agronomy

MDPI

Perspective

Recent Progress in Genetic Transformation and Gene Editing Technology in Cucurbit Crops

Jing Feng [1,†], Naonao Wang [2,†], Yang Li [1], Huihui Wang [1], Wenna Zhang [2], Huasen Wang [1] and Sen Chai [1,*]

[1] Engineering Laboratory of Genetic Improvement of Horticultural Crops of Shandong Province, College of Horticulture, Qingdao Agricultural University, Qingdao 266109, China
[2] Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, China Agricultural University, Beijing 100193, China
* Correspondence: chaisen@qau.edu.cn
† These authors contributed equally to this work.

Abstract: Cucurbits (Cucurbitaceae) include major horticultural crops with high nutritional and economic value that also serve as model plants for studying plant development and crop improvement. Conventional breeding methods have made important contributions to the production of cucurbit crops but have led to a breeding bottleneck because of the narrow genetic bases and low variation rates of these crops. With the development of molecular techniques, innovations in germplasm development through transgenesis and gene editing have led to breakthroughs in horticultural crop breeding. Although the development of genetic transformation and gene editing techniques for cucurbit crops has lagged behind that for other major crops, great progress has been made in recent years. Here, we summarize recent advances in improving the genetic transformation efficiency of cucurbit crops, including the screening of germplasm and the application of physical treatments, morphogenic genes, and selection markers. In addition, we review the application of gene editing technology to cucurbit crops, including CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 (CRISPR-associated nuclease 9)-mediated gene knockout and base editing. This work provides a reference for improving genetic transformation efficiency and gene editing technology for cucurbit crops.

Keywords: cucurbit crops; genetic transformation; gene editing; biotechnology; plant regeneration; breeding; genomics

check for updates

Citation: Feng, J.; Wang, N.; Li, Y.; Wang, H.; Zhang, W.; Wang, H.; Chai, S. Recent Progress in Genetic Transformation and Gene Editing Technology in Cucurbit Crops. *Agronomy* **2023**, *13*, 755. https://doi.org/10.3390/agronomy13030755

Academic Editor: Yang Zhu

Received: 15 February 2023
Revised: 2 March 2023
Accepted: 2 March 2023
Published: 5 March 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Cucurbits (Cucurbitaceae) are a major group of horticultural crops that are cultivated worldwide, including numerous species with extremely high economic and nutritional value. Cucurbit crops such as melon (*Cucumis melo*), pumpkin (*Cucurbita moschata* Duchesne), watermelon (*Citrullus lanatus*), and cucumber (*Cucumis sativus*) are very popular among consumers. Cucurbit plants are also used as model materials for studying plant development and quality improvement because of their variable shapes and abundance of flavor compounds [1]. In the past few decades, conventional genetic breeding technologies (cross breeding and mutation breeding) have played important roles in breeding selection of cucurbit varieties with high yields and quality. However, the low genetic diversity and variation rates in these crops limit the breeding of complex genetic characters and hamper innovation. With the development of modern biotechnology techniques, innovation via transgenesis and gene editing has become an important focus in the development of horticultural crops and has given rise to new breeding approaches such as "de novo domestication", "rapid breeding", "haploid breeding", and "breaking of genetic linkage" [2,3]. Notably, the recent development of transgenic and gene editing techniques for multiple cucurbit species has greatly facilitated the study of the molecular mechanisms underlying

gene function in these crops and should provide new methods for cultivating cucurbit crops with economically desirable traits.

Genetic transformation is a supporting technology for genetic engineering and includes the cloning, delivery, integration, and expression of target genes and the regeneration, screening, and identification of transgene-positive plants. Vector delivery is the first step in transformation, via methods such as protoplast transfection, Agrobacterium (*Agrobacterium tumefaciens*)-mediated transformation, and particle bombardment. Agrobacterium-mediated transformation is currently the most widely used genetic transformation method because of its high efficiency. Agrobacterium-mediated transformation involves the stable integration of T-DNA from a Ti plasmid containing the target gene into plant chromosomal DNA via infection. The transformation process relies on the efficiency of the plant regeneration system. Much progress has been made in the in vitro culture of cucurbit crops via common methods such as organ culture, somatic embryogenesis, anther culture, and protoplast culture [4–7]. The most common transformation method for cucurbit crops uses cotyledons as explants for direct or indirect plant regeneration; indirect regeneration is mainly used for watermelon and direct regeneration for cucumber and melons. Trulson et al. established the first system for Agrobacterium-mediated transformation of cucurbit crops using hypocotyls as explants to achieve the stable expression of the *Neomycin Phosphotransferase II (NPTII)* gene in cucumber [8]. In 1990, Fang et al. successfully transferred the *NPTII* gene into melons [9]. This was followed by the successful transformation of cucurbit crops such as watermelon, followed by pumpkin, opening the door to the genetic transformation of numerous cucurbit crops.

In recent years, the maturity of transgenic technology enables wide application of gene editing technology in many plants. In gene editing, an engineered endonuclease identifies specific DNA sequences or guide RNAs via its DNA-binding domain (DBD) and cuts the target DNA sequence precisely and efficiently, thus editing specific DNA sequences [10]. Researchers have developed many specific gene editing systems, including a class of nucleases that identify their targets via protein-DNA interactions, e.g., mega nucleases (MNs), zinc finger nucleases (ZFNs), and transcription-activator-like effector nucleases (TALENs) [11,12]. The other class of nucleases includes CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated) and CRISPR/Cpf1 (a class 2/type V CRISPR RNA-guided endonuclease), which identify their targets through RNA–DNA base pairing [13]. CRISPR technology is regarded as the optimal method for acquiring genome edited crops given its low cost, high flexibility, and high reliability [14–16]. Gene editing technologies have been applied to cucurbit crops relatively recently, particularly CRISPR, which has been successfully used in cucumber, watermelon, melon, and pumpkin.

Although transformation systems have been established for some cucurbit crops, these systems suffer from low genetic transformation efficiency and poorly developed technology, and there are still gaps between these systems and those for other major crops. Researchers have tried to improve the genetic transformation efficiency of cucurbit crops in various ways and have optimized the genetic transformation steps to improve the transformation efficiency of multiple plant varieties. In addition, researchers have achieved gene knockout and base editing in cucurbit crops using CRISPR technology, and the genes responsible for many important agronomic traits have been explored. Here, we summarize the methods used to improve the genetic transformation efficiency of cucurbit crops and the application of gene editing technology to them.

2. Methods to Improve the Genetic Transformation Efficiency of Cucurbit Crops

Agrobacterium-mediated transformation using cotyledons as explants is currently the major technique adopted for many cucurbit crops. The efficiency of genetic transformation is influenced by many factors, including the regeneration efficiency of the plant, infection method, Agrobacterium strain, screening method, and use of exogenous phytohormones (Figure 1). Researchers have recently optimized the transformation process based on previous work, including genotype screening, Agrobacterium infection methods, applica-

tion of morphogenic genes, and diversification of screening markers, thus improving the transformation efficiency (Figure 1) [17–31].

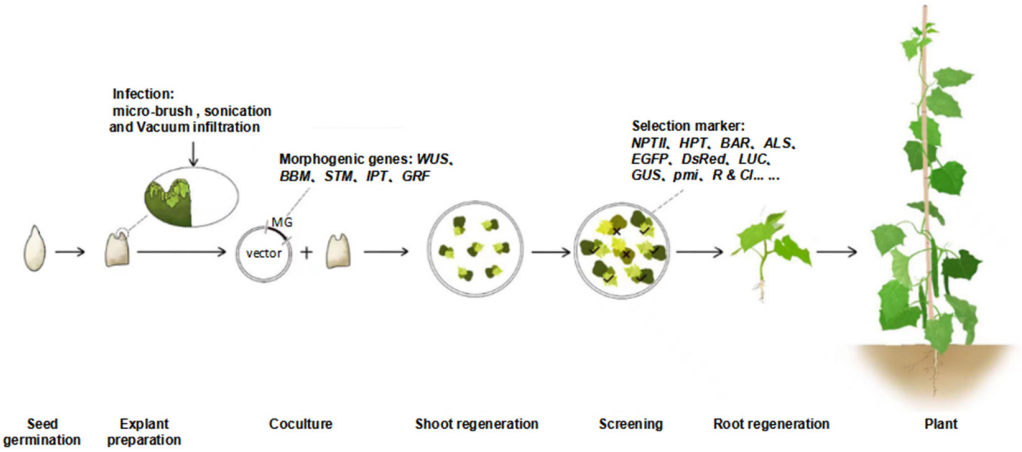


Figure 1. Efficient methods for the genetic transformation of cucurbit crops.

