

## Review

## Applications of CRISPR/Cas to Improve Crop Disease Resistance: Beyond Inactivation of Susceptibility Factors

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## SUMMARY

Current crop production systems are prone to increasing pathogen pressure. Fundamental understanding of molecular plant-pathogen interactions, the availability of crop and pathogen genomic information, as well as emerging genome editing permits a novel approach for breeding of crop disease resistance. We describe here strategies to identify new targets for resistance breeding with focus on interruption of the compatible plant-pathogen interaction by CRISPR/Cas-mediated genome editing. Basically, crop genome editing can be applied in several ways to achieve this goal. The most common approach focuses on the "simple" knockout by non-homologous end joining repair of plant susceptibility factors required for efficient host colonization. However, genome re-writing via homology-directed repair or base editing can also prevent host manipulation by changing the targets of pathogen-derived effectors or molecules beyond recognition, which also decreases plant susceptibility. We conclude that genome editing by CRISPR/Cas will become increasingly indispensable to generate in relatively short time beneficial resistance traits in crops to meet upcoming challenges.

## INTRODUCTION

Biotic stress is besides drought and flooding a major challenge for stable crop yield. Considering only the five main staple crops, Savary et al. (2019) reported for the period from 2010 to 2014 that the global yield losses due to pests reached –21.5% for wheat, –30.0% for rice, –22.5% for maize, –17.2% for potato, and –21.4% for soybean. Furthermore, it has been found that the highest losses were observed in food-deficit regions with fast-growing populations (Savary et al., 2019). Pesticides have been used in the past to efficiently control diseases, but their negative effect on biodiversity requires a drastic reduction. Plants, which are naturally resistant to pathogens, can contribute to pesticide reduction and yield protection. However, constitutively activated pathogen defenses often cause a growth trade-off resulting in reduced growth or even plant dwarfism (Gao et al., 2020; van Butselaar and van den Ackerveken, 2020). Thus, alternative resistance types are welcome, which balance resistance with growth and yield. During co-evolution pathogens adapted to their host and generated specific effectors to manipulate the plants' defense reaction. Effector research and target identification allows not only to develop new biotechnological tools (Doron et al., 2018) but also to disturb this interaction by target mutation to prevent host manipulation and subsequently increase resistance. A recent example is the tomato receptor kinase TARK1, originally identified as a *Xanthomonas* effector (XopN) target, whose loss of function prevented *Pseudomonas syringae* pv tomato DC3000-induced stomatal reopening (Guzman et al., 2020). Genes up-regulated during the plant-pathogen interaction and encoding factors that facilitate host colonization by the pathogen are called susceptibility or compatibility factors, and their loss often results in increased resistance (van Schie and Takken, 2014; Langner et al., 2018). For example, since several years a durable barley resistance to powdery mildew was achieved by a mutation in MLO, which is considered to be a susceptibility factor allowing basic compatibility in several plants (Kusch and Panstruga, 2017). However, loss of MLO also causes a trade-off regarding growth and yield, because it is a negative regulator of plant defense (Pavan et al., 2010; Brown and Rant, 2013). Other susceptibility factors encode immunity suppressors (e.g., cellulose synthases, CESAs) or allow sustained compatibility, e.g., some SWEET sugar transporters (van Schie and Takken, 2014). Knockout (KO) of such susceptibility factors is generally believed to increase the so-called recessive resistance, but could also have negative side effects, which have to be determined. Recessive inheritance also means that a

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complete natural KO is hard to detect in nature because many crops have complex genomes with several alleles responsible for functional redundancy (Zhu et al., 2016). Despite this obstacle most approaches aim to KO such susceptibility factors, because now it is rather easy to accomplish. Here we will show that this is only one option to increase resistance and that genome editing (GE) is a much more flexible tool to boost resistance breeding in several other ways than a “simple” KO of susceptibility genes. Great potential has the recently emerging concept of cross-kingdom RNAi (Wang et al., 2016b), as fungal pathogens deploy small non-coding RNAs (sRNAs) to silence host defense-related genes (Weiberg et al., 2013). Altering the host target site of these fungal sRNAs by GE will be very helpful to prevent compatible interactions. On the other hand, some plants can deploy themselves microRNAs (miRNAs) encoding sRNAs to silence fungal virulence genes (Cai et al., 2018) or viruses (Liu et al., 2017a; Satish et al., 2019). The importance of this interaction is further corroborated by findings that virus-encoded inhibitors can directly interfere with host RNAi (Muhammad et al., 2019) and that oomycete pathogens deploy effectors suppressing cross-kingdom RNAi to promote disease susceptibility (Hou et al., 2019).

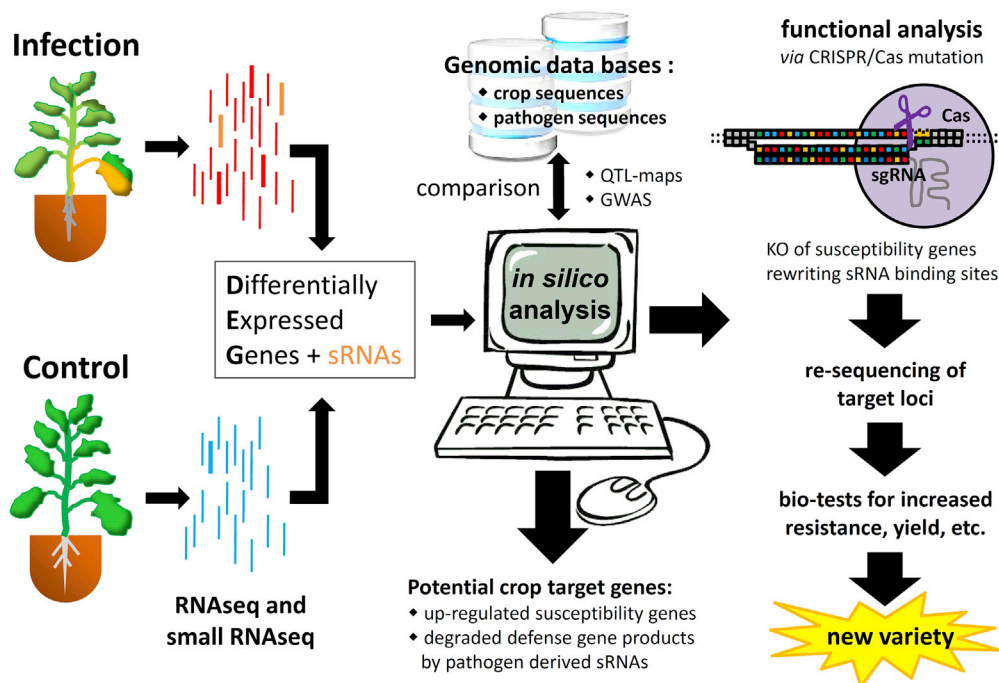
Thus, RNAi can be used to target eukaryotic or viral pests with artificial miRNA genes designed to silence indispensable pathogen-specific genes. This biotechnical adoption is termed host-induced gene silencing (HIGS) and involves miRNAs proven or predicted to fend off viral, fungal, insect, or nematode pathogens (Younis et al., 2014; Huang et al., 2019; Iqbal et al., 2016) and will be discussed later in more detail.

