



CRISPR-Cas-Led Revolution in Diagnosis and Management of Emerging Plant Viruses: New Avenues Toward Food and Nutritional Security

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Plant viruses pose a serious threat to agricultural production systems worldwide. The world's population is expected to reach the 10-billion mark by 2057. Under the scenario of declining cultivable land and challenges posed by rapidly emerging and re-emerging plant pathogens, conventional strategies could not accomplish the target of keeping pace with increasing global food demand. Gene-editing techniques have recently come up as promising options to enable precise changes in genomes with greater efficiency to achieve the target of higher crop productivity. Of genome engineering tools, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) proteins have gained much popularity, owing to their simplicity, reproducibility, and applicability in a wide range of species. Also, the application of different Cas proteins, such as Cas12a, Cas13a, and Cas9 nucleases, has enabled the development of more robust strategies for the engineering of antiviral mechanisms in many plant species. Recent studies have revealed the use of various CRISPR-Cas systems to either directly target a viral gene or modify a host genome to develop viral resistance in plants. This review provides a comprehensive record of the use of the CRISPR-Cas system in the development of antiviral resistance in plants and discusses its applications in the overall enhancement of productivity and nutritional landscape of cultivated plant species. Furthermore, the utility of this technique for the detection of various plant viruses could enable affordable and precise in-field or on-site detection. The futuristic potential of CRISPR-Cas technologies and possible challenges with their use and application are highlighted. Finally, the future of CRISPR-Cas in sustainable management of viral diseases, and its practical utility and regulatory guidelines in different parts of the globe are discussed systematically.

Keywords: CRISPR-Cas, viral resistance, genome editing, diagnostics, management, CRISPR regulatory framework, food and nutritional security

INTRODUCTION

Challenges to Global Food and Nutritional Security and Overview of Plant Disease Resistance as a Multicomponent System

It has been projected that the world human population will reach the 10-billion mark by the year 2057. The current average population increase is estimated at 81 million people per year (1). To meet the ever-increasing population's growing food and nutritional requirements, concerted efforts are needed to intensify food production and increase the nutritional value of foods produced (2). Many factors, such as abiotic and biotic stresses and climatic disruptions, pose significant challenges to food production. Up to 40% of crop yields are lost to pests and diseases worldwide (3). The plant diseases caused by fungi, bacteria, viruses, and nematodes largely burden the global agricultural production in general and food and nutritional security in particular. Among these pathogens, viruses and viroids account for losses of up to 100% in case of severe infections depending on the crop species (4). Due to their unique nature and infection cycle, viruses are considered the most challenging to manage among all plant pathogens. With an array of new infections and novel viruses identified using new tools and techniques, the past few decades have witnessed the emergence of viruses as a severe and challenging threat to agriculture worldwide, amounting to losses of several billion dollars annually (5). Recently, owing to erratic climatic disruptions, there have been several reports on the emergence and re-emergence of viruses, the reason for which ranges from modified cropping practices (monocropping and introduction of monoculture of new crops in different geographical areas), free global trade, to the introduction of infected germplasm coupled with the ability of viruses to rapidly evolve and adapt (6). In response to infections, plants, over the years, have developed a complex and intricate defense mechanism, enabling them to avoid, suppress, or defend against a range of pathogens. A plant defense mechanism relies on pathogen recognition followed by the induction of signaling mechanisms leading to resistance or defense. Plant immunity is analogous with the immunity activation component (IAC) associated with the recognition of molecular patterns, i.e., microbe-/pathogen-associated molecular patterns (MAMPs/PAMPs) or damage-associated molecular patterns (DAMPs), effectors, and the immunity modulation component (IMC) that deals with the regulation of immune response.

Plant viruses, in general, have a narrow host range, and the number of non-host plant species far exceeds that of the host ones. In hosts plants, viruses encounter several defense mechanisms; while some act against all viruses, others are specific to a virus and involve R (resistance) genes. In many cases, R genes do not necessarily confer total resistance where virus replication is observed, albeit in a low titer (7) and, thereby, confer only partial immunity. Non-host resistance is a general, non-specific resistance involving two types of mechanisms (8). While type 1 is associated with the activation of the basal defense mechanism, like modulation of the cell wall or activation of secondary metabolite production, type 2 is associated with necrotic lesions

and is induced after subduing type 1 infection. Type 2 mechanism is associated with the recognition of molecular patterns like MAMP/PAMPs, and activation of PAMP-triggered immunity (PTI) responses (9). Plant viruses also encounter an antiviral silencing barrier of the host known as RNA interference (RNAi) as a first response to infection, where double-stranded (ds) RNA triggers the silencing of viral genes *via* the action of proteins involved in an RNA-induced silencing complex (RISC). Therefore, dsRNA acts as a MAMP/PAMP for an RNAi acting as a PTI. RNA silencing also includes a regulatory mechanism involving the host-encoded micro-RNAs (miRNAs). While fungal and bacterial diseases are generally managed using antifungal or antibacterial agents, the specific and daunting task of viral disease management relies more on preventing viral infection in plants or developing virus-resistant plants using various strategies developed specifically for a particular target virus. Among these strategies, the use of genomic tools holds utmost importance in terms of robustness and specificity. In addition, accurate diagnosis is the first and most essential tool to identify and characterize a virus infection in plants, thereby paving the way for their downstream management.