Seed germination: The sterilized seeds were sown on germination medium and cultured for 24–72 h until the cotyledons swelled and the hypocotyls extended to about 5–7 mm (different plant standards). Explant preparation: The cotyledons of the seeds were divided into two parts along the gap, and 2/3 of the adaxial end was taken as the explants. Physical treatments such as sonication, micro-brush, and vacuum infiltration were applied to assist the infection according to the experimental requirements. Coculture: The transformation vector with morphogenic genes was transformed into Agrobacteria and cocultured with explants for 3–4 days. Shoot regeneration: Explants after coculture were placed on the corresponding induction medium to induce shoots. Screening: Transformed positive shoots were identified using different screening markers. Root regeneration: Rooting was induced on positive shoots. Plant: Positive plants were obtained.

2.1. Germplasm Influences Transformation Efficiency

Differences in the genotypes of the target germplasm lead to significant differences in regeneration efficiency, representing an important factor influencing genetic transformation efficiency (Table 1). Identifying suitable germplasm is a direct method for improving the efficiency of genetic transformation. Germplasm screening has often been employed in watermelon, cucumber, and melon, less so in pumpkins, and rarely in other cucurbit crops. Different watermelon germplasms show significant differences in transformation efficiency. The transformation efficiency reached 8–17% in watermelon varieties such as Arka manik, Sugar baby, Arka muthu, IIHR-14, Feeling, and YL but only approximately 1–2% in China baby and Quality [17–20]. In general, the genetic transformation efficiency of cucumber ranges from 1% to 23%. The genetic transformation efficiency significantly differs among varieties, e.g., up to 23% in Jinyan no.7 but only approximately 1% in Eunchim and 404 [21,22]. The varieties Cu2, Poinsett 76, and Xintaimici are widely used by researchers and show stable genetic transformation efficiencies of 5–20% under different conditions [23–26]. Genetic transformation has been reported in more than 20 varieties of melon, such as Kirkagaç 637, CM-15, Charentais, and Silver Light, but their transformation efficiency is less than 7% in most cases. Fewer melon varieties with high transformation efficiency are currently available [27,32–35]. Finally, although it is difficult to transform pumpkins, successful transformation was recently achieved. Using JingXinZhen No. 4, Xin et al. achieved a transformation efficiency of up to 3.56% [22].

Table 1. Details of some successful reports on Agrobacterium-mediated transformation of cucurbit crops.

Species	Germplasm	Stability	Agrobacterium Strains	Transformation Efficiency (%)	T1	Reference
Watermelon	Feeling, China baby, Quality	Kanamycin	LBA4404	8.5–10%, 1–2.1%, 0–1.3% (PCR)	Reported	[18]
Watermelon	Feeling, China baby, Quality	Kanamycin	LBA4404	7%, 2.22%, 1.25% (PCR and Southern blot)	NR	[17]
Watermelon	the female parent line of Zhengkang No. 6	Kanamycin/cefotaxime	LBA4404	3 plantlets (PCR and Southern blot)	Reported	[36]
Watermelon	PI179878	Hygromycin B	EHA105	1.67% (shoots)	NR	[37]
Watermelon	ZG94	Bialaphos resistance	EHA105	23% (PCR)	Reported	[38]
Watermelon	Arka manik, Sugar baby, Arka muthu, IIHR-14	BASTA	EHA105	17.33%, 12.33%, 16%, 14.66% (GUS, PCR, Southern hybridization)	NR	[19]
Watermelon	TC	NR	EHA105	6.5% (GFP, PCR)	NR	[29]
Watermelon	YL	Glufosinate-ammonium	EHA105	12.5% (PCR)	NR	[20]
Cucumber	Eunchim	Phospinotricin	EHA101	1.7% (Northern blot)	NR	[21]
Cucumber	Poinsett 76	Phosphinothricin	EHA105 LBA4404	21.0%, 8.5% (shoots)	Reported	[24]
Cucumber	Eunsung	Paromomycin	EHA101	4.01% (Southern blot)	Reported	[39]
Cucumber	Xintaimici	Kanamycin	LBA4404	4.8% (PCR, Southern blot, Western blot)	NR	[40]
Cucumber	Shinhokusei 1	Kanamycin	EHA105	11.9 ± 3.5% (plants)	Reported	[41]
Cucumber	Ilan	Kanamycin	EHA105	3 plants (PCR)	Reported	[42]
Cucumber	Cu2	GFP	EHA105	1.32% (plantlets)	Reported	[43]
Cucumber	Xintaimici	Kanamycin	GV3101	8.10% (PCR)	NR	[26]
Cucumber	CCMC	GUS/Hygromycin B	EHA105	0.24% (PCR)	NR	[30]
Cucumber	Cu2, Xintaimici, 404, Eu1	GFP	EHA105	5.18%, 2.20%, 1.97%, 2.46% (GFP)	Reported	[22]
Melon	Silver Light	Kanamycin	LBA4404	Unspecified (2 transgenic lines)	Reported	[44]
Melon	Hetao		Transformation via the pollen-tube pathway	4.3% (PCR)	Reported	[45]
Melon	Védrantis	Kanamycin	GV2260	0.35–3.0% (PCR and GUS histochemical analysis)	NR	[46]
Melon	Hybrid line M01–3		Ovary-injection transformation	2.7% (PCR)	Reported	[47]

Table 1. *Cont.*

Species	Germplasm	Stability	Agrobacterium Strains	Transformation Efficiency (%)	T1	Reference
Melon	Cantaloupe F39 Honeydew 150	Kanamycin/GUS	EHA105	0.3%, 0.5% (PCR, GUS and Southern blot)	NR	[48]
Melon	Geumnodajieunchun	Kanamycin/geneticin	LBA4404	2.9%, 7.1% (PCR)	NR	[49]
Melon	Silver Light	Kanamycin	LBA4404	11 transgenic lines (PCR and Southern blot)	Reported	[50]
Melon	Silver Light	Kanamycin	LBA4404	0.8% (PCR)	Reported	[33]
Melon	CM-23	Kanamycin	EHA105	4% (PCR, Southern hybridization)	NR	[51]
Melon	M-15	Kanamycin	EHA105	13% (PCR)	Reported	[32]
Melon	CM-15	Kanamycin	EHA105	4 plants (PCR)	Reported	[34]
Melon	Charentais, Galia, Piel de sapo, Blanco	Kanamycin	EHA105	1.3%, 1.6%, 3.8%, 2.5% (plants)	Reported	[27]
Melon	Charentais	Hygromycin B	EHA105	5.68% (shoots)	NR	[35]
Melon	m1	GFP	EHA105	3.95% (GFP)	Reported	[22]
Pumpkin	JingXinZhen No. 4	GFP	EHA105	3.56% (GFP)	Reported	[22]

2.2. Auxiliary Physical Treatments Significantly Enhance Agrobacterium Infection

Various strains of Agrobacterium, an efficient natural carrier of transgenes, are widely used for the genetic transformation of plants because of their easy transformation and low cost. The most commonly used Agrobacterium strains for the genetic transformation of cucurbit crops include EHA105, GV3101, and LBA4404; different strains show somewhat different transformation efficiencies in various germplasms [26–28].

In addition to the effect of strain type, the key to successful transformation lies in the transmission process during infection. For cucurbit crops, the most commonly used explants are leaves, whose regenerable cells are located at the U-shaped cut end that is produced when generating explants. However, it is difficult for Agrobacteria to reach this region under routine infection conditions [43]. To achieve effective Agrobacterium infection, various methods are used, such as sonication, micro-brush (KITA, Nanotek Brush) treatment, and vacuum infiltration to promote the penetration of Agrobacteria into cells deep in the explant. Sonication generates many minor wounds on explants to facilitate the entry of Agrobacteria and improve the genetic transformation efficiency. Sonication improved the transformation efficiency of watermelon from the original rate of 8% to 17% [19]. Similarly, slightly scratching cucumber explants to allow better access by Agrobacteria significantly increased the entry of the transgene, as revealed by β-glucuronidase (*GUS*) staining [30]. Vacuum infiltration is another effective way to increase the probability of Agrobacterium entry. This method was first applied in the genetic transformation of soybean (*Glycine max*), Arabidopsis (*Arabidopsis thaliana*), and wheat (*Triticum aestivum*) and has also been successfully utilized in cucurbit crops [52]. A variety of cucumber germplasms were successfully transformed using vacuum infiltration. The "optimal infiltration intensity" strategy, which integrates multiple auxiliary methods, was recently proposed. The researchers used sonication and micro-brush techniques to wound cotyledonary node explants and created vacuum conditions with a sterile syringe, significantly improving the transformation efficiency of cucumber, melon, and pumpkin [22].