Breeding as an ancient process to select favorable agronomic traits depends originally on natural variation caused by mutation, the force that drives evolution. Usually a mutation will only manifest within a population when providing an advantage and will otherwise get lost. In case of recessive traits all target gene alleles are rarely mutated at once in crops with complex genomes, and even if so, the identification of such mutations within a population is laborious, time consuming, and often limited by the population size. Random mutagenesis can increase genetic variation and facilitates screening of artificially mutated populations for a desired trait by TILLING (Targeting Induced Local Lesions IN Genomes; Till et al., 2006). However, naturally, such undirected mutagenesis can cause many unwanted off-target effects, resulting, for example, in decreased yield. With GE based on sequence-specific nucleases (SSNs) it is now possible to introduce specific mutations at target loci of interest, which can be then easily analyzed and tested for improved resistance, e.g., toward biotic stress. Furthermore, it is now also possible to introduce a foreign gene responsible for a resistance phenotype at a defined location within the genome, which can greatly facilitate efficient expression and avoid negative side effects, caused, e.g., by integration into a different gene. With the new GE techniques, it should be feasible to breed pathogen-resistant crops in relatively short time, which could be either transgene-free or transgenic (Borel, 2017). This mini-review focuses on the applications of clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) as the most popular GE technique to improve crop resistance to pathogens. The steadily growing knowledge about the molecular mechanisms underlying plant-pathogen interactions allows creating many hypotheses concerning potential breeding targets and test if their manipulation leads to improved resistance.

## PIPELINE FOR TARGET GENE IDENTIFICATION IN RESISTANCE BREEDING

One major bottleneck in engineering resistance is the identification of suitable target genes, which can be altered without negatively affecting the overall crop performance. Potential target genes can be identified in several ways, e.g., by text mining on effector research, and also by “*de novo*” functional genomics, an approach that is applicable to any plant-pathogen system of interest. Nowadays, next-generation sequencing (NGS) facilitates not only the cost-efficient identification of differentially expressed genes in the plant-pathogen interaction but also of sRNAs, which could be derived from either host or pest. The correct assignment depends on an *in silico* analysis by comparing the transcriptome with the genome sequences of both host and pathogen (as prerequisite for functional genomics). Up-regulated host genes potentially encode, e.g., for susceptibility factors induced by the pathogen or defense-related genes. Small RNAs derived from the pathogen and matching host defense-related genes or derived from the host-to-target-pathogen virulence factors are indicative of cross-kingdom RNAi, which we will discuss in more detail below. Both candidate target types can be exploited by genome engineering to prevent host manipulation by the pathogen. If loss of a susceptibility factor has no negative impact on overall crop performance, a simple KO by GE-mediated mutagenesis will already do fine. The change of an sRNA-binding site by GE is technically more complicated, because it requires an additional repair template encoding the same amino acid sequence, but with a different codon usage as described in the section

# Strategy for target identification



**Figure 1. “De novo” Pipeline for Target Identification to Engineer Biotic Stress Resistance**  
For details see text.

**Potential of CRISPR/Cas-Mediated Genome Editing.** Furthermore, comparing transcriptomic data with existing maps of quantitative trait loci or phenotypes identified by genome-wide association studies can narrow down the list of potentially interesting candidate genes (Wang and Qin, 2017; Alqudah et al., 2019). An outline of the pipeline for *de novo* target identification and verification is presented in Figure 1.

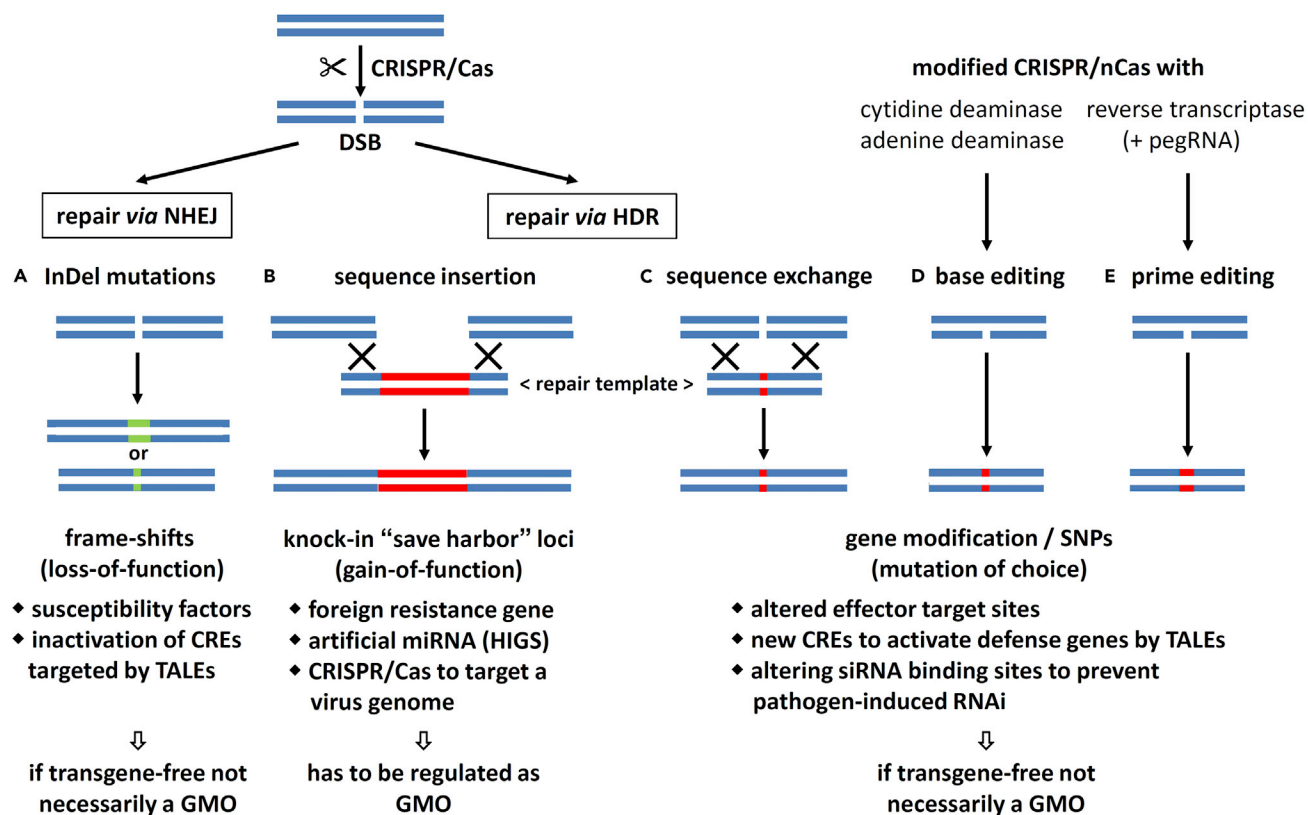
Such an approach was successful to identify CRT1a as a novel susceptibility factor in *Brassica napus* and *Arabidopsis thaliana* infected with *Verticillium longisporum*, albeit its function in endoplasmic reticulum quality control/protein folding could well have secondary effects on the functionality of other yet unknown susceptibility factors. Importantly, the plants appeared to develop normally, because only one “active” CRT1a locus was targeted, whereas other CRT1a, CRT1b, and CRT3 loci were not affected (Pröbsting et al., 2020). Generally, basic research elucidating the molecular mechanisms underlying host-pathogen interactions is also a rich source for several biotechnological applications and resistance resources. During the co-evolution of host and pathogen these mechanisms became highly specific and can be exploited for genetic engineering (Doron et al., 2018). For example, original bacterial defense mechanisms, such as restriction enzymes or the CRISPR/Cas system were adapted as tools for molecular cloning or GE. Similarly, pathogen-derived effectors specifically target host components to suppress the hosts’ innate immunity during co-evolution. The list of described effectors from pathogenic bacteria and fungi is steadily expanding (Lewis et al., 2009; Büttner, 2016; De Wit et al., 2009; Giraldo and Valent, 2013). Once the effector targets have been identified, some of these could be exploited for resistance breeding (Gawehns et al., 2013). Also in this respect functional genomics can be deployed because effectors usually contain common regulatory *cis*-elements in their promoters or secretion signals, which can be searched in pathogen genomes to identify candidate genes for further analysis (Zwiesler-Vollick et al., 2002; Benevenuto et al., 2018; Ai et al., 2020). Altering the host targets to prevent manipulation by pathogen effectors will impair compatibility and render the plant more resistant. In general, such effector targets identified from very specific plant-pathogen interactions are likely highly conserved (Jwa and Hwang, 2017). Thus, altering the amino acid sequence of a given target protein has to be pursued carefully, as this might impact the original function and cause trade-offs. However, these are likely less strong than a complete loss-of-