The Emergence of Virus-Led Epiphytotics and Associated Challenges to Global Food Security

Evolution and diversification in plant pathogens lead to the emergence and re-emergence of new diseases in different cropping systems and geographical areas. Viruses have either DNA or RNA as genetic materials and lack proofreading and correction mechanisms during the replication of their genetic material (particularly in the case of RNA viruses). Frequent occurrences of recombination and mutation lead to the emergence of new variants and strains of viruses (10). It is, however, difficult to describe the exact origin of virus variants, as they co-evolve along with their respective hosts coupled with crop domestication, introduction, and diversification (11). Variation occurs continuously in viruses and their vectors in the adaptation time scale against selection pressure posed by host resistance and immunity (11). Continuous evolution in plant viruses, coupled with frequent occurrence of mixed infections of taxonomically different viruses in the same host, leads to the development of new virulent species/strains, thus reducing host resistance durability. With the advent of technologies such as deep sequencing, the detection and characterization of new and emerging virus variants and species have seen major upsurge during the last 2 decades at the global level. Agricultural systems have, thus, witnessed the emergence of new viruses and their variants, and earlier reported viruses of minor economic significance are now becoming causes of major epiphytotics. Some classical examples of virus-led epiphytotics are discussed here.

Infection of maize streak virus (MSV), a mastrevirus complex having 11 strains reported from different parts of the world that could infect more than 80 species in the *Poaceae* family, is a significant constraint to maize production across the globe and can cause losses of up to 100% (12). Complex

genotypic structure and rapidly evolving MSV population make it challenging to manage, although host resistance has been well-worked out particularly in Africa (12, 13). Single infection of High Plains wheat mosaic virus (HPWMoV), an emaravirus, and its co-infection with wheat streak mosaic virus (WSMV) in wheat have posed a serious challenge to this staple food (14). In pulses, yellow mosaic disease of mungbean, urdbean, and soybean caused by a begomovirus complex is considered a significant threat (15). The pigeonpea sterility mosaic virus (PPSMV), having five segmented RNA genome has emerged as a major threat to pigeonpea production during the recent two decades (16). Cotton leaf curl disease (CLCuD), caused by genetically distinct virulent strains of begomoviruses, has led to the occurrence of multiple epiphytotic in major cotton growing parts of India, Pakistan, China, and the United States (17). The leaf curl resistance developed by the introgression of two genes in cotton cultivars was broken due to the evolution of recombinant resistance-breaking strain, cotton leaf curl Burewala virus (CLCuBuV) in Pakistan and Indian cotton-growing belts (17–19). Interestingly, resistance-breaking strains quickly replaced the earlier strains and caused havoc to cotton production in this region.

Perennial fruit crop species are infected by several viruses. The citrus tristeza virus (CTV) infection has always remained a major challenge for more than a century due to its complex genetic structure and evolving strains (20). Escape of its detection during initial years in quarantine system, introduction and spread of infected planting materials (rootstocks, grafted trees, and scions), efficient insect vectors, and rapidly evolving CTV genetic variants have made it a virus of global importance. Grapevine being a vegetatively propagated fruit plant has been identified as a sink of the plethora of viruses. Infection of 23 viruses and viroids was identified just from three cultivars (21). Widespread occurrence of grapevine leafroll-associated virus (GLRaV)–3 and –2, grapevine rupestris stem pitting-associated virus (GRSPaV), and hop stunt viroid (HSVd) has emerged in grapevines across the globe (22, 23). In bananas, a group of genetically diverse variants of badnaviruses causing streak disease (BSVs) and banana bunchy top virus (BBTV) are the major threat to its production globally (24–27).

Infection of novel virus species and their variants in apple fruit plants was recorded from different apple-growing parts of the world (28, 29). The genetic variants of apple chlorotic leaf spot virus (ACLSV) and apple stem pitting virus (ASPV) cause the devastating ring-shaped rust and green crinkle disease of fruits, respectively (28). Apple necrotic mosaic virus (ApNMV) and apple hammerhead viroid (AHVd) were recently reported in Indian apple groves (30, 31). Similarly, the infection of multiple viruses in pome and stone fruit crops has emerged in the recent decade. Plum pox virus (PPV), transmitted by various species of aphid vectors and infected propagating materials, has emerged as a significant threat in Europe and Asia (32). A similar threat by viruses of different taxonomic groups was reported in apricot, nectarine, plum, cherry, almond, etc. The symptomless decline is caused by a raspberry bushy dwarf virus (RBDV), and blueberry shock virus (BShV) infection is horizontally transmitted in berries, thus making it difficult to control and could emerge as