2.3. Morphogenic Genes Significantly Increase Transformation Efficiency

The genetic transformation efficiency of cucurbit crops can be improved by introducing some auxiliary physical methods. However, exploring and optimizing genetic transformation conditions requires a lot of time and effort, and the same genetic transformation system cannot be universally applied to different genotypes. Many genes capable of promoting or reprogramming cell fate can improve the genetic transformation efficiency of explants. These genes are known as morphogenic genes, including the wounding-associated *Wound-Induced Dedifferentiation 1–4* (*WIND1–4*); epigenetic modification genes, including *Polycomb Repressive Complex1/2* (*PRC1/2*) and *Arabidopsis Trithorax4* (*ATX4)*; growth regulatory genes, including *Indole3-Acetic Acids* (*IAAs*), *YUCCAS* (*YUCs*), and *Arabidopsis Response Regulator7/15* (*ARR7/15*); and developmental regulatory genes, including *Wuschel* (*WUS*), *Shoot Meristemless* (*STM*), and *Wuschel Related Homeobox5/11* (*WOX5/11*) [53–55]. Among morphogenic genes, the *WUS-STM* and *Growth-Regulating Factor* and *GRF-Interacting Factor* (*GRF-GIF*) complexes are commonly used to improve genetic transformation efficiency. For example, co-expressing *WUS* and *STM* greatly improved the in vitro transformation efficiency of monocotyledonous plants such as rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), and some maize (*Zea mays*) inbred lines [56]. Co-expressing *WUS* and *Isopentenyl Transferase* (*IPT*) induced meristem growth and budding in *Nicotiana benthamiana* seedlings [57]. In addition, exogenously expressing *AtGRF5* promoted budding and improved the genetic transformation efficiency of soybean, oilseed rape (*Brassica napus*), and sunflower (*Helianthus annuus*) [58]. Expressing fusion protein of *TaGRF4* and *OsGIF1* improved the regeneration speed and efficiency of wheat and rice and expanded the scope of varieties that could be used for genetic transformation [59].

Expressing morphogenic genes can improve the genetic transformation efficiency of watermelon. Among these genes, *AtGRF5*, *TaGRF4-OsGIF1*, *ZmWUS*, *ZmWUS–ZmBBM*, *ZmWUS–IPT*, and *AtGRF5* showed the best effects, increasing the genetic transformation

efficiency of watermelon cultivar WWl50 approximately 40-fold to 24.73% [28]. In another study, overexpressing the endogenous genes *ClGRF4–ClGIF1* achieved a transformation efficiency of up to 47.02% in watermelon cultivar TC, representing an approximately 9-fold increase compared with the control (with a transformation efficiency of 5.23%). Mutating the *miR396* target site in *ClGRF4* further increased the transformation efficiency to 67.27% and had significant effects in eight other watermelon varieties, such as YL, M08, 148, and 97103 [29]. The role of morphogenic genes in increasing transformation efficiency has been verified in watermelon, for which indirect regeneration is mainly used, but whether these genes can be used for other cucurbit crops such as cucumber and melon (for which direct regeneration is mainly used) remains to be studied.

2.4. Diversification of the Screening Markers Used in Genetic Transformation

Introducing screening marker genes during transformation can facilitate the identification of positive transgenic events and can help determine whether the transformation was successful. Three major categories of screening markers are currently used in the genetic transformation of cucurbit crops. The first category is resistance genes, e.g., *NPTII*, *Hygromycin phosphotransferase (HPT)*, *Phosphinothricin N-acetyltransferase (BAR)*, and *Acetolactate Synthase (ALS)*. The buds of explants harboring a resistance gene can grow normally under selection, whereas buds lacking the resistance gene cannot grow. The second category is reporter genes, e.g., *Enhanced Green Fluorescent Protein* (*eGFP*), *Discosoma striata red Fluorescent Protein* (*DsRed*), *luciferase* (*LUC*), and *β-glucuronidase* (*GUS*). Under specific physical/chemical conditions, buds harboring a reporter gene exhibit a different appearance from buds that have not been transformed. The third category is metabolism-related genes—e.g., *Phosphomannose isomerase* (*pmi*) and *maize R*, *C1*, and *B transcription factor genes*—which allow transformed regenerated buds to grow under specific nutritional conditions [31,60].

Among the resistance genes, *NPTII* has been used as a selection marker, but its effectiveness differs significantly between germplasms. Cotyledon explants of cucumber variety CMCC are extremely sensitive to hygromycin B, with good screening effects even at low concentrations [30]. However, escape is likely to occur when resistance genes are used; that is, buds free of any resistance gene can sometimes grow under selection [27]. In contrast, the use of reporter genes results in no escapes. Under specific physical/chemical conditions, reporter genes such as *eGFP*, *DsRed*, *LUC*, and *GUS* enable transgene-positive buds to exhibit a color different from that of non-transformed buds, thus achieving effective screening and avoiding the disadvantage of escapes. However, reporter genes are not perfect. They may be interfered with by the plants themselves, making it difficult to identify transgenic material under specific conditions. Moreover, high levels of reporter gene expression can damage the plant, leading to abnormal phenotypes. In addition to resistance genes and reporter genes, researchers have also developed metabolism-related genes, which allow transgene-positive buds to grow under specific nutritional conditions. For example, expression of the *pmi* gene enables the conversion of mannose-6-phosphate into fructose-6-phosphate, allowing regenerated buds to grow normally using mannose as a carbon source. Using the mannose selection system in cucumber, effective transgene-positive bud screening was achieved using a medium containing 10 g/L mannose and 10 g/L saccharose, leading to reduced damage and escape compared with the use of reporter genes and resistance genes [61].

2.5. Other Factors

Genetic transformation involves multiple factors and multiple steps. Therefore, various issues are likely to occur during transformation, which differ somewhat in different species. For example, cucumber is rich in endogenous ethylene, but the accumulation of ethylene is unfavorable for cell growth and differentiation and in severe cases leads to the aging of cells. $AgNO_3$ can be added to the medium during transformation to inhibit ethylene production and promote cell differentiation and regeneration. However, Ag^+ is a heavy metal that is somewhat toxic to plants, and the long-term use of $AgNO_3$ can lead to plant deformity [43].

Browning, vitrification, and Agrobacterium contamination are likely to occur during melon transformation. Browning occurs when cells experience metabolic changes upon the activation of polyphenol oxidase in explants. This process can occur because of the germplasm selected, physiological conditions, culture conditions, and damage [62]. Antioxidants such as vitamin C, sodium thiosulfate, and cysteine can be added during melon transformation to inhibit browning. Vitrification is a condition in which the stems and leaves of explants are water-soaked and fragile. Vitrified seedlings tend to grow slowly and show weak differentiation ability. Vitrification may be related to phytohormone levels, culture conditions, and relative humidity. Vitrification of seedlings during transformation can be reduced by increasing the concentrations of agar and saccharose in the medium as appropriate, strengthening ventilation and air exchange, and controlling relative humidity levels. Finally, since Agrobacterium mediation is generally required for transformation, Agrobacterium contamination/overgrowth may occur during tissue culture. For Agrobacterium contamination, cefotaxime, plant preservative mixture (PPM), and Timentin can be added to the medium to inhibit the reproduction of the Agrobacteria and improve the survival rates of plants [43].

3. Application of Gene Editing Technology in Cucurbit Crops

Gene editing technology is an important strategy for gene function studies and molecular breeding. A gene editing system, which is introduced into a plant through genetic transformation, uses engineered endonucleases to edit the specific nucleotide sequences of the plant genome precisely and efficiently. CRISPR technology has become a mainstream gene editing technology used for cucurbit crops thanks to its efficiency, precision, and easy operation. After gene editing, the carrier fragments can be eliminated from the plants through hybridization [63]. CRISPR/Cas9-based knockout and base editing of genes have been successfully achieved in various cucurbit crops (Figure 2 and Table 2).