function approach. Nevertheless, increasing information about effector targets will supply a plethora of potential new candidates that can be tested by alteration of their DNA sequence for increased resistance and thereby facilitate effector-assisted resistance breeding.

## POTENTIAL OF CRISPR/CAS-MEDIATED GENOME EDITING

GE is the basis of the so-called new breeding techniques (NBTs), including oligo-directed mutagenesis (ODM) and SSNs, such as zinc-finger nucleases (ZFN), transcription-activator like effector nucleases (TALEN), or CRISPR/Cas allowing to substitute random mutagenesis thereby greatly reducing the background of unwanted off-target mutations. Now that at least draft sequences of most crop genomes are available, precise GE can be applied to mutate all target gene alleles at once to cause a phenotype even in crops with very complex genomes, such as octoploid strawberry (Wilson et al., 2019), hexaploid wheat (Wang et al., 2014), tetraploid potato (Johansen et al., 2019), and amphidiploid/allotetraploid species such as oilseed rape or cotton (Braatz et al., 2017; Wang et al., 2018c). Although two TALEN can very specifically target a defined genomic locus, it is laborious to program them on the amino acid level and also challenging to deliver them together into the crop nucleus (Langner et al., 2018). Thus, since it became possible to apply the bacterial CRISPR/Cas system in eukaryotic cells, which is easy to program, relatively cheap, and multiplex compatible (Thompson et al., 2018) TALEN have been replaced quickly by CRISPR/Cas as the most popular GE tool. This system consists of only two components: the Cas protein, generally inducing a double-strand break (DSB) in the DNA, and the single guide RNA (sgRNA), which directs the Cas protein to the genomic target locus of interest. The specificity of the system is conferred by a 20-nt-long replaceable guide sequence within the sgRNA, which is complementary to the target sequence. This allows a relatively easy reprogramming of sgRNAs, e.g., by a few consecutive PCR reactions (Li et al., 2013). The most commonly used Cas protein is the Cas9 from the gram-positive bacterium *Streptococcus pyogenes*, which requires additionally a 3-nt-long sequence (NGG) adjacent to the 20-nt-long recognition site of the sgRNA (Doudna and Charpentier, 2014). This sequence is termed the protospacer adjacent motif (PAM) and varies depending on the CRISPR/Cas-system (Leenay and Beisel, 2017). Provided the crop genome sequence is known, it is possible to design a very specific sgRNA by selecting a highly conserved 20-bp sequence within all alleles of a certain gene of interest and distinct from the remaining genomic sequence. Meanwhile, several CRISPR/Cas systems have been described greatly extending the GE toolbox (Komor et al., 2017). Potential off-target effects might be further reduced by deploying mutated Cas proteins. One example is the D10A Cas9 nickase (nCas9), causing not a DSB but just a nick within the target sequence. However, two nicks on opposite strands will also cause a DSB (Schiml et al., 2016). Thus, paired nCas9 binding in close proximity on opposite DNA strands can increase specificity, resembling the binding pattern of two TALEN (Doudna and Charpentier, 2014; Mikami et al., 2016). Other examples for the continuous progress in optimizing Cas9 to reduce off-target effects are SpCas9-HF1 (Kleinstiver et al., 2016), HypaCas9 (Chen et al., 2017), or HiFi Cas9 (Vakulskas et al., 2018), resulting in systems with almost no unintended mutations. Several bioinformatics tools have been developed to design specific sgRNAs for CRISPR/Cas experiments and predict potential off-target sites, which are listed in a recent review by Khatodia et al. (2016). The efficiency of an sgRNA appears to depend on the secondary structure (Kumlehn et al., 2018), and the specificity, on the number of mismatches outside the sgRNA seed region (the first 10–12 bp proximal to the PAM site), which are sometimes tolerated and might cause off-target mutations (Jiang and Doudna., 2017). Next, the cells have to repair the DSB, which can occur via two prominent pathways.

A DSB can result in insertions or deletions (InDels) when repaired by the error-prone non-homologous end joining (NHEJ) mechanism. This would result in a simple random mutation, most likely leading to a frame-shift causing a loss-of-function phenotype. The advantage of CRISPR/Cas is that the system theoretically will not rest until all target sites are mutated so they cannot be recognized by the sgRNA/Cas complex anymore. However, mutations consisting of small InDels with a multiple of three nucleotides will stay in frame and might therefore not lead to the desired loss of function (Figure 2A). In the presence of a repair template with the complementary flanking arms, homology-directed repair (HDR) facilitates the introduction of a sequence of choice (Figure 2B). This enables a knockin into a “safe harbor” eliminating the risk to inactivate an important gene or element required for plant development. Furthermore, selection of euchromatic regions of high transcriptional activity can improve transgene expression and HDR also allows seamless allele replacement at the position of the DSB (Figure 2C). Such “rewriting” of the original gene sequence by introducing specific mutations can also be used to alter a single amino acid to engineer enzyme activity or substrate specificity. However, HDR requires a sufficient amount of repair templates that have to be delivered into the nucleus in addition to the CRISPR/Cas system. Naturally, HDR takes place during the late S or G2 phase of the cell cycle, thus chemicals such as Nocodazole and CCND1 could be used to achieve higher GE efficiencies (Zhang et al., 2017a). Virus-derived vectors also appear to be helpful,



**Figure 2. Potential CRISPR/Cas Applications to Increase Pathogen Resistance**

(A–E) Depending on the repair mechanism the CRISPR/Cas-induced double-strand breaks (DSBs) can lead to simple mutation of a certain sequence (via the error-prone non-homologous end joining [NHEJ] pathway), resulting often in small insertions or deletions (InDels) causing frameshift mutations and thereby loss of function (A). Alternatively, in the presence of a repair template, the DSB can be repaired via homology-directed repair (HDR) allowing the insertion of larger foreign DNA in a genomic locus of choice (B) or modification of a target region by introducing mutations of choice (C). Single-nucleotide mutations can further be induced by deploying cytosine base editors (CBEs) with cytidine deaminase or adenine base editors (ABEs) with adenine deaminase activity (D). Larger defined mutations can be induced by prime editing, which is based on nCas9 fused with reverse transcriptase depending on prime editing gRNA instead of normal sgRNAs (E). For details see text.