a major threat (33, 34). Vegetable and spice crops worldwide are severely affected by the emergence of viral infection. Widespread infection of begomoviruses, particularly tomato yellow leaf curl virus (TYLCV), begomovirus complex in tomato, and begomoviruses, potyviruses in chili has been the ever-emerging constraint in these crops due to the evolution and spread of highly virulent genetic variants (35). They were earlier more prevalent in tropical and subtropical regions but now spreading to other temperate areas. Thrips-transmitted tospoviruses, like tomato spotted wilt virus (TSWV), groundnut bud necrosis virus (GBNV), and capsicum chlorosis virus (CaCV), infecting diverse crops of different plant families, have drawn a concern (36). Cucurbit production is hampered worldwide by several virus diseases caused by potyviruses, cucumoviruses, and other virus groups. Cucumber vein yellowing virus (CVYV) has caused significant havoc in the Middle East and Mediterranean regions (37). The production of cassava, an important staple food for a considerable section of the population in Africa and different parts of other continents, is affected mainly by the evolving begomovirus complex causing cassava mosaic disease (CMD). The spread of CMD-associated begomoviruses (African cassava mosaic virus, Indian cassava mosaic virus, and Sri Lankan cassava mosaic virus) through whiteflies and infected planting materials has caused severe losses in its production (38).

The occurrence, evolution, and emergence of infectious, highly virulent, and pathogenically distinct variants of viruses infecting crops of economic importance have put forth a major burden on global food and nutritional security. The plant host-virus-vector continuum presents a unique combination in the ecosystem, where all three components are continuously evolving under varying natural and posed selection pressure. These reports and experiences of working with viruses showed that dynamically evolving practices of mixed farming, introduction and establishment of new host genotypes/varieties to new geographical pockets, and parallel evolution of vector biotypes put unique synergistic effects on viruses to evolve to more virulent species and strains with higher fitness efficiency in the ecosystem.

An array of genomic tools were invented and utilized to effectively combat the emerging virus and virus-like pathogens associated with epiphytotic. Conventional resistance breeding has seen a paradigm shift to genome-assisted breeding and genetic engineering. The latter involves the wide application of RNAi and virus-derived resistance in the last two decades. These techniques, as a whole, are discussed in brief under the heading “Pre-Genome Editing Era” in this review. In the last decade, virus disease management witnessed a significant shift from the pre-genome editing era to the genome-editing era, where several highly efficient genome editing tools are employed. The subsequent section “Genome Editing Era” of this review discusses the details of genome editing tools and their applications with particular reference to plant virology. We also briefly discuss the parallel applications of genome editing in improving yield and quality that could benefit the global population. The review will provide a holistic view on utilizing and combining the genomic editing technologies to fight the

emerging viruses simultaneously while fulfilling the overall goal of food and nutritional security.