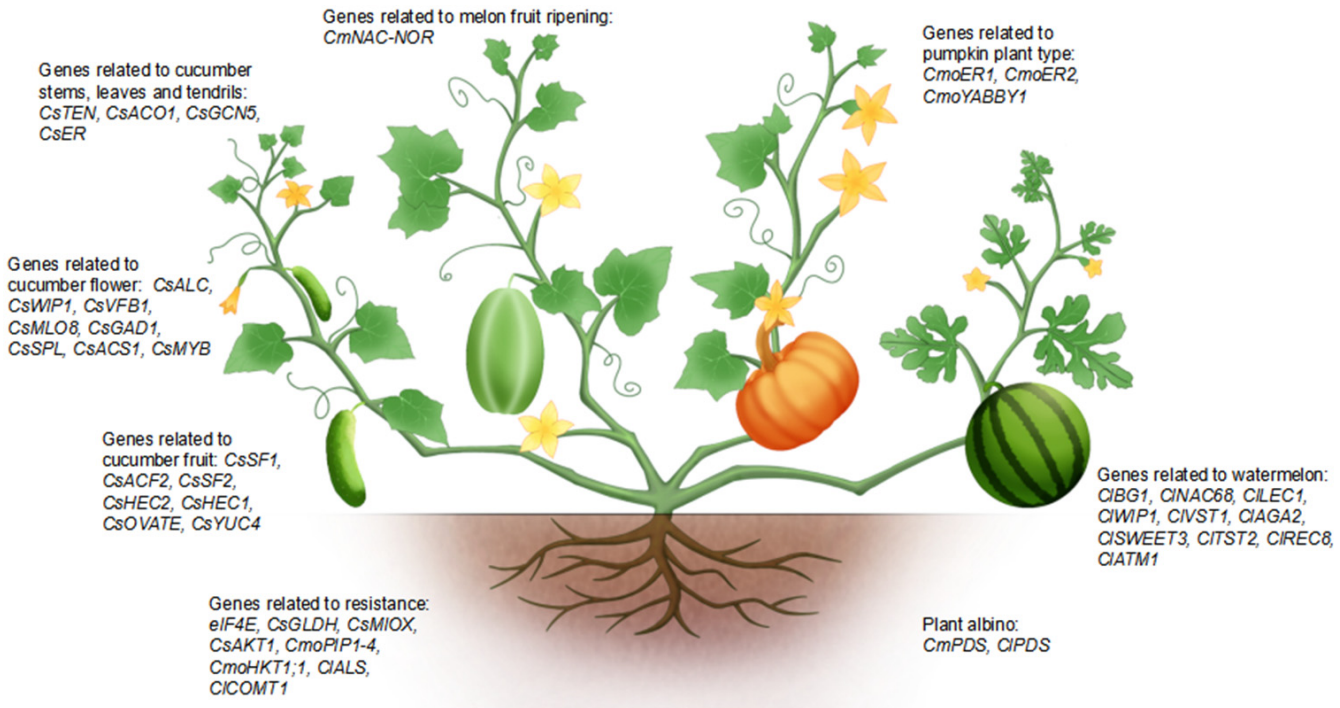


Figure 2. Illustration of editing genes in cucurbit crops.

Table 2. Details of editing genes of cucurbit crops.

Species	Gene	ID	Gene Function or Phenotype	References
Watermelon	*ClALS*	Cla019277	Herbicide resistance	[38]
Watermelon	*ClPDS*	Cla97C07G142100	Albino plants	[28]
Watermelon	*ClPDS*	Cla010898	Albino plants	[37]
Watermelon	*ClBG1*	Cla97C08G153160	Regulation of seed size and seed germination	[64]
Watermelon	*ClNAC68*	Cla97C03G059250	Regulation of fruit sugar content and seed development	[65]
Watermelon	*ClLEC1*	Cla97C10G188900	Reproductive development	[29]
Watermelon	*ClWIP1*	Cla008537	Sex determination	[66]
Watermelon	*ClVST1*	Cla97C02G031010	Vacuolar sugar transporter	[67]
Watermelon	*ClAGA2*, *ClSWEET3*, *ClTST2*	Cla97C04G070460, Cla97C01G000640, Cla97C02G036390	Carbohydrate partitioning	[68]
Watermelon	*ClREC8*	Cla97C07G132920	Seedless watermelon	[69]
Watermelon	*ClCOMT1*	Cla97C10G188660	Tolerance to abiotic stresses	[70]
Watermelon	*ClATM1*	Cla010576	Male sterility	[71]
Cucumber	*CsALC*	Csa2G356640.1	Pollen tube emergence	[72]
Cucumber	*eIF4E*	XM_004147349	Antivirus	[42]
Cucumber	*CsWIP1*, *CsVFB1*, *CsMLO8*, *CsGAD1*	Csa4M290830, Csa4M641640, Csa5M623470, Csa5M348050	Gynoecious inbred lines	[43]
Cucumber	*CsSPL*	Csa3M850670	Male and female fertility and ovule development	[73]
Cucumber	*CsSF1*, *CsACF2*	Csa2G174140, Csa1G580750	Cucurbit-specific RING-type E3 ligase, Rate-limiting enzyme for ethylene biosynthesis	[74]
Cucumber	*CsSF2*	Csa2G337260	Fruit elongation	[75]
Cucumber	*CsTEN*, *CsACO1*	Csa5P644520.1, Csa6G160180	Identity and mobility of tendrils	[76]
Cucumber	*CsHEC2*	Csa2G285890	Fruit wart	[77]
Cucumber	*CsACS1*, *CsMYB*	CsaV3_6G044400, CsaV3_6G044410	Female floral development	[78]
Cucumber	*CsHEC1*, *CsOVATE*, *CsYUC4*	Csa4G639900, Csa4G038760, Csa2G379350	Fruit neck length	[79]
Cucumber	*CsGCN5*	Csa6G527060	Gene transcription and plant development	[30]
Cucumber	*CsER*	CsaV3_4G036080	Compact plant architecture	[22]
Cucumber	*CsGLDH*, *CsMIOX*	Csa4M236360.1, Csa2M000640	Ascorbic acid biosynthesis	[80]
Cucumber	*CsAKT1*	CsaV3_1G029650	Salt tolerance	[81]
Melon	*CmPDS*	MELO3C017772.2	Albino plants	[35]
Melon	*CmNAC-NOR*	MELO3C016540.2	Fruit ripening	[82]
Melon	*CmNAC-NOR*	MELO3C016540	Fruit coloring and ripening	[83]
Melon	*eIF4E*	MELO3C002698.2	Antivirus	[84]
Pumpkin	*CmoER1*, *CmoER2*	CmoCh09G003660, CmoCh01G017570	Compact plant architecture	[22]
Pumpkin	*CmoPIP1-4*	CmoCh04G011950	Salt stress	[85]
Pumpkin	*CmoHKT1;1*	CmoCh10G003830	High-affinity K+ transporter1	[86]
Pumpkin	*CmoYABBY1*	CmoCh15G012090	Architecture regulation	[1]

3.1. CRISPR/Cas9-Based Gene Knockout

CRISPR/Cas9 is a microbial adaptive immune mechanism that achieves precise gene editing based on the RNA-guided Cas9 nuclease within the Type II prokaryotic CRISPR/Cas system [87]. The present CRISPR-Cas9 systems are modifications of bacterial CRISPR/Cas9, comprising modified Cas9 endonuclease and a single guide (sg)RNA [88]. Under the guidance of a specific sgRNA, Cas9 specifically identifies the protospacer adjacent motif (PAM, NGG) in its target sequence and produces blunt-ended double-strand breaks approximately 3 bp upstream of the PAM to trigger the HDR or NHEJ pathway, thus achieving precise editing of plant genes [89].

In 2016, CRISPR/Cas9 was successfully applied to cucurbit crops for the first time. The researchers used CRISPR/Cas9 to mutate the DNA sequence at the target site of *eukaryotic translation initiation factor 4E* (*eIF4E*) at its N′ and C′ termini in cucumber. Two homozygous *eIF4E* knockout mutants exhibited immunity to *Cucumber vein yellowing virus* (*Ipomovirus*) infection and resistance to the *potyviruses Zucchini yellow mosaic virus* and *Papaya ring spot mosaic virus-W* [42]. Subsequently, Hu et al. used the cucumber U6 promoter to drive sgRNA to knock out *WIP Domain Protein 1* (*CsWIP1*) using CRISPR/Cas9 technology and successfully created a gynoecious cucumber line [43]. Thereafter, Liu et al. explored the role of *Sporocyteless* (*CsSPL*) in ovule development and reproduction [73]. Since then, CRISPR/Cas9 has been widely used in cucumber. Researchers have successfully used CRISPR/Cas9 in breakthrough studies on the functions of genes for sex, fruit length, tendril formation, and stress resistance in cucumber [22,30,75–81].

In addition to the successful application of CRISPR/Cas9 in cucumber, gene knockout using CRISPR/Cas9 has also been reported in watermelon. Tian et al. knocked out the *phytoene desaturase* (*ClPDS*) gene in watermelon and successfully obtained albino regenerated buds, demonstrating the feasibility of applying CRISPR/Cas9 technology to watermelon [37]. In 2019, researchers used CRISPR/Cas9 to knock out the *ClWIP1* gene and obtained a gynoecious line to explore the molecular mechanism that determines the sex of watermelon [66]. Researchers also used CRISPR/Cas9 to successfully knock out genes (e.g., a key component of the cohesin complex in meiosis (*ClREC8*), *caffeic acid O-methyltransferase* (*ClCOMT1*), *β-glucosidase* (*ClBG1*), *NAC transcription factor68* (*ClNAC68*), *vacuolar sugar transporter (ClVST1)*, *alkaline alpha-galactosidase (ClAGA2)*, *Citrullus lanatusSugars Will Eventually Be Exported Transporter 3 (ClSWEET3)*, *Tonoplast Sugar Transporter (ClTST2)*, *Citrullus lanatus Abnormal Tapetum 1 (ClATM1)*, and *nuclear transcription factor Y subunit B-9-like* (*ClLEC1*)) and achieved good results, increasing seed fertility, stress resistance, seed size, and sugar transport in watermelon [29,64–66,68–71]

Compared with that in cucumber and watermelon, the application of gene editing technology in other cucurbit crops is lagging. For melons, researchers successfully used CRISPR/Cas9 to knock out the *CmPDS* gene in 2019 and obtained albino regenerated buds [35]. Researchers subsequently used CRISPR/Cas9 to knock out *eIF4E*, *ERECTA* (*CmER*), and the NAC transcription factor gene *Nonripening* (*CmNAC-NOR*) and revealed their functions in virus resistance, plant architecture, and fruit ripening, respectively [22,82–84]. In pumpkin, researchers used *Agrobacterium rhizogenes* as an infection tool to develop an efficient CRISPR/Cas9-based root transformation system to analyze stem–root communication in cucurbit crops and successfully knocked out *high-affinity K + transporter 1* (*CmoHKT1;1*) and *Plasma membrane intrinsic proteins* (*CmoPIP1–4*) [85,86]. Besides the root transformation system, Xin et al. obtained a knockout mutant of *CmoER* and achieved stable gene editing in pumpkin [22]. This team recently used CRISPR/Cas9 technology to achieve domain B knockout of *CmoYABBY1*, thus strengthening the translation of this gene and inhibiting stem growth proportionally in a dose-dependent manner, creating a pumpkin plant with a dominant bushy trait (Figure 2) [1].

