Transgenic Improvement for Biotic Resistance of Crops

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Abstract: Biotic constraints, including pathogenic fungi, viruses and bacteria, herbivory insects, as well as parasitic nematodes, cause significant yield loss and quality deterioration of crops. The effect of conventional management of these biotic constraints is limited. The advances in transgenic technologies provide a direct and directional approach to improve crops for biotic resistance. More than a hundred transgenic events and hundreds of cultivars resistant to herbivory insects, pathogenic viruses, and fungi have been developed by the heterologous expression of exogenous genes and RNAi, authorized for cultivation and market, and resulted in a significant reduction in yield loss and quality deterioration. However, the exploration of transgenic improvement for resistance to bacteria and nematodes by overexpression of endogenous genes and RNAi remains at the testing stage. Recent advances in RNAi and CRISPR/Cas technologies open up possibilities to improve the resistance of crops to pathogenic bacteria and plant parasitic nematodes, as well as other biotic constraints.

Keywords: biotic; crop; improvement; transgene

1. Transgenic Improvement Is Effective to Control Biotic Constraints

1.1. Biotic Constraints to Crops

As sessile organisms, crop plants have to cope with other living organisms in their environment. Except for a few, such as rhizobia, which are beneficial to their hosts, most of the others cause some damage to crop plants such as pathogenic fungi, viruses, bacteria, herbivory insects, as well as parasitic nematodes. They are classified as biotic constraints to crop plants [1–5]. Pathogenic fungi and bacteria are mostly intracellular pathogens. They invade different organs of the plant during the interaction with the host, live inside and kill the host cells using toxic enzymes or compounds such as necrotrophs or initially feed on plants parasitically as biotrophs or hemi-biotrophs, leading to vascular wilts, leaf spots, and cankers, among other symptoms [4,5]. Pathogenic viruses produce not only local lesions but also systemic damage that causes stunting, chlorosis, and malformations, although they rarely kill their hosts. They have a very efficient system of dissemination through vector transmission by insects, arthropods, and nematodes [6]. Herbivory insects, as well as mites, damage plants through feeding or egg-laying. Piercing–sucking insects can also act as virus vectors, transmitting them to plants through their styles [1,7]. Plant-parasitic nematodes attack the root system, withdraw the nutrients of plant cells, and alter plant development, physiology, and immunity, resulting in leaf necrosis and chlorosis, plant wilting, and stunting, and enhanced susceptibility to other pathogens [8–10]. Several economically important genera parasitize various crop plants. The root-knot, root lesion, and cyst nematodes of Heteroderidae are the three most economically damaging genera of plant-parasitic nematodes and lead to significant yield loss [10]. In order to deal with these biotic stresses, plants have evolved an advanced immune system, such as physical barriers (waxes, thick cuticles, and specialized trichomes) to prevent pathogen settling and herbivory feeding, chemical compounds to protect against herbivory and pathogen infection, and immune responses that activate plant defense mechanisms in a much more effective way [11–15]. However, these mechanisms cannot completely resist pathogen and herbivory damage, which still affect crop development and lead to a yield loss of 17–30%
(Figure 1) and significant quality deterioration, and are irrefutably the most serious threat to worldwide food security [1,7,16–19]. In the *Brassica* crops, which can be invaded and fed by numerous pathogens and herbivory insects, the yield loss is estimated as high as 50–60% [20–22].