PRE-GENOME EDITING ERA

Plant Breeding and Genomic Techniques for Developing Disease-Resistant Plants

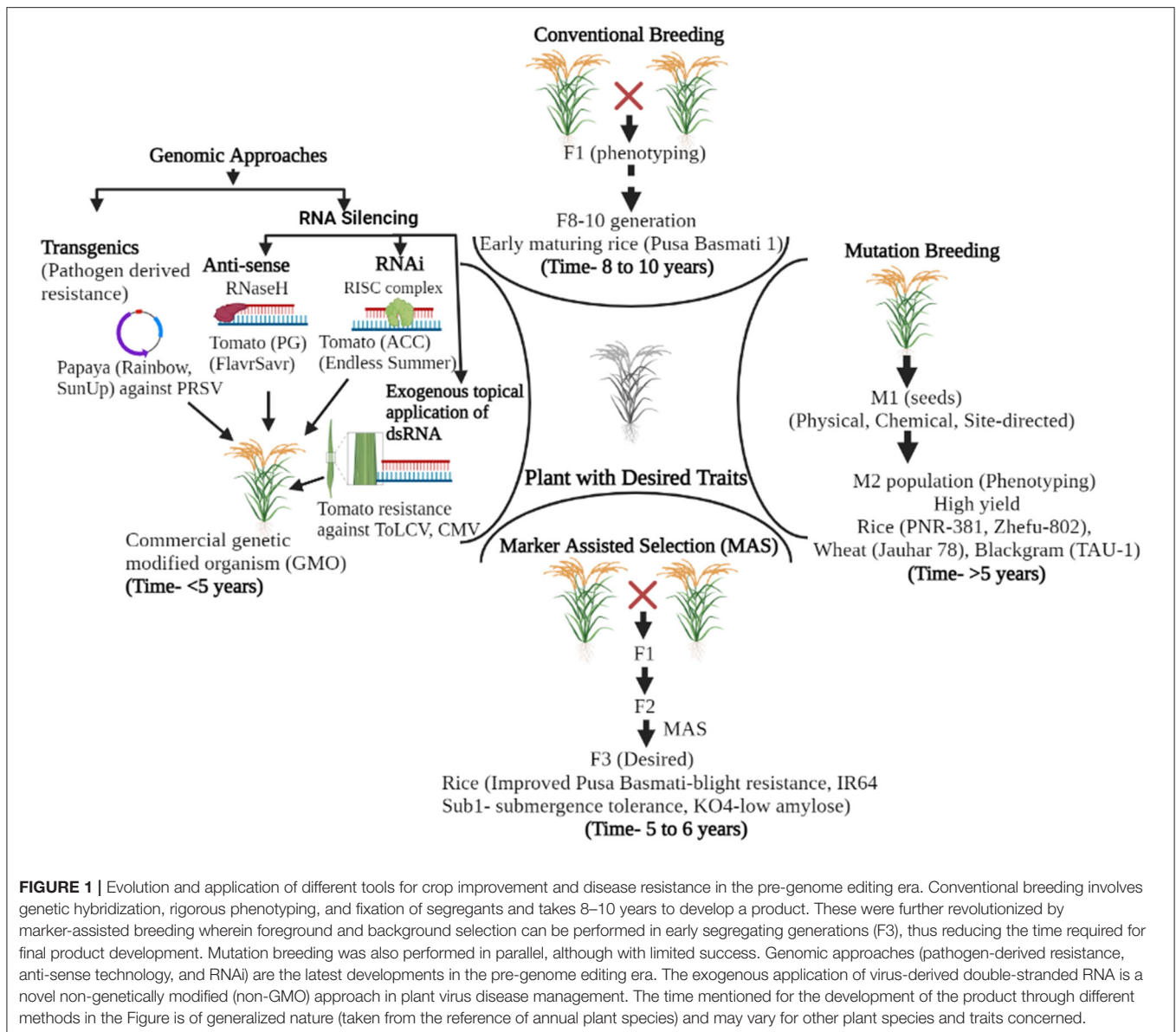
Several management practices were employed to tackle the losses caused by plant pathogens in different agricultural and horticultural crops; genetic resistance signifies the utmost economical tactic among them. The breakthrough in the field of disease resistance came with the understanding of the gene for gene hypothesis (39, 40). Since then, conventional breeding approaches have comprehensively improved plant cultivars and imparted resistance to plant diseases. The drawbacks of cultural practices and chemical control put the concept of resistant varieties on a better front, and plant varieties with genes resistant to viral infections were utilized as the most sustainable route for their management (41, 42). For now, several dominant and recessive genes involved in viral resistance have been identified and isolated for further deployment in viral resistance programs (7, 43). However, with traditional resistance breeding approaches alone, it is challenging for researchers to keep pace with the reckless evolutionary potential of plant viruses and the increasing demand for the development of disease-resistant varieties. In addition, drawbacks, like non-specificity and being time-consuming and laborious, of these technologies make it challenging to develop economically efficient disease-resistant plants at a pace to tackle the evolving plant viruses (Figure 1). Brief illustrations of different methods in the pre-genome editing era and their attributes are presented in Figure 1, Table 1. Mutation breeding, with limited success, was also employed for imparting desired traits in plant species.

The introduction of molecular markers during the 1980s and 1990s has opened new vistas in crop improvement in general and resistance breeding in particular. The progress in DNA molecular markers and the unceasing advancement of molecular techniques have directed the innovation of marker-assisted breeding (MAB). Furthermore, recombinant DNA technology helped transfer foreign DNA into a host through direct gene transfer (physical, chemical, and electrical methods) and indirect methods, which involves *Agrobacterium* as a biological vector (Figure 1). Transgenic approaches have been used against viral diseases in many crops like tomato, potato, rice, legumes, cucurbits, and others (66) (Table 1).

After that, several viral protein-coding genes, such as replicase (Rep), movement protein (MP), and proteases, were employed for pathogen-derived resistance (PDR) (67–69), but the coat protein (CP) approach is preferred over other techniques due to the durability of protection (70). In 1986, Beachy's lab piloted a revolutionary study on coat-protein-mediated resistance against tobacco mosaic virus (TMV) (59), employing the concept of PDR (71). The transgenic tobacco expressing the coat protein prevented the assembly of TMV (59, 72). The successful attempt on the utilization of CP-mediated resistance was also replicated against potato virus X (PVX), alfalfa mosaic virus (AMV),

cucumber mosaic virus (CMV), and tobacco rattle virus (TRV) (72, 73). Similarly, reports on the development of virus-resistant plants using genes like the dominant *SX-5* gene in *Solanum* sp. resulted in tomato spotted wilt virus-resistant plants (74). Cosson et al. (75) stated that proteins encoded by dominant resistance genes like *RTM1* and *RTM2* were involved in suppressing the movement of tobacco etch virus (TEV) in several genotypes of *Arabidopsis*. The recessive genes *rym4/5* in *Hordeum vulgare* were reported to confer resistance to barley yellow mosaic virus (BaYMY) (76). Hart and Griffiths (77) highlighted the association of the *bc-3* gene of *Phaseolus vulgaris* in exhibiting resistance to clover yellow vein virus (CYVV).