as they provide lots of repair templates increasing the chance of successful HDR events (Baltes et al., 2014; Cermak et al., 2015). Gene modification via HDR using a repair template might even become obsolete when considering that targeted base editing was already successful in rice, tomato, and cotton by employing dead Cas9 (dCas9) or better nCas9 fused with cytidine deaminase or adenine deaminase (Komor et al., 2016; Li et al., 2017b; Shimatani et al., 2017; Qin et al., 2020; Molla et al., 2020). nCas9 fusions with cytidine or adenine deaminase such as engineered TadA are termed cytosine base editors (CBEs) enabling C–G to T–A conversion or adenine base editors (ABEs) altering an A–T base pair into a G–C pair, respectively (Gaudelli et al., 2017; Marzec and Hensel, 2018; Rees and Liu, 2018; Molla and Yang, 2019). In plants, such induced point mutations resulted, e.g., in the generation of herbicide-resistant rice (Shimatani et al., 2017). This application is depicted in Figure 2D. If single mutations are not sufficient, e.g., to mutate a 20-bp-long sRNA target site, prime editing might be the method of choice for precise GE. This new technique has the advantage that no "repair template" is required as in the case of HDR-mediated sequence exchange (Anzalone et al., 2019). Prime editing utilizes engineered Cas9 nickase fused to reverse transcriptase paired with a prime editing gRNA encoding the desired edit (Marzec and Hensel, 2020). Also, this system has already been successfully applied in plants, such as rice and wheat (Lin et al., 2020), and will become a more versatile tool in resistance breeding than "simple" KO applications (Figure 2E).

Thus, CRISPR/Cas enables the simultaneous mutation of all target gene copies, of one specific allele, or even of multiple target genes simultaneously (multiplexing with several sgRNAs), with highly reduced off-target effects, which can be largely avoided by careful sgRNA design (Hahn and Nekrasov, 2019). So far the off-target rates in plants were found to be very low (Langner et al., 2018) and can be largely avoided

by deploying optimized Cas proteins as described earlier. From a breeders' perspective, even a few off-target mutations are well tolerable when considering the extensive amount of such unwanted mutations arising from undirected ethyl methanesulfonate (EMS) or X-ray mutagenesis. This allows now to produce a visible phenotype from recessive resistance genes, something which was beyond imagination for crops with complex genomes before TALEN and CRISPR/Cas were established.

However, another bottleneck in many crops is often technical difficulties concerning transformation efficiency with *Agrobacterium tumefaciens* or other methods to deliver the CRISPR/Cas system into plant tissue or protoplasts, as well as the regeneration of whole plants. However, also in these respects novel techniques such as *de novo* induction of meristems might help one day to bypass such limitations, making all crops amenable to GE (Maher et al., 2020).

## APPROACHES TO ENGINEER DISEASE RESISTANCE

In the following we will summarize theoretical and already tested CRISPR/Cas applications to increase plant resistance toward pathogens. The plant-pathogen interaction is very complex and consists of several layers. Constitutive barriers form a preformed defense, which—when overcome by the pathogen—is complemented by the pattern-triggered immunity (PTI). During PTI highly conserved microbe/pathogen-associated molecular patterns are recognized by plant pattern recognition receptors (PRRs) to launch a defense response. In such a case some pathogens have evolved means to overcome PTI by deploying, for example, effectors to manipulate plant defense signaling thereby facilitating host colonization or inducing, e.g., susceptibility genes. Plants can respond to these effectors by recognizing them, for example, directly (receptor-ligand model) or indirectly (guard hypothesis) and launch another strong defense response, which is called effector-triggered immunity (ETI). In this evolutionary arms race pathogens develop constantly new effectors to overcome PTI and ETI (Jones and Dangl, 2006). Basic research elucidating the molecular mechanisms underlying this plant-pathogen interaction becomes now extremely useful, because the growing knowledge enables us to speed up host evolution by mutating/altering the pathogen's targets in crops. Thus, the modified targets cannot be recognized and manipulated by the pathogen-deployed effectors anymore thereby providing a temporal advantage for the crop until the pathogens adjust to the new situation. In the following we introduce examples of how CRISPR/Cas applications can increase pathogen resistance and distinguish between approaches, resulting either in transgene-free crops, which (on a product-based evaluation) would not resemble genetically modified organisms (GMOs), or stable transgenic crops, which by conventional criteria need to be regulated as GMOs.

### Transgene-Free Approaches to Increase Biotic Stress Resistance by CRISPR/Cas-Induced Mutations

Transgene plants can be initially identified by PCR and further certified by whole-genome sequencing, which also helps to describe the mutations induced at the target sites as well as detecting potential off-target mutations (Kim and Kim, 2016). In the United States CRISPR-mutated crops are already evaluated on product-based legislation and do not fall under GMO regulations (Waltz, 2018), whereas the situation in Europe is more difficult because it relies on a process-based legislation (Sprink et al., 2016). However, because it is virtually impossible to retrace how a small mutation was originally induced (naturally, chemically by EMS, physically by X-ray, ODM, ZFN, TALEN, or CRISPR/Cas), the attempt to regulate CRISPR-mediated mutations in the European Union appears to be a farce (Urnov et al., 2018). Because SSNs such as CRISPR/Cas act *in trans*, the induced mutation can be inherited independently from the construct integration site (e.g., when Cas9 and sgRNA are delivered by the transfer DNA of *Agrobacterium tumefaciens*). This means that even if the CRISPR technique involves a transgenic intermediate state, it allows relative easy segregation of mutated loci from the CRISPR/Cas construct insertion locus to produce transgene-free crops, which are sexually propagated. However, vegetatively propagated or perennial crops such as potatoes, apple, grape vine, banana, sugarcane, cassava, or strawberries do not allow such easy out-segregation posing another obstacle for plant breeders, which might be resolved by transient expression of CRISPR/Cas systems (Zhang et al., 2016a; Nadakuduti et al., 2018). Another option to avoid the GMO status of edited crops by abstaining from any transgenic intermediate is the deployment of biolistics or methods to transfect plant protoplasts with ribonucleoprotein complexes, consisting only of the Cas9 protein and the sgRNA, but this is merely a theoretical solution (Kim and Kim, 2016; Metje-Sprink et al., 2019). Nevertheless, such "DNA-free" systems have already been successfully applied in several plant species, such as *Chlamydomonas reinhardtii* (Baek et al., 2016), maize (Svitashev et al., 2016), petunia (Subburaj et al., 2016), wheat (Liang et al., 2017), apple, grape vine (Malnoy et al., 2016), lettuce, rice, tobacco, and

*Arabidopsis* and might also help to further reduce potential off-target mutations, as has been shown for lettuce (Woo et al., 2015).