**Figure 1.** Global yield loss to pathogens and pests on major food crops.

### 1.2. Conventional Management

To meet the food demand of the booming world population, sustainable agriculture makes great efforts to reduce the yield loss and quality deterioration of crops without compromising the biotic constraints [1,23]. In agricultural practice, the conventional approaches are traditional breeding for resistance and application of agrochemicals (fungicides, bactericides, nematicides, and insecticides), assisted by comprehensive field management and biological control [24]. The application of resistant cultivars with broad-spectrum and durable resistance is the most cost-effective, environmentally friendly, and less labor-intensive [25–28]. However, traditional breeding heavily depends on germplasm resources. It is effective for the improvement of vertical resistance to specific parasitic pathotypes of biotrophic pathogens, such as rust fungi *Puccinia striiformis*, *P. triticina*, and *P. graminis* (pathogens of stripe, leaf, and stem rust of wheat, respectively) and *Phytophthora infestans* (pathogen of potato late-blight) [29–32]. This kind of resistance is complete but can be easily evaded due to the continuous accumulating mutations of pathogens, leading to the breakdown of resistant varieties after their commercial utilization [33–36]. For improvement of horizontal resistance to non-specific parasitic pathotypes of saprophytic pathogens, such as *Bhizoctonia solani* and *Hyaloperonospora parasitica* (pathogens of maize sheath blight and rape downy mildew, respectively), as well as pathogenic viruses and phytophagous insects, it is a great challenge to pyramid the multiple dominant resistant genes into a single cultivar for developing broad-spectrum and durable resistance [37–42]. This challenge occurs even with the advances in distant hybridization, artificial mutation, double haploid technology, somatic hybrid, and DNA marker-assisted genotyping and selection [38,39,43–49], due
to the limitation of germplasm resources, the genetic background of minor polycyenes, and negative correlation with yield and quality characteristics [50–53]. The application of agrochemicals is effective for the control of herbivory insects, but increases production costs and causes environmental pollution [54–57]. For the control of pathogenic fungi and bacteria, especially plant parasitic nematodes, conventional management is much more difficult. Comprehensive field management procedures, such as field sanitation, soil sterilization, crop rotation and fallowing, rouging, and the manual removal of infected plants, are only applied as assistive approaches, because of their labor input and limits in disease and insect eradication [16,58,59]. Biological control may not be compatible with the crop production system or the regulatory environment and remains at the testing stage, except for a few successful stories [60,61].

1.3. Transgenic Improvement

Transgenic technology overcomes the hybridization barriers, utilizes a broader and more diverse range of desirable genes from genetically distant species, and provides a direct and directional approach to improve crops for biotic resistance in a careful target manner with minimal effect on beneficial soil microbes and environment [62–64]. It is praised as a second Green Revolution, hopeful for improving biotic resistance and other agronomical characteristics of crops and making a great contribution to food security [65,66]. Transgenic cultivars of crops are generated by the cloning of desirable genes, construction of expression vectors, genetic transformation of recipient cultivars, and identification of transgenic lines, so as to improve the original undesirable traits or endow them with new beneficial traits [65–67]. Transgenic technology is also used to modify or knock out some suboptimal genes to change their undesirable phenotypes [68–71]. After a strict assessment for food and environmental safety, transgenic cultivars with significant improvement in biotic resistance and other agronomical characteristics are authorized for cultivation and market [72]. According to the survey carried out by the International Service for the Acquisition of Agri-Biotech Applications (ISAAA, http://www.isaaa.org/gmapprovdatabase/default.asp, accessed on 22 September 2022), 292 transgenic events and hundreds of varieties and hybrids between them of 32 cereals, vegetables, fruits, fibers, oils, trees, and forage crops have been authorized for cultivation and market, cultivated for 176.85 million hectares in more than thirty countries, and providing great profitability by increasing yield and reducing input in pesticides, labor, and machinery. They are harmless to humans, environmentally friendly, and favored by farmers worldwide [62,73,74]. A meta-analysis shows that the application of herbicide-resistant and insecticidal transgenic crops reduces the use of synthetic herbicide and pesticides by 2.43% and 41.67%, and their cost by 25.29% and 43.43%, increases the yield of crops by 9.29% and 24.85%, and benefits farmers by 64.29% and 68.78%, respectively (Figure 2) [75].