Although the transgenic approach has yielded promising results in conferring virus resistance in plants, strict regulatory guidelines for the commercial cultivation and instability of the transgene were the associated limitations (Figure 1). Hence, only a limited area is cultivated under genetically modified (GM) crops (190.4 million hectares) (78). Classical examples of commercial GM crops include the Rainbow and Sun-Up varieties of papaya against papaya ringspot virus (PRSV) and GM squash variety against three different mosaic viruses that were released in the United States (14, 79, 80) (Table 1, Figure 1). The transgenic approach provides durable resistance but is not widely accepted due to apprehensions of adverse effects on untargeted organisms, interference with the function of other essential genes due to insertion of transgenes, being costly, and other regulatory issues. Recently, RNA silencing (RNAi or post-transcriptional gene silencing; PTGS) has evolved as a practical measure against viral diseases. RNA silencing leads to antiviral defense in plants in response to virus infections (81). RNAi is triggered by dsRNA, resulting in high efficiency of gene silencing through specific RNA degradation (82) (Figure 1, Table 1). So far, RNA silencing technology has successfully been applied to target over 60 species of economically important plant viruses. In the bean common mosaic virus (BCMV), two genomic regions (N1b and CP) were targeted, which induced a protection level of about 85–92% in *N. benthamiana* and cowpea through RNAi-inducing dsRNA molecules (83). RNAi-inducing constructs targeting the CP coding region of plum pox virus (PPV) (84), P3 coding region of soybean mosaic virus (SMV) (85), CP coding region of sorghum mosaic virus (SrMV) (86), and proteinase co-factor coding region of cowpea severe mosaic virus (CPSMV) (87) have been used successfully to develop transgene-free virus-resistant plants. MicroRNA (miRNA)-guided silencing was also performed to control virus infection in plants (88). Jiang et al. (89) reported that miRNAs regulated the defense system in *Nicotiana benthamiana* upon co-infection of tobacco curly shoot virus (TbCSV) and its betasatellite (TbCSB). In rice, the expression of miR319 targeting the *TCP21* gene positively acts as plant defense against rice ragged stunt virus (RRSV) infection (90), while in cotton plants, the symptom expression of cotton leafroll dwarf virus (CLRVDV) was correlated with the downregulation of specific miRNAs (91). Although these approaches were successful in various plant virus-host combinations, issues of being laborious, time-consuming, expensive, and associated public acceptance hindered their wide popularity and acceptability (Figure 1, Table 1). The exogenous



application of naked dsDNA, proven to trigger the RNA silencing pathway against pathogenic viruses, was then attempted (92–94). This dsRNA application approach, although non-transgenic, also has the limitation of having a short virus protection window of only 5 days post application. Most dsRNA-based strategies have been principally limited to either the laboratory stages or restricted field trials (Figure 1). Recently, the topical application of clay-based delivery of pathogen-specific dsRNA has given an affirmative inflection point toward RNAi. The virus-specific dsRNA coated with layered double hydroxide clay nanosheets was successfully employed for virus protection by spraying on the plants and providing prolonged protection. With just a single application of bioclay, the plants were protected from cucumber mosaic virus (CMV) infection in cowpea, and pepper mild mottle virus (PMMoV) infection in tobacco. Recent work in different laboratories to prolong the durability of dsRNA using

nano bioclay and their field applications for achieving durable protection against plant viruses has opened new avenues. This approach of translating bioclay-based dsRNAs for viral disease control seems to have a massive potential in the future.

GENOME-EDITING ERA

Genome editing refers to the process of inducing a precise variation in the targeted part of a genome. The tools used for inducing such targeted mutations are known as genome/gene editing techniques (GETs), which offer precise modification in different forms such as insertions and/or deletions (indels) or base substitutions in target sequences (95). Different genome editing tools, such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases

TABLE 1 | Different plant breeding techniques used to develop virus-resistant plants and their drawbacks.