- A) Susceptibility factor-encoding genes are generally interesting targets, because a simple KO can already enhance resistance (Zaidi et al., 2018). A well-known gene in many crop species is the MLO locus conferring mildew resistance when all alleles are non-functional (recessive trait). For example, by mutating MLO in tomato, plants also became more resistant toward powdery mildew (Nekrasov et al., 2017). Problematic was the targeted inactivation in polyploid crops with complex genomes and several existing alleles until Wang et al. (2014) demonstrated the potential of the NBTs TALEN and CRISPR/Cas to mutate all six wheat MLO alleles, resulting in enhanced resistance to the fungal pathogen *Blumeria graminis f. sp. tritici*. Rice resistance to the bacterial pathogen *Xanthomonas oryzae pv. oryzae* was engineered by mutating SWEET susceptibility genes or their promoters (Zhou et al., 2015; Oliva et al., 2019; Xu et al., 2019) and to the rice blast fungus *Magnaporthe oryzae* by mutating ERF922 (Wang et al., 2016a). Mutation of the recessive *eIF4E* cucumber gene resulted, for example, in resistance to cucumber vein yellowing virus (Chandrasekaran et al., 2016). DMR6 (downy mildew resistance 6) has been described as a susceptibility gene in *Arabidopsis* (van Damme et al., 2008) and potato (Sun et al., 2016). In tomato, a DMR6 KO was reported in a pre-print to cause increased disease resistance against different pathogens, including *Pseudomonas syringae pv. tomato* (Pto), *Phytophthora capsici*, and *Xanthomonas* spp. (de Toledo Thomazella et al., 2016). Thus, CRISPR/Cas has been successfully implemented in generating plant resistance toward viral, bacterial, and fungal pathogens. However, susceptibility factors are likely also somehow important for plant development or responses to other stresses. Any change, especially complete loss-of-function approaches, which increase pathogen resistance, might have secondary effects on other traits and should be evaluated carefully to avoid fitness costs or reduced yield.
- B) Also, deletion, rewriting, or introduction of cis-elements in promoters of susceptibility or defense genes might result in increased tolerance. Effector-targeted cis-elements in susceptibility genes can be deleted with the advantage that the gene itself remains intact and can still fulfill its natural function in plant development. One example is the rice OsSWEET14 gene promoter cis-element targeted by *Xanthomonas oryzae* transcription activator-like effector (TALE) AvrXa7. The deletion of this cis-element resulted in less-severe disease symptoms, although this was achieved via a TALEN-based approach, hoisting the bacteria with their own petard (Li et al., 2012). Meanwhile, the promoters of OsSWEET11 and OsSWEET13 have also been targeted with CRISPR/Cas (Jiang et al., 2013; Oliva et al., 2019). Likewise, modification of the PthA4 effector-binding elements in the CsLOB1 promoter resulted in increased resistance of Duncan grapefruit toward *Xanthomonas citri* (Jia et al., 2016). It would be technically more difficult to modify a promoter by rewriting cis-elements via HDR using a repair template, but this could ensure that InDels do not interfere with spacing of regular cis-elements required by the host plant for proper gene regulation. On the other hand, the introduction of a new cis-element into a defense gene promoter could enable expression in response to new stress signals/pathogens. In nature, expression of the pepper resistance gene *Bs3* is unintendedly activated by the *Xanthomonas campestris* effector *avrBS3* (Rivas and Genin, 2011; Boch et al., 2014). Thus, activating host genes involved, e.g., in the hypersensitive response by introduction of a TALE-binding site in their promoters could lead to resistance against biotrophic pathogens producing the respective TALE. Thus, by this approach it should be possible to bypass fitness costs. Such small changes might not be regulated as GMO, provided the gain/loss is not exceeding 20 bp, which is considered to occur also spontaneously in nature (ZKBS (German Biosafety Commission), 2012).
- C) Engineering specific mutations in the coding sequence of host defense factors via HDR will also become possible, although efficiencies are naturally lower than for NHEJ because repair templates are needed in sufficient amounts at the moment of repair. Owing to the degenerated nature of the genetic code, triplets can be replaced in the open reading frame without changing the original amino acid sequence. This allows to prevent degradation of mRNA encoding, for example, plant defense genes, which are targeted by pathogen-derived sRNAs by hijacking of the hosts RNAi machinery ("cross-kingdom RNAi"). Potential targets for such an approach were identified in the tomato-*Botrytis cinerea* interaction, affecting, for example, the defense signaling component MAPKKK4 (Weiberg et al., 2013), or in the *Arabidopsis Botrytis cinerea* interaction, targeting, e.g., AtWRKY7, AtPMR6, and AtFEI2 (Wang et al., 2017). Also, *Verticillium dahliae* appears to utilize sRNA effectors,

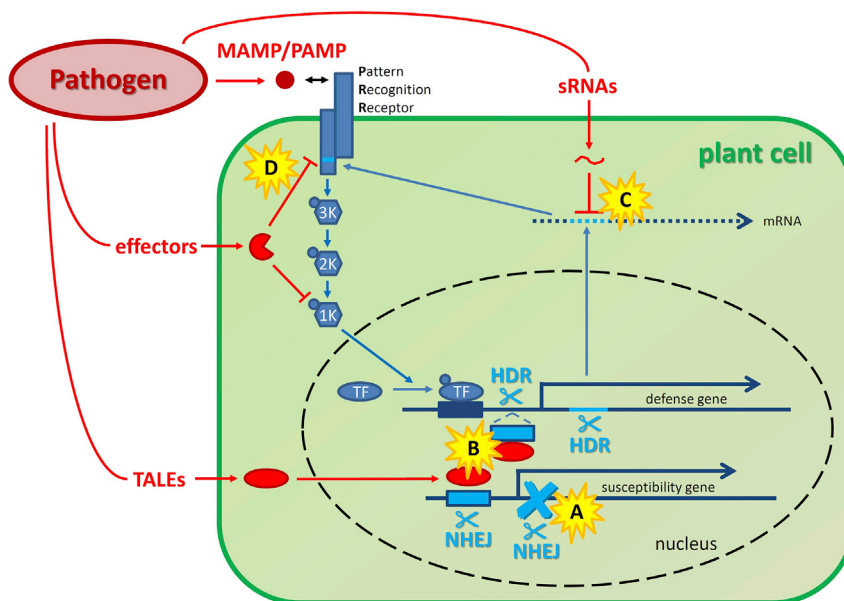
which have been identified in complex with *Arabidopsis* AGO1 (Wang et al., 2016b). This approach allows to disarm any small RNA effector deployed during cross-kingdom RNAi by the pathogen, and NGS of sRNAs produced during host-pathogen interactions will undoubtedly reveal many more potential sequences for alteration within the host genomes. In this case there is also the advantage that no fitness costs are to be expected.

- D) Last but not least it should also be possible to change certain amino acids in a plant target protein that are required for recognition and/or cleavage by pathogen effectors. For example, effector proteases (EPs) from *Pseudomonas syringae*, e.g., HopB1 cleaving the co-receptor BAK1 (Li et al., 2016) or AvrPphB cleaving the signaling components PBS1, PBL1, PBL2, PBL6, and BIK1 (Zhang et al., 2010) could be exploited, because even a single amino acid change in the cleavage motif prevents cleavage by AvrPphB. However, a double change in BIK1 (G230A/D231A) resulted in a dominant-negative effect on flg22-induced defense signaling, indicating that these amino acid residues are functionally important (Zhang et al., 2010). Thus, it is critically important that the amino acid change does not completely abolish the natural function of the target. On the other hand, it is also possible to enhance the disease response by mutations as has been demonstrated for a wheat R-gene by Stirnweis et al. (2014). Substitutions of two amino acids in the nucleotide-binding site domain enhanced the hypersensitive response, and thereby resistance against biotrophic powdery mildew. RIN4 is a target of at least four bacterial effectors, two of which induce RIN4 hyperphosphorylation weakening the RIN4-ROC1 interaction (Rodriguez et al., 2016). Some of the amino acids hyperphosphorylated might be replaced to dampen the effector-triggered susceptibility. The serine 141 phosphorylation in response to FLS2 activation is required for proper function of RIN4 in de-repression of immune responses, and phosphorylation of threonine 166 is mediated by the effector AvrB (Chung et al., 2014). The pathogen-induced callose deposition was diminished by AvrB in WT plants, whereas this suppression was lost in mutant plants expressing non-phosphorylatable RIN4 (T166A). This demonstrates that there are many things to consider, but that it should be generally possible to substitute amino acids in effector targets to render plants more resistant. In this respect, targeted base editing by CBEs or ABEs will become a powerful tool, enabling molecular biologists to exchange specific amino acids to disrupt effector-target interactions.