![Figure 2. Benefits of herbicide-resistant (a) and insecticidal (b) transgenic crops.](image-url)
2. Commercial Release for Transgenic Resistance to Herbivory Insects, and Pathogenic Viruses, and Fungi

2.1. Resistance to Herbivory Insects

One of the most successful transgenic improvements for biotic resistance is the wide application of transgenic events resistant to herbivory insects, which used to be the major biotic constraint that caused a serious reduction in crop productivity and posed a great threat to food security in the world [7]. According to the survey carried out by ISAAA (http://www.isaaa.org/gmapprovaldatabase/default.asp, accessed on 22 September 2022), 93 single transgenic events (not included hybrids between them. The same below.) resistant to herbivory insects of lepidopteran, coleopteran, as well as hemipteran orders, have been authorized for cultivation and market. From them, hundreds of cotton, cowpea, eggplant, maize, potato, rice, soybean, sugarcane, and tomato varieties and hybrids have been developed and authorized for cultivation and market. Their application has reduced pesticide usage and increased yield and the benefits of crop production [73–79]. Ninety of these ninety-three transgenic events were transformed by the heterologous expression of one or more (for pyramiding resistance) of the insecticidal genes \textit{Cry} (δ-endotoxin) from different strains of soil bacterium \textit{Bacillus thuringiensis} [80], and one was transformed by the heterologous expression of the vegetative insecticidal protein gene \textit{vip3} also from \textit{B. thuringiensis}. Ten were simultaneously transformed by orthologs of the \textit{Cry} gene from the other strains of \textit{B. thuringiensis} or for pyramiding resistance. Three were simultaneously transformed by vegetative insecticidal protein genes \textit{vip3}, \textit{CpTI}, and \textit{API}, as well as double-stranded RNA transcript of gene \textit{Snf7} from Western corn rootworm (\textit{Diabrotica virgifera}), respectively, for pyramiding resistance (Table 1) [81–84].

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Crop & Gene Introduced & Doner Species & Target Insect & Released Event \\
\hline
Cotton & \textit{Cry} & \textit{B. thuringiensis} & 23 (1) & 1 & 25 \\
& \textit{vip3} & \textit{B. thuringiensis} & 1 & \\
& \textit{CpTI} & \textit{V. unguiculata} & (1) & \\
\hline
Cowpea & \textit{Cry} & \textit{B. thuringiensis} & 1 & 1 & \\
\hline
Eggplant & \textit{Cry} & \textit{B. thuringiensis} & 1 & 1 & \\
\hline
Maize & \textit{Cry} & \textit{B. thuringiensis} & 15 (2) & 8 (3) & 25 \\
& \textit{vip3} & \textit{B. thuringiensis} & 2 (1) & \\
& RNAi of \textit{Snf7} & \textit{D. virgifera} & (1) & \\
\hline
Potato & \textit{Cry} & \textit{B. thuringiensis} & 30 & 30 & \\
\hline
Rice & \textit{Cry} & \textit{B. thuringiensis} & 3 (2) & 3 & \\
\hline
Soybean & \textit{Cry} & \textit{B. thuringiensis} & 4 (2) & 1 & 4 \\
\hline
Sugarcane & \textit{Cry} & \textit{B. thuringiensis} & 3 & 3 & \\
\hline
Tomato & \textit{Cry} & \textit{B. thuringiensis} & 1 & 1 & \\
\hline
\hline
Total & & & 54 & 38 & 1 & 93 \\
\hline
\end{tabular}
\caption{Number of transgenic events of insect resistance authorized for cultivation and market.}
\end{table}

Note: The brackets indicate the numbers of the genes introduced for pyramiding.