3.2. Precise Editing via Cytosine Base Editors

In addition to the gene knockout achieved in cucurbit crops, precise single-nucleotide editing of genes in watermelon has been achieved using cytosine base editors (CBEs). CBEs

consist of a Cas9 nickase (nCas9) with a D10A mutation, which deactivates RuvC (one of the two Cas9 nuclease domains), fused with a cytidine deaminase and a uracil DNA glycosylase (UDG) inhibitor (UGI). CBEs induce a C:G > T:A base change. Tian et al. used a novel Cas9 variant fused with CBE3 to achieve a single-nucleotide conversion from C to T in the Pro190 (CCG) codon of the *ALS* gene of watermelon without merging the DNA templates of the donor, thereby creating a non-GM herbicide-resistant watermelon variety [38]. It is worth noting that the first successful application of CRISPR/Cas9 technology in watermelon was also achieved by the same team [37].

4. Discussion and Future Prospects

In this review, we systematically summarized recent innovations and applications of genetic transformation and gene editing technology in cucurbit crops. Significant improvements have been made, but there are still many outstanding issues in the genetic transformation of cucurbit crops.

4.1. Establish Genotype-Free Transformation Systems

Genotypes play a pivotal role in transformation. Extensive studies have been performed on cucurbit crops, and the germplasms examined have varied widely among research teams. In contrast to other species, no widely used germplasm is currently available for the stable transformation of cucurbit crops. A uniform genotype would help researchers explore the functions of genes and analyze their genetic relationships. Therefore, testing the genetic transformation efficiency of the existing germplasms and identifying the most suitable receptor germplasms could help improve the genetic transformation efficiency of cucurbit crops. Certainly, for future molecular breeding, striving to establish a transformation system that is not limited by genotype is our ultimate goal, e.g., establishment of a genotype-free genetic transformation system using morphogenic genes. The use of morphogenic genes can significantly improve genetic transformation efficiency, as verified in multiple plant varieties [28,29]. However, the strong expression of morphogenic genes can affect the growth and development of plants, making it crucial to eliminate the abnormal phenotypes of regenerated seedlings when morphogenic genes are used. Various studies have used inducible *35s-MdBBM-GR* vectors or the heat-induced Cre/*loxP* gene editing system during specific periods to reduce the damage caused by morphogenic genes [90,91]. In the future, innovative ways to solve this problem should continue to be explored.

4.2. Further Development of Gene Editing Technologies in Cucurbit Crops

The development prospects of gene editing technology are very broad. For example, the CRISPR-Cas9 system in tissue-specific or induced promoter control can help with spatial or temporal genomic alterations [92,93]. CRISPR-Cas9 also enables rapid functional identification of genes by generating gRNA libraries. As a result, CRISPR-Cas9 has become an efficient, simple, and fast tool for growing agriculturally improved crops that can both add better traits and remove undesirable traits [94]. Certainly, one of the best applications of the CRISPR-Cas9 system is the use of label-free "cisgene" plants with improved agronomic traits. These plants will eventually be immune to current transgene regulation because CRISPR-associated RNA-guided nucleases (RGENs) or ribonucleoproteins (RNPs) induce genomic mutations without any foreign DNA [95]. However, the development of gene editing technology in cucurbit crops is lagging, with successful results (including gene knockout) achieved only through CRISPR/Cas9 and base editing [37,38]. Efficient editing systems, such as "precise editing" via prime editing and gene editing systems optimized for the characteristics of cucurbit crops, remain to be further explored.

In conclusion, some progress has been made in the genetic transformation and gene editing of cucurbit crops, but many issues remain to be resolved.

Author Contributions: Conceptualization, J.F., N.W. and S.C.; formal analysis, J.F. and N.W.; investigation, J.F. and N.W.; resources, J.F., N.W. and H.W. (Huihui Wang); data curation, Y.L.; writing—original draft preparation, J.F., N.W. and S.C.; writing—review and editing, W.Z., N.W. and S.C.; visualization, J.F. and N.W.; supervision, H.W. (Huasen Wang) and S.C.; project administration, S.C.; funding acquisition, S.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (32102404 to S.C.), This work was also supported by the Natural Science Foundation of Shandong Province (ZR2020QC157 to S.C.).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wang, S.; Wang, K.; Li, Z.; Li, Y.; He, J.; Li, H.; Wang, B.; Xin, T.; Tian, H.; Tian, J.; et al. Architecture design of cucurbit crops for enhanced productivity by a natural allele. *Nat. Plants* **2022**, *8*, 1394–1407. [CrossRef] [PubMed]
2. Nanasato, Y.; Tabei, Y. A method of transformation and current progress in transgenic research on cucumbers and Cucurbita species. *Plant Biotechnol. (Tokyo Jpn.)* **2020**, *37*, 141–146. [CrossRef]
3. Li, H.; Yang, X.; Shang, Y.; Zhang, Z.; Huang, S. Vegetable biology and breeding in the genomics era. *Sci. China Life Sci.* **2023**, *66*, 226–250. [CrossRef]
4. Debeaujon, I.; Branchard, M. Somatic embryogenesis in Cucurbitaceae. *Plant Cell Tiss. Org. Cult.* **1993**, *34*, 91–100. [CrossRef]
5. Hooghvorst, I.; Nogués, S. Opportunities and Challenges in Doubled Haploids and Haploid Inducer-Mediated Genome-Editing Systems in Cucurbits. *Agronomy* **2020**, *10*, 1441. [CrossRef]
6. Jarl, C.I.; Bokelmann, G.S.; De Haas, J.M. Protoplast regeneration and fusion in Cucumis: Melon × cucumber. *Plant Cell Tiss. Org. Cult.* **1995**, *43*, 259–265. [CrossRef]
7. Kurtar, E.S.; Seymen, M. Another Culture in Cucurbita Species. *Methods Mol. Biol.* **2021**, *2289*, 111–121. [CrossRef]
8. Trulson, A.J.; Simpson, R.B.; Shahin, E.A. Transformation of cucumber (*Cucumis sativus* L.) plants with Agrobacterium rhizogenes. *Theor. Appl. Genet.* **1986**, *73*, 11–15. [CrossRef]
9. Fang, G.; Grumet, R. Agrobacterium tumefaciens mediated transformation and regeneration of muskmelon plants. *Plant Cell Rep.* **1990**, *9*, 160–164. [CrossRef]
10. Wyman, C.; Kanaar, R. DNA double-strand break repair: All's well that ends well. *Annu. Rev. Genet.* **2006**, *40*, 363–383. [CrossRef]
11. Puchta, H.; Fauser, F. Synthetic nucleases for genome engineering in plants: Prospects for a bright future. *Plant J.* **2014**, *78*, 727–741. [CrossRef]
12. Voytas, D.F.; Gao, C. Precision Genome Engineering and Agriculture: Opportunities and Regulatory Challenges. *PLoS Biol.* **2014**, *12*, e1001877. [CrossRef] [PubMed]
13. Zetsche, B.; Gootenberg, J.S.; Abudayyeh, O.O.; Slaymaker, I.M.; Makarova, K.S.; Essletzbichler, P.; Volz, S.E.; Joung, J.; van der Oost, J.; Regev, A.; et al. Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. *Cell* **2015**, *163*, 759–771. [CrossRef] [PubMed]
14. Liu, Y.; Zhang, C.; Wang, X.; Li, X.; You, C. CRISPR/Cas9 technology and its application in horticultural crops. *Hortic. Plant J.* **2022**, *8*, 395–407. [CrossRef]
15. Mao, Y.; Botella, J.R.; Liu, Y.; Zhu, J.-K. Gene editing in plants: Progress and challenges. *Natl. Sci. Rev.* **2019**, *6*, 421–437. [CrossRef]
16. Chen, K.; Wang, Y.; Zhang, R.; Zhang, H.; Gao, C. CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. *Annu. Rev. Plant Biol.* **2019**, *70*, 667–697. [CrossRef] [PubMed]
17. Huang, Y.-C.; Chiang, C.-H.; Li, C.-M.; Yu, T.-A. Transgenic watermelon lines expressing the nucleocapsid gene of Watermelon silver mottle virus and the role of thiamine in reducing hyperhydricity in regenerated shoots. *Plant Cell Tissue Organ Cult. (PCTOC)* **2010**, *106*, 21–29. [CrossRef]
18. Yu, T.A.; Chiang, C.H.; Wu, H.W.; Li, C.M.; Yang, C.F.; Chen, J.H.; Chen, Y.W.; Yeh, S.D. Generation of transgenic watermelon resistant to Zucchini yellow mosaic virus and Papaya ringspot virus type W. *Plant Cell Rep.* **2011**, *30*, 359–371. [CrossRef]
19. Vasudevan, V.; Sathish, D.; Ajithan, C.; Sathish, S.; Manickavasagam, M. Efficient Agrobacterium-mediated in planta genetic transformation of watermelon [Citrullus lanatus Thunb.]. *Plant Biotechnol. Rep.* **2021**, *15*, 447–457. [CrossRef]
20. Cao, L.; Wei, W.; Shen, J.; Xu, Z.; Li, Z. Study on the optimization of transformation systems in watermelon. *Veg. Res.* **2022**, *2*, 1–9. [CrossRef]
21. Kim, H.-A.; Min, S.-R.; Choi, D.-W.; Choi, P.-S.; Hong, S.-G. Development of transgenic cucumber expressing TPSP gene and morphological alterations. *J. Plant Biotechnol.* **2010**, *37*, 72–76. [CrossRef]
22. Xin, T.; Tian, H.; Ma, Y.; Wang, S.; Yang, L.; Li, X.; Zhang, M.; Chen, C.; Wang, H.; Li, H.; et al. Targeted creating new mutants with compact plant architecture using CRISPR/Cas9 genome editing by an optimized genetic transformation procedure in cucurbit plants. *Hortic. Res.* **2022**, *9*, uhab086. [CrossRef]
23. Vasudevan, A.; Selvaraj, N.; Ganapathi, A.N.; Choi, C.W. Agrobacterium-mediated Genetic Transformation in Cucumber (*Cucumis sativus* L.). *Am. J. Biochem. Biotechnol.* **2007**, *3*, 24–32. [CrossRef]