These four possibilities to deploy CRISPR/Cas in resistance engineering are also summarized in Figure 3. It is noteworthy that in all these cases resistance would be only race-specific against the pathogen deploying the respective effector. Introducing several changes (“mutation stacking”) in the host genome can also help to slow down pathogen adaptation to the new crop variety and establish a more durable type of plant immunity.

- E) Negative regulators of innate immunity might be generally less well suited for a targeted KO, as constitutively activated defenses are usually resulting in smaller “dwarfed” plants and therefore imply a yield penalty, which could be, for example, attributed to increased salicylic acid (SA)-dependent defense responses (Huot et al., 2014). This is also reported for the disruption of rice SEC3A, which displayed increased SA content, enhanced senescence, and induced plant defense responses and the typical mini-plant phenotype (Ma et al., 2018). However, in *Arabidopsis*, the CRISPR/Cas-mutated negative regulators *ian9* and *iap1* did not show any obvious developmental phenotype, suggesting that they are involved rather in inducible immunity than constitutive immune responses (Wang et al., 2018b). Also, the *edr1* mutant does not show constitutively activated defense responses, thus it was selected by Zhang et al. (2017b) as a target to improve resistance to powdery mildew. Whether the observed increased resistance to *Phytophthora tropicalis* after mutation of the cacao negative regulator NPR3 has no negative side effects on regenerated whole cacao plants remains to be elucidated (Fister et al., 2018).
- F) Manipulation of central players in plant defense, such as WRKY transcription factors, might be another option to increase resistance. The KO of *Brassica napus* WRKY70 was recently reported to increase resistance to the necrotroph *Sclerotinia sclerotiorum* (Sun et al., 2018b). As a positive activator of SA-dependent defense genes and a repressor of jasmonic acid (JA)-regulated genes WRKY70 is a node of convergence affecting defenses against both biotrophic and necrotrophic pathogens, respectively (Li et al., 2006). Thus, a WRKY70 KO might have negative effects on resistance to biotrophic pathogens. This is corroborated by a report showing that the knockdown of *Arabidopsis* WRKY75 reduced SA production and resistance to the biotrophic pathogen *Pseudomonas*





**Figure 3. Potential Targets for CRISPR/Cas Applications to Engineer “Non-transgenic” Disease Resistance in Plants**

(A–D) Upon pathogen recognition, the plant-induced defense reactions involve, for example, a phosphorylation cascade via MAP kinases, leading to transcription factor activation and defense gene expression. (A) Open reading frame disruption via NHEJ-induced frameshift mutation of pathogen-induced susceptibility genes; (B) cis-element elimination by NHEJ to prevent activation of susceptibility genes by TALEs/cis-element introduction by HDR leading to TALE-triggered defense gene activation; (C) inhibition of cross-kingdom RNAi by HDR-mediated rewriting of pathogen-derived small interfering RNA target sequences; (D) HDR-mediated rewriting of the coding sequence to replace amino acid residues required for protein recognition by pathogen-originated effectors, thus preventing, for example, cleavage or modification. For details see text.

*syringae* and showed, on the other hand, delayed leaf senescence, which allows to extend the grain filling period under abiotic stress conditions (Guo et al., 2017; Abdelrahman et al., 2018). Furthermore, this indicates that there is a defense trade-off, resulting from SA-JA hormonal antagonism, and that a negative impact on plant defense can positively affect other important agronomic traits. Such effects should be considered when manipulating a crop by GE. Interestingly, by CRISPR inactivation of tomato JAZ2, it was possible to avoid such potential defense trade-offs by simply preventing stomatal reopening in response to coronatine produced by *Pseudomonas syringae*, without negatively affecting the defense response to the necrotroph *Botrytis cinerea* (Ortigosa et al., 2018).

### Transgenic Approaches Deploying CRISPR/Cas to Increase Resistance

GE also enables the transfer of heterologous genes involved in resistance mechanisms. The difference from former approaches via transfer DNA transfer is that CRISPR/Cas-HDR precisely allows to select an “empty” space between existing defense genes, which are highly expressed, e.g., under pathogen stress. This option should improve the regulatory accessibility of transferred resistance-conferring genes and enhance their expression. In this way, potential negative side effects on other host genes close to the integration site might also be reduced. Sequence integration in such genomic regions also allows to identify positive events without the use of selection markers, because the flanking regions are known and thus allow PCR-based locus amplification and sequencing. First-generation GMOs have been often criticized in the past especially due to random integration of transgenes causing such potential side effects and because of their antibiotic resistance genes required for selection of positive events (Gepts, 2002). This can now be largely avoided by targeted integration into “safe harbor” genomic loci, but the insertion of a foreign DNA definitely requires regulation as GMO, a time- and cost-intensive process, which often goes along with reduced consumer acceptance (Waltz, 2018).

A) One example how CRISPR/Cas can be used in resistance breeding is the HDR-mediated transfer of resistance-conferring genes into a stress-responsive and actively expressed chromatin region of

susceptible hosts. Such gain-of-function approaches could be facilitated with CRISPR/Cas GE. For example, tightly linked R genes in a head-to-head configuration allow co-regulation ensuring they can cooperatively confer resistance. The expression of R-genes in such coregulatory modules might reduce the probability of dysregulation and further reduce fitness costs (Karasov et al., 2017). However, R-genes such as nucleotide-binding site leucine-rich repeat (NLR) genes often function as pairs consisting of a helper NLR (activating the defense signaling) and a sensor NLR recognizing the pathogen effector and controlling the helper to prevent autoimmunity in the absence of the pathogen (Wu et al., 2017). Transferring R-genes to crops and expressing them in response to biotic stress could improve pathogen resistance, but the number of suitable R-genes is limited and may first require the identification of interacting helper NLRs (Wang et al., 2019). Furthermore, dominant R-gene-mediated resistance is less durable than recessive resistance and is usually restricted to a few race-specific isolates, which can quickly overcome this resistance by their higher mutation rate (Dangl et al., 2013; Pandolfi et al., 2017; Kourelis and van der Hoorn, 2018). Thus, to achieve a more durable resistance, trait stacking/pyramiding has been applied and could be further facilitated by CRISPR/Cas-induced HDR (Pandolfi et al., 2017). On the other hand, the transfer of PRRs would confer per se a much broader and more durable resistance, which has been successfully shown between *Arabidopsis* and tomato (Lacombe et al., 2010), rice and banana (Tripathi et al., 2014), or *Arabidopsis* and wheat (Schoonbeek et al., 2015). This is possible, because the immune signaling pathways are conserved between monocots and dicots (Holton et al., 2015). The advantage of these approaches is that plants are enabled to detect a new pest and launch a defense response only when being challenged with the respective pathogen, thus also reducing the potential yield penalty (Karasov et al., 2017). Furthermore, it is known that stress can induce priming through establishment of permissive chromatin states allowing a more rapid defense gene expression when facing a second challenge with the same stress (Karasov et al., 2017). Such a genomic region can potentially have a positive impact on heterologous expression of R-genes or PRRs. The expanding list of potential candidate genes also includes antimicrobial peptides, signaling components and transcription factors, or structural genes to produce antimicrobial secondary metabolites.