2.2. Resistance to Pathogenic Viruses

In the early years, the transgenic improvement for resistance to pathogenic viruses was focused on pathogen-derived resistance (PDR), whereby a sequence or part of the viral genome was introduced into the host plant [85–89]. After its discovery in the model non-parasitic nematode \textit{Caenorhabditis elegans} and demonstration in transgenic improvement of vial resistance [90,91], RNA interference (RNAi) technology has rapidly emerged as the most effective strategy of transgenic improvement for resistance to plant viruses [91]. Synthetic DNA sequences expressing double-stranded RNAs (dsRNAs)
for interference of the housekeeping genes of pathogenic viruses, such as the genes of the coat protein (CP) [92–94], the replication protein (Rep) [95–97], as well as the movement protein (MP) [98,99]. According to the survey carried out by ISAAA (http://www.isaaa.org/gmapprovaldatabase/default.asp, accessed on 22 September 2022), twenty-five transgenic events of bean, papaya, plum, potato, squash, sweet pepper, and tomato resistant to pathogenic viruses were generated and authorized for cultivation and market (Table 2). All of them were transformed by double-stranded sequences transcribing the coat protein, replicase, or helicase of the pathogenic viruses themselves for RNAi [94,100–102]. In recent years, clustered regularly interspaced short palindromic repeats-cas (CRISPR/Cas) technology and its new advances attracted much attention in transgenic improvement for rival resistance [103–106]. Transgenic potato lines with broad-spectrum resistance were developed by introduction of a multiple gRNA cassette of CRISPR/Cas13 system to target six genes of three potato virus strains [106].

Table 2. Number of RNAi transgenic events of viral resistance authorized for cultivation and market.

| Crop     | Gene Introduced                  | Target Virus                  | Released Event |
|----------|----------------------------------|-------------------------------|----------------|
| Bean     | Replicase gene bgmv_ac1          | Bean golden mosaic virus      | 1              |
| Papaya   | Coat protein gene prsv_cp        | Papaya ringspot virus         | 3              |
|          | Replicase gene prsv_rep          |                               | 1              |
| Plum     | Coat protein gene ppv_cp         | Plum pox virus                | 1              |
| Potato   | Coat protein gene pvy_cp         | Potato virus Y                | 8              |
|          | Replicase gene plrv_orf1         | Potato leaf roll virus        | 7              |
|          | Helicase gene plrv_orf2          |                               | (7)            |
| Squash   | Coat protein gene zymv_cp        | Zucchini yellow mosaic virus   | 2              |
|          | Coat protein gene cmv_cp         | Cucumber mosaic virus          | 1              |
| Tomato   | Coat protein gene cmv_cp         | Cucumber mosaic virus          | 1              |
| Total    |                                  |                               | 25             |

Note: The parentheses and square brackets indicate the numbers of the genes introduced for resistance pyramiding or broadening, respectively.

2.3. Resistance to Pathogenic Fungi

A lot of attempts have been made to battle against a wide range of pathogenic fungi by transgenic improvement [107–115]. However, most these attempts were generated by the overexpression of endogenous genes resistant to pathogenic fungi [109,116,117]. Unfortunately, their resistance improvement was usually non-significant, highly specific against a few pathogens, and even showed reduced yield or growth vigor, due to the complex mechanisms and signaling networks of disease resistance [107,109,118]. Almost all of them remain at testing stage and few commercial applications have yet been achieved [107,119]. Much effort has been also paid to the heterologous expression of exogenous resistant genes. The immune receptor gene AtRLP23 was introduced into potato and resulted in smaller lesions and reduced pathogen colonization when infected with Phytophthora infestans or Sclerotinia sclerotiorum [120]. The heterologous expression of the immune receptor genes SIVc1 from tomato and SmELR from wild potato also enhanced resistance to Verticillium wilt in tobacco and cotton, and Phytophthora infestans in potato, respectively [121,122]. According to the survey carried out by ISAAA (http://www.isaaa.org/gmapprovaldatabase/default.asp, accessed on 22 September 2022), only four transgenic events for the resistance improvement for pathogenic fungi have been authorized for cultivation and market for their significant improvement of resistance to the potato late-blight pathogen (Phytophthora infestans). One (event SP951) was transformed by the heterologous expression of exogenous resistant genes RB from distant species Solanum bulbocastanum of the nightshade family. The resistance
of the other three (W8, X17, and Y9) were conferred by the heterologous expression of exogenous gene Rpi-vnt from distant species Solanum venturi of the nightshade family, and pyramided by RNAi of endogenous genes Asn1, Ppo5, Phl, R1, and Vinv related to pathogenesis [123]. In recent times, a more efficient strategy was developed to combine multiple resistance genes in an expression cassette. These combined genes not only increased the resistance spectrum to fungal rust pathogen in the transgenic wheat lines, but also inherited a single genetic locus without segregation [124].