24. Selvaraj, N.; Kasthurirengan, S.; Vasudevan, A.; Manickavasagam, M.; Choi, C.W.; Ganapathi, A. Evaluation of green fluorescent protein as a reporter gene and phosphinothricin as the selective agent for achieving a higher recovery of transformants in cucumber (Cucumis sativus L. cv. Poinsett76) via Agrobacterium tumefaciens. *Vitr. Cell. Dev. Biol.-Plant* **2010**, *46*, 329–337. [CrossRef]
25. Wang, Y.; Wang, J.; Shi, B.; Yu, T.; Qi, J.; Meyerowitz, E.M.; Jiao, Y. The Stem Cell Niche in Leaf Axils Is Established by Auxin and Cytokinin in Arabidopsis. *Plant Cell* **2014**, *26*, 2055–2067. [CrossRef] [PubMed]
26. Du, C.; Chai, L.a.; Liu, C.; Si, Y.; Fan, H. Improved Agrobacterium tumefaciens-mediated transformation using antibiotics and acetosyringone selection in cucumber. *Plant Biotechnol. Rep.* **2022**, *16*, 17–27. [CrossRef]
27. García-Almodóvar, R.C.; Gosalvez, B.; Aranda, M.A.; Burgos, L. Production of transgenic diploid Cucumis melo plants. *Plant Cell Tissue Organ Cult. (PCTOC)* **2017**, *130*, 323–333. [CrossRef]
28. Pan, W.; Cheng, Z.; Han, Z.; Yang, H.; Zhang, W.; Zhang, H. Efficient genetic transformation and CRISPR/Cas9-mediated genome editing of watermelon assisted by genes encoding developmental regulators. *J. Zhejiang Univ. Sci. B* **2022**, *23*, 339–344. [CrossRef]
29. Feng, Q.; Xiao, L.; He, Y.; Liu, M.; Wang, J.; Tian, S.; Zhang, X.; Yuan, L. Highly efficient, genotype-independent transformation and gene editing in watermelon (*Citrullus lanatus*) using a chimeric ClGRF4-GIF1 gene. *J. Integr. Plant Biol.* **2021**, *63*, 2038–2042. [CrossRef]
30. Zhao, Z.; Qi, Y.; Yang, Z.; Cheng, L.; Sharif, R.; Raza, A.; Chen, P.; Hou, D.; Li, Y. Exploring the Agrobacterium-mediated transformation with CRISPR/Cas9 in cucumber (*Cucumis sativus* L.). *Mol. Biol. Rep.* **2022**, *49*, 11481–11490. [CrossRef]
31. Sundar, I.K.; Sakthivel, N. Advances in selectable marker genes for plant transformation. *J. Plant Physiol.* **2008**, *165*, 1698–1716. [CrossRef]
32. Zhang, H.J.; Gao, P.; Wang, X.Z.; Luan, F.S. An efficient regeneration protocol for Agrobacterium-mediated transformation of melon (*Cucumis melo* L.). *Genet Mol. Res.* **2014**, *13*, 54–63. [CrossRef] [PubMed]
33. Bezirganoglu, I.; Hwang, S.Y.; Shaw, J.F.; Fang, T.J. Efficient production of transgenic melon via Agrobacterium-mediated transformation. *Genet Mol. Res.* **2014**, *13*, 3218–3227. [CrossRef] [PubMed]
34. Zhang, H.; Luan, F. Overexpression of the CmACS-3 gene in melon causes abnormal pollen development. *Genet Mol. Res.* **2015**, *14*, 10433–10443. [CrossRef]
35. Hooghvorst, I.; López-Cristoffanini, C.; Nogués, S. Efficient knockout of phytoene desaturase gene using CRISPR/Cas9 in melon. *Sci. Rep.* **2019**, *9*, 17077. [CrossRef]
36. Liu, L.; Gu, Q.; Ijaz, R.; Zhang, J.; Ye, Z. Generation of transgenic watermelon resistance to Cucumber mosaic virus facilitated by an effective Agrobacterium-mediated transformation method. *Sci. Hortic.* **2016**, *205*, 32–38. [CrossRef]
37. Tian, S.; Jiang, L.; Gao, Q.; Zhang, J.; Zong, M.; Zhang, H.; Ren, Y.; Guo, S.; Gong, G.; Liu, F.; et al. Efficient CRISPR/Cas9-based gene knockout in watermelon. *Plant Cell Rep.* **2017**, *36*, 399–406. [CrossRef] [PubMed]
38. Tian, S.; Jiang, L.; Cui, X.; Zhang, J.; Guo, S.; Li, M.; Zhang, H.; Ren, Y.; Gong, G.; Zong, M.; et al. Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing. *Plant Cell Rep.* **2018**, *37*, 1353–1356. [CrossRef]
39. Jang, H.-A.; Kim, H.-A.; Kwon, S.-Y.; Choi, D.-W.; Choi, P.-S. The use of cotyledonary-node explants in Agrobacterium tumefaciensmediated transformation of cucumber (*Cucumis sativus* L.). *J. Plant Biotechnol.* **2011**, *38*, 198–202. [CrossRef]
40. Wang, J.; Zhang, S.; Wang, X.; Wang, L.; Xu, H.; Wang, X.; Shi, Q.; Wei, M.; Yang, F. Agrobacterium-mediated transformation of cucumber (*Cucumis sativus* L.) using a sense mitogen-activated protein kinase gene (CsNMAPK). *Plant Cell Tissue Organ Cult. (PCTOC)* **2012**, *113*, 269–277. [CrossRef]
41. Nanasato, Y.; Okuzaki, A.; Tabei, Y. Improving the transformation efficiency of Cucurbita species: Factors and strategy for practical application. *Plant Biotechnol.* **2013**, *30*, 287–294. [CrossRef]
42. Chandrasekaran, J.; Brumin, M.; Wolf, D.; Leibman, D.; Klap, C.; Pearlsman, M.; Sherman, A.; Arazi, T.; Gal-On, A. Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Mol. Plant Pathol.* **2016**, *17*, 1140–1153. [CrossRef]
43. Hu, B.; Li, D.; Liu, X.; Qi, J.; Gao, D.; Zhao, S.; Huang, S.; Sun, J.; Yang, L. Engineering Non-transgenic Gynoecious Cucumber Using an Improved Transformation Protocol and Optimized CRISPR/Cas9 System. *Mol. Plant* **2017**, *10*, 1575–1578. [CrossRef] [PubMed]
44. Wu, H.W.; Yu, T.A.; Raja, J.A.J.; Christopher, S.J.; Wang, S.L.; Yeh, S.D. Double-Virus Resistance of Transgenic Oriental Melon Conferred by Untranslatable Chimeric Construct Carrying Partial Coat Protein Genes of Two Viruses. *Plant Dis.* **2010**, *94*, 1341–1347. [CrossRef]
45. Hao, J.; Niu, Y.; Yang, B.; Gao, F.; Zhang, L.; Wang, J.; Hasi, A. Transformation of a marker-free and vector-free antisense ACC oxidase gene cassette into melon via the pollen-tube pathway. *Biotechnol. Lett.* **2011**, *33*, 55–61. [CrossRef] [PubMed]
46. Chovelon, V.; Restier, V.; Giovinazzo, N.; Dogimont, C.; Aarrouf, J. Histological study of organogenesis in Cucumis melo L. after genetic transformation: Why is it difficult to obtain transgenic plants? *Plant Cell Rep.* **2011**, *30*, 2001–2011. [CrossRef] [PubMed]
47. Shan, W.; Zhao, C.; Fan, J.; Cong, H.; Liang, S.; Yu, X. Antisense suppression of alcohol acetyltransferase gene in ripening melon fruit alters volatile composition. *Sci. Hortic.* **2012**, *139*, 96–101. [CrossRef]
48. Ren, Y.; Bang, H.; Curtis, I.S.; Gould, J.; Patil, B.S.; Crosby, K.M. Agrobacterium-mediated transformation and shoot regeneration in elite breeding lines of western shipper cantaloupe and honeydew melons (*Cucumis melo* L.). *Plant Cell Tissue Organ Cult. (PCTOC)* **2011**, *108*, 147–158. [CrossRef]