B) Also, non-coding RNAs, namely, polymerase II- expressed miRNAs, can confer resistance. The underlying mechanism is RNAi, which generally functions in eukaryotic cells. Small RNAs are mobile between the host and pathogen (Hua et al., 2018) and have been identified in nature causing “cross-kingdom RNAi”. That is, pathogen-derived sRNAs have been shown to target host mRNAs encoding for defense-related genes for degradation by RNAi (Weiberg et al., 2013), whereas plant sRNAs have been shown to be differentially expressed in response to pathogens (Zhang et al., 2011; Shen et al., 2014) and some can even target virulence factors within the pathogen (Zhang et al., 2016b). Technically, the RNAi system is naturally present in crops, which means we only need to harness this machinery to target pathogenic RNAs via transformation of a short, artificial miRNA with homologies to the virus genome. Popular targets are, for example, viral coat proteins or replicases (Dong and Ronald, 2019). Also, stable constitutive expression of the CRISPR/Cas system can target viral genomes by suitable sgRNAs, thereby establishing a novel immune system (Ji et al., 2015; Zhang et al., 2018a; Mushtaq et al., 2020). The CRISPR/Cas system requires transformation of both the Cas protein and the short virus-specific sgRNA. When the host cells are infected by a virus, either system is suitable to degrade the pathogen’s genome or replication intermediates. However, such miRNAs or sgRNAs should be designed carefully not to target accidentally host or human nucleic acids. As the principal resistance mechanism (degradation of essential pathogen derived nucleic acids) is almost identical and application of both systems (HIGS and CRISPR/Cas) will result in GM crops, the system choice will depend on following arguments:

- 1) miRNAs/RNAi can target only RNA molecules for cleavage, whereas CRISPR/Cas can target both DNA (e.g., via Cas9) and RNA via Cas13 (Wolter and Puchta, 2018; Ali et al., 2018).
- 2) CRISPR/Cas works only within the host cell to control, e.g., virus infection, whereas HIGS can also affect eukaryotic pests by cross-kingdom RNAi.
- 3) The processing of sRNAs for RNAi cannot be controlled as exactly as the designed CRISPR/Cas sgRNA, resulting in potentially more off-target effects (Smith et al., 2017).
- 4) At least some plant viruses have evolved already counter-defense measures (suppressor proteins) against the host RNAi mechanism (Voinnet, 2005), which would also affect HIGS during co-infection events, but not the bacterial CRISPR/Cas system. However, it should be considered that

CRISPR/Cas9 might also affect virus evolution, as has been proposed for cassava infected with geminiviruses (Rybicki, 2019; Mehta et al., 2019).

Taking these considerations into account, it appears that overall CRISPR/Cas might be the preferable method for virus control.

Although constitutive expression of positive regulators in innate immunity also will theoretically increase plant resistance, we can expect similar trade-offs concerning yield and growth when compared with the KO of negative regulators. Only under tight control with respect to strictly stress-induced expression pattern such genes are suitable candidates for HDR-mediated integration into a crop genome. All in all, it appears that CRISPR/Cas can be deployed in many ways to increase pathogen resistance, and a list of successful applications is presented in Table 1.

### CONCLUDING REMARKS

Regardless of GMO regulation issues, it is obvious that GE will be very useful to speed up resistance breeding considering the ever-expanding CRISPR toolbox. Developing CRISPR systems with increased and broadened specificity will make virtually any desired modification possible. To date CRISPR technology has been deployed mainly to inactivate host susceptibility factors and address fungal (18 reports) viral (16 reports), and bacterial (10 reports) diseases, counting only the studies selected for Table 1. To our knowledge there are almost no reports about the successful generation of insect or nematode resistance by CRISPR/Cas, although it is discussed to be possible (Douglas, 2018; Bisht et al., 2019; Ali et al., 2019). One report exists, describing that loss of function of rice CYP71A1 leads to increased resistance toward the brown planthopper (*Nilaparvata lugens* Stål) and striped stem borer (*Chilo suppressalis*), but unfortunately this mutation had negative effects on grain yield in field trials (Lu et al., 2018). Effector mining studies elucidating the repertoire in insects (Zhao et al., 2015; Rao et al., 2019) and nematodes (Vieira et al., 2018; Gardner et al., 2018) as well as their function will definitely help to identify potential host targets to create insect and nematode resistance. Systems biology and functional genomics based on the NGS-facilitated generation of vast genomic and transcriptomic data will help to identify novel breeding targets allowing the generation of new hypotheses regarding how to prevent host manipulation by pathogens. These hypotheses can be addressed even by smaller breeding companies, because CRISPR/Cas-mediated GE is not that expensive and relatively easy to handle. Importantly, GE techniques allow a very specific KO of certain alleles, whereas a knockdown often causes off-target effects resulting in a different phenotype maybe due to lost genetic compensation (Gao et al., 2015; Unniyampurath et al., 2016; Smith et al., 2017; Kumar et al., 2018; Kim et al., 2019; Pröbsting et al., 2020). Pathogen resistance is just one important trait that can be addressed with GE in many different crops (Table 1), and CRISPR/Cas is currently also deployed to encounter citrus greening, a bacterial disease threatening sweet orange (Ledford, 2017). For some other crops, such as banana or cacao, GE might be even the only protection against currently spreading diseases (Maxmen, 2019; Goergen, 2020). A first success in banana breeding is the application of CRISPR/Cas to inactivate the endogenous banana streak provirus in the banana B genome (Tripathi et al., 2019). Especially for crops being vegetatively propagated, such as banana or potato, GE is a solution for adaption to changing environments. Transgenic expression of RGA2 from wild banana in the commercial variety Cavendish resulted in increased resistance to *Fusarium oxysporum*, but the authors argue that increasing the expression level of endogenous Cavendish homologs by GE of the promoter might provide non-transgenic resistance (Dale et al., 2017). However, the acceptance of such new varieties will depend largely on the consumer decision. If GE crops have to be labeled as GMO as currently the case in the European Union, registration of GE mutated varieties will become too expensive for smaller companies. How far greenhouse results can be confirmed under field conditions is another issue affected by restrictive legislation, but a necessary step in breeding. Whether or not any new resistance will be durable depends furthermore not only on the nature of the crop modification and mutation rate of the respective pathogen but also on the field management, including tillage operations, sufficient crop rotation, and the renouncement of extensive monocultures. CRISPR/Cas-induced mutations can help to create pathogen-resistant crop ideotypes when resistance resources in natural variation or wild relatives are scarce. Theoretically, crops with higher resistance would also require less pesticide treatments, an important issue considering that pesticides also become less available or lose their effect (Borel, 2017). However, for a real reduction of chemicals in agriculture it needs especially a rethinking of how conveniently we want to produce our food and how much we care about biodiversity in an intact, healthy environment.