3. Exploration for Transgenic Resistance to Bacteria and Nematodes

3.1. Resistance to Pathogenic Bacteria

A lot of efforts have also been devoted to the creation of transgenic resistance to bacterial diseases [107,125,126]. In fact, antibacterial proteins, widely present in animals, plants, and bacteriophages, have a bactericidal action on a large range of Gram-negative and -positive bacteria. Their encoding genes have been cloned from genetic distant species and heterogeneously expressed in crops in attempts to confer resistance to bacterial diseases [125]. For example, the genes of cecropins (lytic peptides) were cloned from the giant silk moth and expressed in potato and tobacco plants. The transgenic lines showed delayed symptoms and reduced mortality following inoculation with Ralstonia solanacearum and Pseudomonas solanacearum, and Pseudomonas syringae (pathogen of bacterial wilt), respectively [127,128]. The heterogenous expression of the genes of lysozymes, lactoferrin, chitinase, the effectors of salicylic acid and jasmonic acid signaling, as well as other antibacterial peptide or phytoalexins genes, and silencing the expression of toxins, pectic enzymes, and exopolysaccharides genes of pathogenic bacteria have also been tried in the transgenic improvement of crops for bacterial resistance [129–135]. For example, the heterologous expression of the EF-Tu receptor gene AtEFR of Arabidopsis in tomato, potato, tobacco, wheat, and rice enhanced resistance to different pathogenic bacteria [136–142]. However, the safety assessment for the efficacy, durability, absence of toxicity, and low environmental impact of these transgenic events are much more difficult than other transgenes. None of them have been authorized for cultivation and market [125].

3.2. Resistance to Parasitic Nematodes

Although overexpression of endogenous and exogenous resistance genes, such as the Mi from tomato for resistance against Meloidogyne incognita, the pro-1 from sugar beet against Heterodera schachtii, the Gpa-2 from potato against Globodera allida, and the Hero from tomato against Globodera rostochiensis, as well as the genes of cowpea trypsin inhibitor, cystatins, and serine proteases, showed some enhancement to the resistance of the transgenic lines [143]; the most effective strategy of transgenic improvement for resistance to parasitic nematodes is RNAi, which was first discovered in the model non-parasitic nematode C. elegans and also demonstrated in plant-parasitic nematodes [144–146]. Crop plants are transformed by RNAi constructs to express dsRNA molecules with sequences derived from the target genes related to parasitism and the development of nematodes, such as dual oxidase, splicing factor, integrase, cathepsin L cysteine proteinase, proteasome integrity-related protein, signal peptidase, tyrosine phosphatase, neuropeptide, as well as aspartic, serine, cysteine proteases, and effector in M. incognita [147–156], Pratylenchus vulnus [157], and Meloidogyne chitwoodi [158], and protease and cyst formation- and reproduction-associated proteins, as well as three genes associated with reproduction to fitness in Heterodera glycines [159,160]. Among them, the effector genes should be a worthwhile group of target genes for in planta RNAi strategy as effectors generally lack high homology with the genes of organisms from other taxa, thereby diminishing the potential for problems related to off-target effects [161].

4. Strategy Option of Transgenic Improvement

4.1. Three Strategies of Transgenic Manipulation

The heterologous expression of exogenous genes from genetically distant species, overexpression of endogenous genes from the same species or homologous genes from
sexually compatible species, and silencing of the expression of suboptimal endogenous, pathogenic, or pest genes by RNAi or CRISPR/Cas are three strategies of transgenic improvement. The first strategy is the transformation by exogenous genes from genetically distant species. The second strategy is the transformation of endogenous genes from the same species or homologous genes from sexually compatible species. The third strategy is silencing the expression of undesirable endogenous or pathogenic and pest genes by RNAi) or CRISPR/Cas technology [162].