49. Choi, J.Y.; Shin, J.S.; Chung, Y.S.; Hyung, N.-I. An efficient selection and regeneration protocol for Agrobacterium-mediated transformation of oriental melon (*Cucumis melo* L. var. makuwa). *Plant Cell Tissue Organ Cult. (PCTOC)* **2012**, *110*, 133–140. [CrossRef]
50. Bezirganoglu, I.; Hwang, S.-Y.; Fang, T.J.; Shaw, J.-F. Transgenic lines of melon (*Cucumis melo* L. var. *makuwa* cv. 'Silver Light') expressing antifungal protein and chitinase genes exhibit enhanced resistance to fungal pathogens. *Plant Cell Tissue Organ Cult. (PCTOC)* **2013**, *112*, 227–237. [CrossRef]
51. Zhang, H.; Gao, P.; Wang, X.; Luan, F. An improved method of Agrobacterium tumefaciens-mediated genetic transformation system of melon (*Cucumis melo* L.). *J. Plant Biochem. Biotechnol.* **2013**, *23*, 278–283. [CrossRef]
52. Zhang, Z.; Li, X.; Ma, S.; Shan, N.; Zhang, X.; Sui, X.; Zhang, Z.; Sui, X. A Protocol for Agrobacterium-mediated Transformation of Cucumber (*Cucumis sativus* L.) from cotyledon explants. *Protoc. Exch.* **2017**. [CrossRef]
53. Mendez-Hernandez, H.A.; Ledezma-Rodriguez, M.; Avilez-Montalvo, R.N.; Juarez-Gomez, Y.L.; Skeete, A.; Avilez-Montalvo, J.; De-la-Pena, C.; Loyola-Vargas, V.M. Signaling Overview of Plant Somatic Embryogenesis. *Front. Plant Sci.* **2019**, *10*, 77. [CrossRef]
54. Zhang, X.; Xu, G.; Cheng, C.; Lei, L.; Sun, J.; Xu, Y.; Deng, C.; Dai, Z.; Yang, Z.; Chen, X.; et al. Establishment of an Agrobacterium-mediated genetic transformation and CRISPR/Cas9-mediated targeted mutagenesis in Hemp (*Cannabis Sativa* L.). *Plant Biotechnol. J.* **2021**, *19*, 1979–1987. [CrossRef] [PubMed]
55. Maren, N.A.; Duan, H.; Da, K.; Yencho, G.C.; Ranney, T.G.; Liu, W. Genotype-independent plant transformation. *Hortic. Res.* **2022**, *9*, uhac047. [CrossRef]
56. Lowe, K.; Wu, E.; Wang, N.; Hoerster, G.; Hastings, C.; Cho, M.J.; Scelonge, C.; Lenderts, B.; Chamberlin, M.; Cushatt, J.; et al. Morphogenic Regulators Baby boom and Wuschel Improve Monocot Transformation. *Plant Cell* **2016**, *28*, 1998–2015. [CrossRef]
57. Maher, M.F.; Nasti, R.A.; Vollbrecht, M.; Starker, C.G.; Clark, M.D.; Voytas, D.F. Plant gene editing through de novo induction of meristems. *Nat. Biotechnol.* **2020**, *38*, 84–89. [CrossRef] [PubMed]
58. Kong, J.; Martin-Ortigosa, S.; Finer, J.; Orchard, N.; Gunadi, A.; Batts, L.A.; Thakare, D.; Rush, B.; Schmitz, O.; Stuiver, M.; et al. Overexpression of the Transcription Factor GROWTH-REGULATING FACTOR5 Improves Transformation of Dicot and Monocot Species. *Front. Plant Sci.* **2020**, *11*, 572319. [CrossRef]
59. Debernardi, J.M.; Tricoli, D.M.; Ercoli, M.F.; Hayta, S.; Ronald, P.; Palatnik, J.F.; Dubcovsky, J. A GRF-GIF chimeric protein improves the regeneration efficiency of transgenic plants. *Nat Biotechnol* **2020**, *38*, 1274–1279. [CrossRef] [PubMed]
60. Miki, B.; McHugh, S. Selectable marker genes in transgenic plants: Applications, alternatives and biosafety. *J. Biotechnol.* **2004**, *107*, 193–232. [CrossRef]
61. He, Z.; Duan, Z.; Liang, W.; Chen, F.; Yao, W.; Liang, H.; Yue, C.; Sun, Z.; Chen, F.; Dai, J. Mannose selection system used for cucumber transformation. *Plant Cell Rep.* **2006**, *25*, 953–958. [CrossRef]
62. Hesami, M.; Tohidfar, M.; Alizadeh, M.; Daneshvar, M.H. Effects of sodium nitroprusside on callus browning of Ficus religiosa: An important medicinal plant. *J. For. Res.* **2020**, *31*, 789–796. [CrossRef]
63. Soda, N.; Verma, L.; Giri, J. CRISPR-Cas9 based plant genome editing: Significance, opportunities and recent advances. *Plant Physiol. Biochem.* **2018**, *131*, 2–11. [CrossRef] [PubMed]
64. Wang, Y.; Wang, J.; Guo, S.; Tian, S.; Zhang, J.; Ren, Y.; Li, M.; Gong, G.; Zhang, H.; Xu, Y. CRISPR/Cas9-mediated mutagenesis of ClBG1 decreased seed size and promoted seed germination in watermelon. *Hortic. Res.* **2021**, *8*, 70. [CrossRef]
65. Wang, J.; Wang, Y.; Zhang, J.; Ren, Y.; Li, M.; Tian, S.; Yu, Y.; Zuo, Y.; Gong, G.; Zhang, H.; et al. The NAC transcription factor ClNAC68 positively regulates sugar content and seed development in watermelon by repressing ClINV and ClGH3.6. *Hortic. Res.* **2021**, *8*, 214. [CrossRef] [PubMed]
66. Zhang, J.; Guo, S.; Ji, G.; Zhao, H.; Sun, H.; Ren, Y.; Tian, S.; Li, M.; Gong, G.; Zhang, H.; et al. A unique chromosome translocation disrupting ClWIP1 leads to gynoecy in watermelon. *Plant J. Cell Mol. Biol.* **2020**, *101*, 265–277. [CrossRef] [PubMed]
67. Ren, Y.; Sun, H.; Zong, M.; Guo, S.; Ren, Z.; Zhao, J.; Li, M.; Zhang, J.; Tian, S.; Wang, J.; et al. Localization shift of a sugar transporter contributes to phloem unloading in sweet watermelons. *New Phytol.* **2020**, *227*, 1858–1871. [CrossRef]
68. Ren, Y.; Li, M.; Guo, S.; Sun, H.; Zhao, J.; Zhang, J.; Liu, G.; He, H.; Tian, S.; Yu, Y.; et al. Evolutionary gain of oligosaccharide hydrolysis and sugar transport enhanced carbohydrate partitioning in sweet watermelon fruits. *Plant Cell* **2021**, *33*, 1554–1573. [CrossRef]
69. Cao, L.; Li, C.; Li, H.; Wang, Z.; Jiang, Y.; Guo, Y.; Sun, P.; Chen, X.; Li, Q.; Tian, H.; et al. Disruption of REC8 in Meiosis I led to watermelon seedless. *Plant Sci.* **2022**, *323*, 111394. [CrossRef] [PubMed]
70. Chang, J.; Guo, Y.; Yan, J.; Zhang, Z.; Yuan, L.; Wei, C.; Zhang, Y.; Ma, J.; Yang, J.; Zhang, X.; et al. The role of watermelon caffeic acid O-methyltransferase (ClCOMT1) in melatonin biosynthesis and abiotic stress tolerance. *Hortic. Res.* **2021**, *8*, 210. [CrossRef] [PubMed]
71. Zhang, R.; Chang, J.; Li, J.; Lan, G.; Xuan, C.; Li, H.; Ma, J.; Zhang, Y.; Yang, J.; Tian, S.; et al. Disruption of the bHLH transcription factor Abnormal Tapetum 1 causes male sterility in watermelon. *Hortic. Res.* **2021**, *8*, 258. [CrossRef] [PubMed]
72. Cheng, Z.; Liu, X.; Yan, S.; Liu, B.; Zhong, Y.; Song, W.; Chen, J.; Wang, Z.; Che, G.; Liu, L.; et al. Pollen tube emergence is mediated by ovary-expressed ALCATRAZ in cucumber. *Nat. Commun.* **2023**, *14*, 258. [CrossRef] [PubMed]
73. Liu, X.; Ning, K.; Che, G.; Yan, S.; Han, L.; Gu, R.; Li, Z.; Weng, Y.; Zhang, X. CsSPL functions as an adaptor between HD-ZIP III and CsWUS transcription factors regulating anther and ovule development in *Cucumis sativus* (cucumber). *Plant J.* **2018**, *94*, 535–547. [CrossRef] [PubMed]