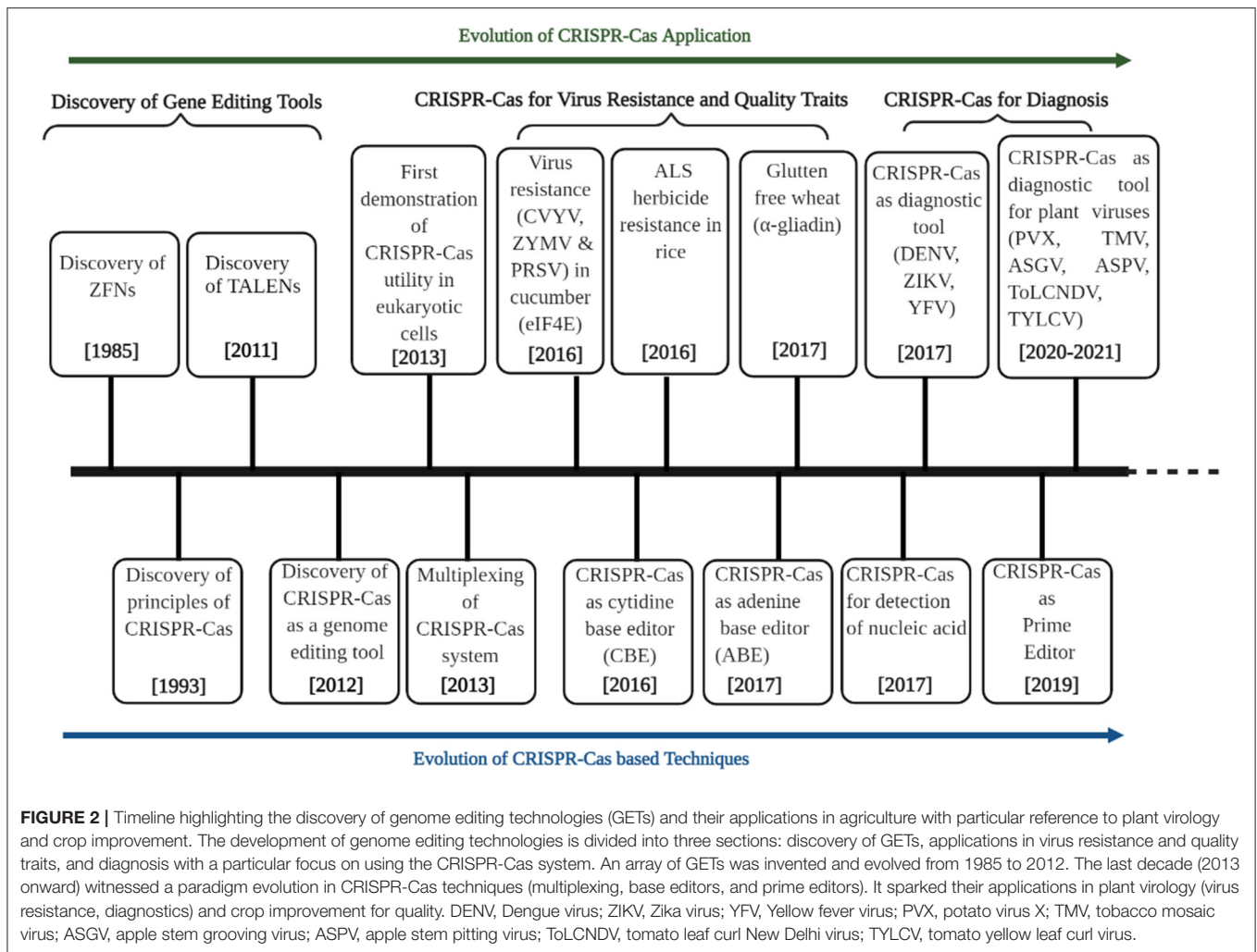
Techniques	Examples	Drawbacks	References
Gene introgression: Transfer of resistance genes into susceptible host species from wild species	Potato virus X (PVX), potato virus Y (PVY) and potato leaf roll virus (PLRV) in <i>Solanum tuberosum</i>	<ul style="list-style-type: none"> • Incompatibility between species due to ploidy level and endosperm balance number • Pre and post fertilization barriers 	(44)
Wide hybridization through bridging species	PLRV, PVY and PVX from the non-tuberous <i>S. brevidens</i> into <i>S. tuberosum</i>	<ul style="list-style-type: none"> • Time-consuming • Low efficiency • Resources demanding 	(45, 46)
Mutation breeding Involving physical and chemical mutagens	Mungbean yellow mosaic virus (MYMV) in mungbean and soybean	<ul style="list-style-type: none"> • Less frequency of desirable mutations • Mostly recessive in nature • Pleiotropic effects 	(47, 48)
Meristem-Tip Culture <i>In vitro</i> culture of shoot tip/apex from mother plant to eradicate viruses associated with phloem	<ul style="list-style-type: none"> • Sugarcane yellow leaf virus (SCYLV) in sugarcane • Peanut stripe virus (PStV) in patchouli • Piper yellow mottle virus (PYMoV) in black pepper • Sugarcane mosaic virus (SCMV), chlorotic streak disease, white leaf disease 	<ul style="list-style-type: none"> • Costly approach • Problem of acclimatization • Development of variability • Cultural contamination 	(49–52)
Somatic hybridization via protoplast fusion	PLRV, PVY, and PVX from <i>S. brevidens</i> into <i>S. tuberosum</i> hybrids	<ul style="list-style-type: none"> • Identification problem • Genetic instability 	(53–55)
Marker assisted breeding	<ul style="list-style-type: none"> • Tobacco mosaic virus (TMV) and bamboo mosaic virus (BMV) in tobacco • Rice yellow mottle virus (RYMV) • Barley yellow mosaic virus (BaYMV) in winter barley crops • Soybean mosaic virus (SMV) 	<ul style="list-style-type: none"> • High cost • Low reliability 	(56–58)
Genetic engineering			
Pathogen derived resistance	<ul style="list-style-type: none"> • Tobacco mosaic virus (TMV) • Plum pox virus (PPV) 	Legislation problems related to biosafety issues	(59, 60)
RNA silencing	<ul style="list-style-type: none"> • Papaya ringspot virus (PRSV) • Maize streak virus (MSV) • Banana bunchy top virus (BBTV) • Tomato yellow leaf curl virus (TYLCV) 	Difficulty in evaluating resistance efficiency	(61–64)
Cross protection	Papaya ringspot virus (PRSV)	Exact molecular mechanism is unclear	(65)

(TALENs), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR-Cas) systems were developed (96) (Figure 2). This section of the review focuses on CRISPR-based genome editing and its mechanism and subsequently discusses its applications in detail.