4.2. Heterologous Expression of Exogenous Genes

The exogenous transgenes were promoted by the constitutive promoters and the possible codon usage bias was usually overcome by codon optimization of the transgene sequences [163,164]. Therefore, their heterologous expression was usually not regulated on the transcriptional level, although the expression levels might be affected by genetic background and growth stage of the recipient cultivars, as well as environmental conditions [77,165–167]. The investigations in transgenic insecticidal cotton (r = 0.762, \( p < 0.001 \)) and rice (r = 0.742, \( p < 0.01 \)) show that the accumulation of the Cry protein in leaves is non-linearly correlated with the heterologous transcription levels of the exogenous Cry genes, although varying with growth and development [168–171]. Therefore, the novel functions conferred by the exogenous transgenes, such as resistance to herbivory insects [74,76–80], usually performed in pathways divergent from the endogenous metabolism of the recipient crops [172–174]. This strategy should be hopeful for transgenic improvement for broad-spectrum resistance to pathogenic fungi with the detailed elucidation of the mechanism of the hypersensitive response mediated by the non-host resistant or avirulence genes [175–178].

4.3. Overexpression of Endogenous Genes

Biochemical reactions are reversible and regulated in complex networks [80,179]. The activity of any step enhanced by overexpression of the gene encoding the enzyme may not desirably result in increased flux through the reaction that it catalyzes [77,180]. Moreover, transformation may generate completely new interactions between the transgenes, making them function differently from what is expected. According to the survey carried out by ISAAA (http://www.isaaa.org/gmapprovaldatabase/default.asp, accessed on 22 September 2022), 93 of the 118 singular transgenic events of biotic resistance authorized for cultivation and market were created by the first strategy and transformed by bacterial insect-resistant genes [77,179–181], except one exogenous gene from a sexually incompatible plant species was introduced into one event for pyramiding herbicide resistance [84]. The other 25 events were created by the third strategy and transformed by synthetic sequences transcribing antisense or double-stranded RNAs for silencing the expression of housekeeping genes of pathogenic viruses [92–94,97,100–102,182–185]. Of course, antibiotic or herbicide-resistant genes from bacteria were also introduced into almost all of these events as selection markers of transformant screening [186]. Although a lot of the literature has documented the improvement of biotic resistance by overexpression of endogenous genes or homologous genes [187–192], all of them remain at the testing stage and none of them have been released commercially through safety evaluation. It is questionable whether this strategy will ever be widely used in transgenic improvement for biotic constraints [107].

4.4. RNA Interference

RNAi triggered by antisense or double-stranded RNAs described by transformed synthetic DNA sequences is a versatile, effective, safe, and eco-friendly technology for crop protection against viruses and other pathogens as well as herbivory insect and nematodes [71,193–195]. Because of the advantages, as it does not produce any functional foreign proteins and targets organisms in a sequence-specific manner, host-delivered RNAi has become a powerful tool of transgenic improvement for biotic constraints [71,193–196].
Up to now, 25 transgenic events of resistant to pathogenic viruses have been authorized for cultivation and market (Table 2) [93,100–102]. In particular, the transgenic papaya cultivars resistant to ringspot virus, that is a devastating disease, have rescued the entire production of papaya [182]. The creation of a topical application of dsRNA using layered double-hydroxide clay nanosheets opens up possibilities to exploit such innovations for specific and combinatorial resistance against herbivory insects and plant parasitic nematodes [197]. However, off-target effects can originate due to sequence similarity between dsRNA and non-target mRNA transcripts resulting in an unintended silence of non-target genes and an unexpected phenotype [198–200]. This is an important consideration for biosafety assessment of transgenic events of host-generating RNAi. Therefore, proper designing of the dsRNA sequence to preventing the off-target effects, as well as probable non-target effects, is crucial for the wider employment of RNAi. The advancement of genome sequence data, functional genomics availability, and new bioinformatic tools have become helpful for the precise design of dsRNA sequence [201]. RNAi strategy is also hopeful for transgenic improvement for resistance to pathogenic fungi and bacteria with the detailed elucidation of the crucial factors of pathogenicity [202–204].