CRISPR/Cas-approaches increasing resistance by direct targeting of viral genomes				
plant species	target region	resistance to virus / disease	comment	reference
Arabidopsis / <i>N. benthamiana</i>	IR, CP, Rep	Beet Severe Curly Top Virus (BSCTV)	Cas9	Ji et al., 2015
Arabidopsis / <i>N. benthamiana</i>	1A, CP, 3'UTR-A	Cucumber Mosaic Virus (CMV) / Tobacco Mosaic Virus (TMV)	FnCas9 > RNA	Zhang et al., 2018a
Barley	MP/CP, Rep/RepA, LIR	Wheat Dwarf Virus (WDV)	Cas9	Kis et al., 2019
<i>Nicotiana benthamiana</i>	RBS, IR hairpin, 3x Rep	Bean Yellow Dwarf Virus (BeYDV)	Cas9	Baltes et al., 2015
	IR, CP, RCR1I of Rep	Tomato Yellow Leaf Curl Virus (TYLCV)	Cas9	Ali et al., 2015
	IR, CP, RCR1I of Rep	Gemini viruses (CLCuKoV, MeMV)	Cas9	Ali et al., 2016
	IR, C1	Cotton Leaf Curl Multan virus (CLCuMuV)	Cas9	Yin et al., 2019
	CP, Hc-Pro	Turnip Mosaic Virus (TuMV)	Cas13a > RNA	Aman et al., 2018
Potato	P3, CI, Nib, CP	Potato Virus Y (PVY)	Cas13a > RNA	Zhan et al., 2019
Tomato	CP, Rep	Tomato Yellow Leaf Curl Virus (TYLCV)	Cas9	Tashkandi et al. 2018
CRISPR/Cas-approaches increasing pathogen and pest resistance by targeting a host gene				
plant species	target gene	resistance to pathogen / pest	comment	Reference
Arabidopsis	<i>AtEIF(iso)4E</i>	Turnip Mosaic Virus (TMV)	Cas9	Pyott et al., 2016
	<i>AtIAN9</i>	<i>Pseudomonas syringae</i> (bacterial speck)	Cas9	Wang et al., 2018b
	<i>At2OGO</i> (DMR6-like)	<i>Fusarium graminearum</i> (head blight)	Cas9	Low et al., 2020
	<i>AtErf019</i>	<i>Phytophthora parasitica</i>	Cas9	Lu et al., 2020
Apple	<i>MpDIPM-1/2/4</i>	<i>Erwinia amylovora</i> (fire blight)	Cas9 RNP	Malnoy et al., 2016
Barley	<i>HvMorc1</i>	<i>Blumeria graminis</i> (Bgh) / <i>Fusarium graminearum</i>	Cas9	Kumar et al., 2018
Cacao	<i>TcNPR3</i>	<i>Phytophthora tropicalis</i>	Cas9	Fister et al., 2018
Cassava	<i>MenCBP-1/2</i>	Cassava brown streak virus (CBSV)	Cas9	Gomez et al., 2019
Cotton	<i>Gh14-3-3d</i>	<i>Verticillium dahliae</i>	Cas9	Zhang et al., 2018b
Cucumber	<i>CseIF4E</i>	Several viral diseases: CVYV, ZYMV, PRSV-W	Cas9	Chandrasekaran et al., 2016
Duncan grapefruit	<i>CsLob1</i>	<i>Xanthomonas citri</i>	Cas9 promoter	Jia et al., 2016

**Table 1. Application of CRISPR/Cas to Increase Plant Resistance**

(Continued on next page)

CRISPR/Cas-approaches increasing pathogen and pest resistance by targeting a host gene				
plant species	target gene	resistance to pathogen / pest	comment	Reference
Grape	VvMLO-7	<i>Erysiphe necator</i> (powdery mildew)	Cas9 RNP	<a href="#">Malnoy et al., 2016</a>
	VvWRKY52	<i>Botrytis cinerea</i> (gray mold)	Cas9	<a href="#">Wang et al., 2018a</a>
Maize	ZmNLB18 (WAK)	<i>Setosphaeria turcica</i> (Northern Leaf Blight)	Cas9 HDR	<a href="#">USDA/APHIS Letter, 2017 (DuPont Pioneer)</a>
<i>Nicotiana benthamiana</i>	CLC-Nb1a/b	Reduced PVY intracellular replication	Cas9	<a href="#">Sun et al., 2018a</a>
Oilseed rape	BnWRKY70	<i>Sclerotinia sclerotiorum</i> (stem rot)	Cas9	<a href="#">Sun et al., 2018b</a>
	BnCRT1a	<i>Verticillium longisporum</i> (stem striping)	Cas9	<a href="#">Pröbsting et al., 2020</a>
Rice	OsSWEET11/14	<i>Xanthomonas oryzae</i> (bacterial blight)	Cas9 promoter	<a href="#">Jiang et al., 2013</a>
	OsSWEET13	<i>Xanthomonas oryzae</i>	Cas9	<a href="#">Zhou et al., 2015</a>
	OsERF922	<i>Magnaporthe oryzae</i> (rice blast)	Cas9	<a href="#">Wang et al., 2016a</a>
	OsBsr-d1	<i>Magnaporthe oryzae</i>	Cas9	<a href="#">Li et al., 2017a</a>
	OsSec3A	<i>Magnaporthe oryzae</i>	Cas9	<a href="#">Ma et al., 2018</a>
	OseIF4G	Rice Tungro Spherical Virus (RTSV)	Cas9	<a href="#">Macovei et al., 2018</a>
	OsCYP71A1	Insects: brown planthopper and striped stem borer	Cas9	<a href="#">Lu et al., 2018</a>
	OsXa13	<i>Xanthomonas oryzae</i>	Cas9 promoter	<a href="#">Li et al., 2019a</a>
	OsPi21, OsXa13	<i>Magnaporthe oryzae</i> / <i>Xanthomonas oryzae</i>	Cas9 multiplex	<a href="#">Li et al., 2019b</a>
	Os8N3	<i>Xanthomonas oryzae</i>	Cas9	<a href="#">Kim et al., 2019</a>
	OsCul3a	<i>Xanthomonas oryzae</i> / <i>Magnaporthe oryzae</i>	Cas9	<a href="#">Gao et al., 2020/Liu et al., 2017b</a>
Soybean	GmF3H1/2, FNSII-1	Soybean Mosaic Virus (SMV)	Cas9	<a href="#">Zhang et al., 2019</a>
Tomato	SIMLO1	<i>Oidium neolycopersici</i> (powdery mildew)	Cas9	<a href="#">Nekrasov et al., 2017</a>
	SIJAZ2	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Cas9	<a href="#">Ortigosa et al., 2018</a>
	SIDMR6	<i>P. syringae</i> , <i>Phytophthora capsici</i> , <i>Xanthomonas</i> spp.	Cas9	<a href="#">de Toledo Thomazella et al., 2016</a>
	SIPMR4	<i>Oidium neolycopersici</i> (powdery mildew)	Cas9	<a href="#">Santillán Martínez et al., 2020</a>

Table 1. Continued

(Continued on next page)

CRISPR/Cas-approaches increasing pathogen and pest resistance by targeting a host gene				
plant species	target gene	resistance to pathogen / pest	comment	Reference
Watermelon	<i>Clpsk1</i>	<i>Fusarium oxysporum</i>	Cas9	Zhang et al., 2020
Wheat	<i>TaMLO</i>	<i>Blumeria graminis</i> (powdery mildew)	Cas9	Wang et al., 2014
	<i>TaEDR1</i>	<i>Blumeria graminis</i>	Cas9	Zhang et al., 2017b
	<i>TaNFXL1</i>	<i>Fusarium graminearum</i> (head blight)	Cas0	Brauer et al., 2020

Table 1. Continued

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## AUTHOR CONTRIBUTIONS

D.S. wrote the paper and prepared the figures, and D.C. conceptually designed and approved the final version.

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