CRISPR-Cas System: An Efficient Tool for Targeted Gene Editing

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated-(Cas) system consists of two parts: CRISPR array and operon of CRISPR-associated (Cas) genes. CRISPR array is a region in the bacterial genome with short and palindromic DNA repeats with spacer DNAs in between. Spacers in the CRISPR array represent the immunological memory of earlier infections (97). Ishino discovered it first in 1987 in *Escherichia coli* as a defense mechanism against viruses, foreign DNA/RNA, and mobile genetic elements (98). However, Mojica took a sincere note of such repeats and named them first as short regularly spaced repeats (SRSRs) and later as CRISPR (99–101). Its function in providing adaptive immunity was also hypothesized by Lander (100) and Mojica et al. (101). Jansen et al. (99) identified Cas genes associated with DNA repeats in prokaryotes. Later discoveries suggested that the proteins expressed by these Cas genes play an important role in tandem

with the CRISPR array, in providing immunity (Figure 2). It was found that during the first attack of any foreign viral or plasmid DNA, Cas proteins help in targeting the specific segment of invading DNA to be inserted in the array. A complex of Cas proteins starts surveillance for a specific short 2–4-bp motif in the target DNA molecule, known as a protospacer adjacent motif (PAM). The Cas proteins, when coming across such a distinct motif, introduce a double-strand break (DSB) in the target DNA and release a protospacer segment that gets inserted between the two repeats of the array to become an additional spacer. These DSBs are induced by utilizing site-specific nucleases, and desired modifications get repaired through an error-prone endogenous DSB repair machinery. Among different site-specific nucleases, CRISPR-Cas9 is more appealing, because it can simultaneously modify several plant genes (102–106). There are exceptions where some CRISPR-Cas systems acquire a spacer, i.e., from the RNA transcript of the invading DNA using a reverse transcriptase enzyme encoded in the CRISPR-Cas locus and most often fused to the Cas1 protein. This process of acquiring a specific segment of invading DNA from the CRISPR array is known as adaptation. The Cas1 and Cas2 proteins play a role in the adaptation process. Cas1 protein cleaves the protospacer containing target DNA and CRISPR array, whereas Cas2 provides structural support to the complex. However, in some CRISPR-Cas systems, there is an



involvement of additional Cas proteins (107). In the next step, the CRISPR array gets transcribed to a long pre-CRISPR RNA (pre-crRNA), which, after processing, gets converted into smaller mature crRNAs. The crRNAs are then assembled with one or more Cas proteins into CRISPR ribonucleoprotein (crRNP) complexes. The final stage of the CRISPR-Cas-mediated immune response is interference wherein crRNA-directed cleavage of invading viral or plasmid DNAs occurs. In this stage, the crRNA that remains bound to the crRNP acts as the guide to identify the protospacer sequence in the invading viral or plasmid genome. Once the recognition is met, the invading DNA is cleaved and inactivated by a Cas nuclease (107).

Clustered regularly interspaced short palindromic repeats (CRISPR) can be found in both nucleoids and plasmids. A CRISPR locus contains an array of short repeated sequences (21–48 bp) intervened by spacer sequences (26–72 bp) that are often acquired from plasmids and viruses. The natural mechanism of the immune system can be divided into three stages: adaptation, expression, and interference (108). Based on signature protein, there are six types of systems: types I, II, III, IV, V, and VI. Many initial studies were carried out to employ this bacterial

immune system in genome editing tools. In the type II system, Cas9 alone can degrade an invading DNA that complements a single guide RNA. The CRISPR-Cas9 type II bacterial immune system came into the limelight in 2005, with the discovery of the extrachromosomal origin of spacer sequence (109). The ability of targeted genome editing of the CRISPR-Cas9 system is due to the structure and conformation of the Cas9 protein. Cas9 is a bilobed protein containing a large recognition lobe (RecA) and a small nuclease lobe (NUC) connected by a helix bridge. The nuclease lobe has two nuclease domains, RuvC and HNH, and a PAM-interacting domain (110). The Cas9-sgRNA complex scans the pairing site between sgRNA and targeted DNA. As it finds the target site, cleavage of RNA-DNA hybrid occurs, HNH is responsible for cleaving the target site, and RuvC cleaves other non-target sites, resulting in double-strand break (DSB). DSB is repaired by a non-homologous end joining (NHEJ) and homology-directed repair (HDR) mechanism, which causes insertion/deletion (INDEL) and frame-shift mutations with just a few base pair (bp) variance, resulting in premature translation termination and loss of function (111, 112). Compared to other genome editing tools like zinc finger nucleases (ZFN)

and transcription activator-like effector nucleases (TALENs), CRISPR-Cas9 is easier to multiplex and design the target construct, as it is an RNA-based approach and it does not work in pairs (113) (**Figure 2**).

