contrast, the European Union (EU) has adopted a cautious approach to GMOs by imposing stringent regulations. In 2018 the European Court of Justice (ECJ) issued a significant ruling stating that organisms obtained through genome-editing techniques, such as CRISPR-Cas9, must be treated as genetically modified organisms (GMOs) under European Union (EU) regulations [187,188]. This ruling clarified that these genetically edited crops and organisms would be subject to the same regulatory framework and labelling requirements as traditional GMOs. The decision emphasised that the exemption previously applied to mutagenesis techniques did not extend to genome editing.

This ruling had a substantial impact on the regulation of genetically edited crops and organisms in the EU, aligning them with the precautionary approach to GMOs. It meant that developers and producers of genetically edited crops had to undergo rigorous safety assessments and comply with labelling and traceability requirements, similar to conventional GMOs. The ECJ's decision aimed to ensure transparency and consumer choice regarding genetically edited products in the European market and reflected the EU's commitment to maintaining strict controls over genetically modified organisms. Intellectual property and access to genetic resources are critical legal considerations in plant genome editing. Therefore, it is important to address the issue of intellectual property rights and access to genetic resources. As scientists create new plant varieties through this method, questions arise regarding ownership of the rights to these innovations and their accessibility to others. The Nagoya Protocol, under the Convention on Biological Diversity [189], provides a framework for accessing and distributing benefits linked to genetic resources. It aims to ensure equitable distribution of such benefits, thereby promoting agricultural equity and sustainability.

Beyond the legal framework, ethics plays a significant role in the use of plant genome editing in agriculture. Questions concerning the ethical implications of creating genetically modified crops, potential environmental impacts and equitable benefit sharing are central to the discourse surrounding genome editing. Various organisations and institutions have developed ethical guidelines and principles for scientists and stakeholders involved in genome editing in response to ethical concerns. These guidelines prioritise transparency, risk assessment and the responsible utilisation of this influential technology. Even when using sophisticated tools such as CRISPR, there is a need to monitor unintended mutations. Understanding the genome landscape, potential locations of atypical mutations and techniques for their detection are of key importance [190]. Edited plants may crossbreed with wild relatives, which may have unintended consequences, thus knowledge of reproductive biology, pollen dispersal and hybridisation potential is essential [191]. Recognising the interactions of edited crops can aid in the safe placement of edited crops [192]. Edited crops can influence the agricultural economy, trade and farmers' choices. Anticipating these impacts and providing equitable benefits can promote sustainable progress [193]. Although genome-edited plants may not have introduced foreign DNA, distinguishing them from traditional genetically modified organisms (GMOs) is the subject of regulatory and public debate. Understanding international regulations, ethical issues and public perceptions can guide research directions and applications [194].

In summary, the legal aspects of utilising plant genome editing in agriculture involve various international agreements, national regulations, intellectual property rights and ethical considerations. Successfully operating in this complex field requires a comprehensive understanding of relevant legal frameworks and a strong commitment to ethical and responsible conduct. As plant genome editing evolves and shapes the future of agriculture,

it is crucial that scientists, policymakers and stakeholders collaborate to establish a cohesive regulatory framework that balances innovation and security.

5. Conclusions and Prospects

Cucurbits are of particular interest owing to their high nutritional and economic value. They are also models for the study of plant development and the refinement of yield improvement strategies. However, while traditional breeding approaches are valuable, they are limited due to the reduced genetic diversity and lower rates of variation in cucurbit species. A broad scope of genome editing has been presented in this review, which covers different aspects of genome editing, including resistance to biotic stresses (pathogenic fungi and viruses) and abiotic stresses (climate change, drought and salinity). It also focused on improving plant quality, optimising plant architecture and influencing cucurbit sex determination. In conclusion, this comprehensive review provides valuable insights into the latest developments in genome editing in cucurbits. It serves as an important reference for the advancement of genome-sequencing and gene-editing technologies in the Cucurbitaceae family and has the potential to have a significant impact on the improvement of horticultural crops.

The prospects for the recent innovations in genome sequencing and gene editing in the context of plant science are promising and offer significant potential for various applications. In particular, they have the capacity to improve crop traits to address pressing global issues such as food security. By manipulating genes responsible for yield, disease resistance and nutritional content, these technologies could revolutionise crop production and ensure an abundant and healthy food supply for a growing population. These innovations also go beyond traditional crop improvement. They can help protect biodiversity, particularly in demanding crops such as cucurbits. By creating new mutants and precisely modifying plant genomes, genetic diversity can be preserved, helping to protect important plant species and ecosystems. Genome editing is at the forefront of sustainable agriculture. Its precision and reduced side effects lessen reliance on chemical interventions. This not only minimises the environmental impact but also increases the resilience of crops to the ever-increasing challenges of abiotic and biotic stresses, making agriculture more sustainable and environmentally friendly. In addition, these technologies are taking research on plants such as cucurbits to a new level. They enable a deeper understanding of plant developmental processes, shedding light on the intricacies of growth, adaptation and response to environmental factors. Finally, the application of gene-editing tools extends to many horticultural crops. By using these technologies, breeders and farmers can improve quality, yield and adaptability, ensuring that these plants meet consumer demands and adapt to changing conditions. In summary, genetic transformation and gene editing offer many benefits, from revolutionising agriculture to deepening the understanding of plant biology.

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