4.5. Gene Edition

After zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN), CRISPR/Cas technology emerged as a precise, simple, and cost-effective tool of RNA-guided genome editing and is now becoming widely applied to edit the genomes of a diverse range of crop plants [205–208]. Without incorporating any foreign DNA, it is even proposed as non-transgenic genetic modification fostering public acceptance [209,210]. At the beginning, most studies focused more on the concept proofing of the CRISPR/Cas system [211–216]. In recent years, the probable off-target effects caused by the imperfect matches with gRNA and the unpredictable efficiency among different DNA target sites and PAM were effectively reduced with the advances in high attractive sgRNA, high fidelity Cas 9, and transformant screening [206,217]. This system has also been applied to improve crops for biotic resistance, such as overexpressing hypersensitivity-responsive genes of host crops, suppressing effector genes of pathogens and susceptibility genes of host crops, such as virus resistance [112,218–233]. For example, bacterial blight disease, caused by Xanthomonas oryzae pv. oryzae, is a major disease that affects rice production. The expression of sucrose-transporter genes SWEET1, SWEET3, and SWEET14 causes disease susceptibility. CRISPR/Cas-editing inhibited the activation of the susceptibility gene (S) of X. oryzae and prevented its encoding for transcription activator-like effectors (TALES). The SWEET genes could not be activated by the binding of the TALES to their effector binding elements (EBEs) during their promoters and prevented the establishment of host susceptibility [234,235]. In recent years, more and more biotic resistance has been included in the ranks of CRISPR/Cas-mediated improvements (Table 3) [104,208,234–242]. However, only a very few events have been in the pipeline of safety assessment up to now [222,224]. Several events have successfully skipped the regulation of government and entered the market because of their safety assurance, and some more events have been in the pipeline of safety assessment [227,228,243]. Recently, CRISPR/Cas13 was characterized as an RNA-guided RNA editing system with high cleavage activities to foreign RNAs [244]. This system can precisely target plant RNA viruses to impart resistance, and even can be multiplexed by designing a streamlined multiple gRNA cassette [105,245]. By this strategy, three transgenic potato lines with broad-spectrum resistance were developed by the introduction of a multiple gRNA cassette of CRISPR/Cas13 system to target six genes of three potato virus strains [106].
Table 3. CRISPR-Cas edited improvement of crops for disease resistance.

| Crop   | Gene Modified          | Resistance Improved                  | Reference |
|--------|------------------------|--------------------------------------|-----------|
| Citrus | CsLOB1                  | Citrus canker                        | [236]     |
| Cassava| nCBP-1, nCBP-2          | Cassava brown streak disease         | [237]     |
| Cucumber| eIF4E                  | Cucumber vein yellowing virus        | [238]     |
| Grape  | VvWRKY52, Bsr-k1, OsSWEET11, 13, 14 | Botrytis cinerea, Broad spectrum resistance, Bacterial blight | [239], [234], [235] |
| Tomato | Pmr4, Jaz2              | Powdery mildew, Bacterial speck disease | [240], [241] |
| Wheat  | TaMlo-A1, -B1, -D1, TaEdr1 (three homologs) | Powdery mildew | [220], [242] |

5. Conclusions

Transgenic manipulation is a direct and directional approach to improve crops for biotic resistance. The heterologous expression of exogenous genes from genetically distant species, as well as silencing the expression by RNAi and CRISPR/Cas is more effective than overexpression of endogenous genes. Recent advances in RNAi and CRISPR/Cas technologies open up possibilities to improve the resistance of crops to pathogenic bacteria and plant parasitic nematodes, as well as the other biotic constraints, and enable the preclusion of public concerns and engendering of public acceptance.

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