74. Xin, T.; Zhang, Z.; Li, S.; Zhang, S.; Li, Q.; Zhang, Z.H.; Huang, S.; Yang, X. Genetic Regulation of Ethylene Dosage for Cucumber Fruit Elongation. *Plant Cell* **2019**, *31*, 1063–1076. [CrossRef]
75. Zhang, Z.; Wang, B.; Wang, S.; Lin, T.; Yang, L.; Zhao, Z.; Zhang, Z.; Huang, S.; Yang, X. Genome-wide Target Mapping Shows Histone Deacetylase Complex1 Regulates Cell Proliferation in Cucumber Fruit. *Plant Physiol.* **2020**, *182*, 167–184. [CrossRef]
76. Yang, X.; Yan, J.; Zhang, Z.; Lin, T.; Xin, T.; Wang, B.; Wang, S.; Zhao, J.; Zhang, Z.; Lucas, W.J.; et al. Regulation of plant architecture by a new histone acetyltransferase targeting gene bodies. *Nat. Plants* **2020**, *6*, 809–822. [CrossRef]
77. Wang, Z.; Wang, L.; Han, L.; Cheng, Z.; Liu, X.; Wang, S.; Liu, L.; Chen, J.; Song, W.; Zhao, J.; et al. HECATE2 acts with GLABROUS3 and Tu to boost cytokinin biosynthesis and regulate cucumber fruit wart formation. *Plant Physiol.* **2021**, *187*, 1619–1635. [CrossRef]
78. Zhang, H.; Li, S.; Yang, L.; Cai, G.; Chen, H.; Gao, D.; Lin, T.; Cui, Q.; Wang, D.; Li, Z.; et al. Gain-of-function of the 1-aminocyclopropane-1-carboxylate synthase gene ACS1G induces female flower development in cucumber gynoecy. *Plant Cell* **2021**, *33*, 306–321. [CrossRef]
79. Wang, Z.; Zhou, Z.; Wang, L.; Yan, S.; Cheng, Z.; Liu, X.; Han, L.; Chen, G.; Wang, S.; Song, W.; et al. The CsHEC1-CsOVATE module contributes to fruit neck length variation via modulating auxin biosynthesis in cucumber. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2209717119. [CrossRef]
80. Liu, P.; Li, Q.; Wang, H.; Lu, T.; Li, Y.; Sui, X.; Yu, H.; Jiang, W. Ethylene signaling plays an important role in UV-B-induced ascorbic acid accumulation in *Cucumis sativus* leaves. *Authorea* **2022**. [CrossRef]
81. Peng, Y.; Chen, L.; Zhu, L.; Cui, L.; Yang, L.; Wu, H.; Bie, Z. CsAKT1 is a key gene for the CeO2 nanoparticle's improved cucumber salt tolerance: A validation from CRISPR-Cas9 lines. *Environ. Sci. Nano* **2022**, *9*, 4367–4381. [CrossRef]
82. Liu, B.; Santo Domingo, M.; Mayobre, C.; Martín-Hernández, A.M.; Pujol, M.; Garcia-Mas, J. Knock-Out of CmNAC-NOR Affects Melon Climacteric Fruit Ripening. *Front. Plant Sci.* **2022**, *13*, 878037. [CrossRef]
83. Wang, J.; Tian, S.; Yu, Y.; Ren, Y.; Guo, S.; Zhang, J.; Li, M.; Zhang, H.; Gong, G.; Wang, M.; et al. Natural variation in the NAC transcription factor NONRIPENING contributes to melon fruit ripening. *J. Integr. Plant Biol.* **2022**, *64*, 1448–1461. [CrossRef] [PubMed]
84. Pechar, G.S.; Donaire, L.; Gosalvez, B.; García-Almodovar, C.; Sánchez-Pina, M.A.; Truniger, V.; Aranda, M.A. Editing melon eIF4E associates with virus resistance and male sterility. *Plant Biotechnol. J.* **2022**, *20*, 2006–2022. [CrossRef]
85. Sohail, H.; Noor, I.; Nawaz, M.A.; Ma, M.; Shireen, F.; Huang, Y.; Yang, L.; Bie, Z. Genome-wide identification of plasma-membrane intrinsic proteins in pumpkin and functional characterization of CmoPIP1-4 under salinity stress. *Environ. Exp. Bot.* **2022**, *202*. [CrossRef]
86. Geng, S.; Sohail, H.; Cao, H.; Sun, J.; Chen, Z.; Zhou, L.; Wang, W.; Ye, R.; Yang, L.; Bie, Z. An efficient root transformation system for CRISPR/Cas9-based analyses of shoot-root communication in cucurbit crops. *Hortic. Res.* **2022**, *9*. [CrossRef] [PubMed]
87. Garneau, J.E.; Dupuis, M.-È.; Villion, M.; Romero, D.A.; Barrangou, R.; Boyaval, P.; Fremaux, C.; Horvath, P.; Magadán, A.H.; Moineau, S. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* **2010**, *468*, 67–71. [CrossRef] [PubMed]
88. Sorek, R.; Lawrence, C.M.; Wiedenheft, B. CRISPR-mediated adaptive immune systems in bacteria and archaea. *Annu. Rev. Biochem.* **2013**, *82*, 237–266. [CrossRef] [PubMed]
89. Li, J.; Yu, X.; Zhang, C.; Li, N.; Zhao, J. The application of CRISPR/Cas technologies to Brassica crops: Current progress and future perspectives. *Abiotech* **2022**, *3*, 146–161. [CrossRef] [PubMed]
90. Chen, J.; Tomes, S.; Gleave, A.P.; Hall, W.; Luo, Z.; Xu, J.; Yao, J.L. Significant improvement of apple (Malus domestica Borkh.) transgenic plant production by pre-transformation with a Baby boom transcription factor. *Hortic. Res.* **2022**, *9*. [CrossRef]
91. Luo, W.; Tan, J.; Li, T.; Feng, Z.; Ding, Z.; Xie, X.; Chen, Y.; Chen, L.; Liu, Y.G.; Zhu, Q.; et al. Overexpression of maize GOLDEN2 in rice and maize calli improves regeneration by activating chloroplast development. *Sci. China Life Sci.* **2023**, *66*, 340–349. [CrossRef] [PubMed]
92. Hyun, Y.; Kim, J.; Cho, S.W.; Choi, Y.; Kim, J.-S.; Coupland, G. Site-directed mutagenesis in Arabidopsis thaliana using dividing tissue-targeted RGEN of the CRISPR/Cas system to generate heritable null alleles. *Planta* **2015**, *241*, 271–284. [CrossRef]
93. Wang, Z.-P.; Xing, H.-L.; Dong, L.; Zhang, H.-Y.; Han, C.-Y.; Wang, X.-C.; Chen, Q.-J. Egg cell-specific promoter-controlled CRISPR/Cas9 efficiently generates homozygous mutants for multiple target genes in Arabidopsis in a single generation. *Genome Biol.* **2015**, *16*, 144. [CrossRef] [PubMed]
94. Belhaj, K.; Chaparro-Garcia, A.; Kamoun, S.; Nekrasov, V. Plant genome editing made easy: Targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods* **2013**, *9*, 39. [CrossRef]
95. Abbott, A. Europe's genetically edited plants stuck in legal limbo. *Nature* **2015**, *528*, 319–320. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.