Among various genome editing tools, the CRISPR-Cas system is the most popular due to its advantages over other contemporary tools. As much advanced research on the CRISPR-Cas system has been conducted, many modified versions of the CRISPR-Cas system have also come up (**Figure 2**). Based on the effector nuclease gene's functionality, the CRISPR-Cas system is divided into two classes. Class I includes types I, III, and IV, and Class II includes types II, V, and VI. The main drawback of the CRISPR-Cas system is the off-target issue, which needs to be taken care of *via* off-target detection and high-fidelity editing. Off-target analysis can be performed using *in silico* tools like Cas-OFFinder, Guide-seq, and Digenome-seq (114). To reduce the chances of off-targets in the CRISPR-Cas system, Cas proteins or guides (gRNAs) need to be engineered. In addition to this, improvement of non-specific base editing is also required (e.g., cytosine/adenine base editor) (115). The large size of Cas protein, however, poses limitations in insert gene size for gene delivery system. To address this limitation, lightweight members like Cas14 are considered the best option (116). For efficient delivery, viral vector systems, such as adeno-associated viruses (AAVs), were employed. The range of editable targets can be expanded using PAM variants, as each Cas protein prefers its PAM sequence, e.g., CRISPR-Cas type II recognizes a G-rich sequence, whereas type V recognizes a T-rich sequence, respectively (117). The science of CRISPR-Cas has evolved at a much greater pace. In addition to discovering an array of sequence-specific nucleases, the options of CRISPR-Cas-led multiplex target have sparked its applications in agriculture (113, 118) (**Figure 2**). The recent discovery of Cas9-based editing tools known as based editors (BEs) and prime editors (PEs) could lead to desired changes in the target genome with DSB and offers a 10–100-fold higher efficiency in obtaining the desired mutations up to single-base-resolution provided for more flexible applications of the CRISPR-Cas system (118, 119) (**Figure 2**).

To effectively use CRISPR-Cas-based gene-editing tools, information on molecular functions of target genes and genome sequences is a prerequisite. With the revolution of genomics, genomes sequences of many crop species have been deciphered, and genes associated with traits of economic interest have been characterized. Once the function of a gene is identified, it can then be subjected to targeted genome manipulation using the CRISPR-Cas system (120). After modifications are made, it is crucial to identify the edited plants by comparing them to wild types.

Identification of CRISPR-Cas-Mutated Plants: Techniques and Methodologies

Once a targeted modification is induced in a genetic locus using the CRISPR-Cas system, it is crucial to ascertain the mutants. To quickly detect/identify CRISPR-Cas induced mutations, various molecular methods have been developed, such as enzymatic mismatch cleavage (EMC), high-resolution melting

curve analysis (HRMA), modified migration-based heteroduplex mobility assay (HMA), and traditional polymerase chain reaction (PCR) combined with ligation detection reaction (LDR) (121–123). These methods are discussed in the next part of this section.

Enzymatic Mismatch Cleavage (EMC)

Enzymatic mismatch cleavage (EMC) is the most widely used technique to confirm site-specific editing in CRISPR-mutated plants. It takes advantage of enzymes that can cleave hetero-duplex DNA at mismatches created by single or multiple nucleotides (122). This method is more suited for larger indels, as its cleavage efficiency is affected by several factors, such as sequence, flanking sequence among two DNA strands, and length of mismatch pairs (122, 124). Furthermore, although it is simple to use, its sensitivity is relatively poor (125), and it cannot discriminate homozygous and heterozygous mutants from wild-type and biallelic mutants, respectively (126). Endonuclease enzymes, T7 endonuclease 1 (T7E1), and surveyor nuclease are mainly utilized in the EMC assay to cleave one or more base pair mismatches in the heteroduplex DNA, and agarose gel electrophoresis may then be used to examine mutations that occur from these minor mismatches (122).

High-Resolution Melting Curve Analysis (HRMA)

High-resolution melting curve analysis (HRMA) is a fluorescence-based technique that involves quantitative-PCR (qPCR) amplification of DNA sequences covering around 90–200 bp of the genomic target, fluorescent dye incorporation, and amplicon melt curve study. HRMA analyzes the melting activity of hetero-duplex and homoduplex DNA fragments to determine the melting temperature (T_m) of a specific PCR component and identify the mutant (121). Since the process is non-destructive, the whole procedure of preparing genomic DNA and detecting mutations takes <2 h. HRMA is a simple method and compatible with the high-throughput screening format (96-well-microliter plates). It is fast, unrestrictive, and suitable for detecting low-level chimeric mutants and single nucleotide polymorphisms (SNPs), but it requires special software and is not ideal for broad indel (> 100 bp) detection (121, 123, 127).

Multiplex Ligation-Dependent Probe Amplification (MLPA)

In conventional PCR, in conjunction with ligation detection reaction (LDR) assay, two pairs of primers are generally used for each target amplification and visualized by gel electrophoresis. However, since this method relies on indel detection by agarose gel electrophoresis, its sensitivity to detect mutants with just a few base pair genetic variations is limited, while in the amplicon labeling-based method, i.e., multiplex ligation-dependent probe amplification (MLPA), tri-primers (additional universal 6-FAM 5'-labeled) were utilized for the target amplification and detected by DNA capillary electrophoresis. The MLPA-based method allows for the detection of CRISPR-Cas9-induced on- and off-target mutations (Indel) and naturally occurring mutations. Additionally, an MLPA-based assay can accurately define indel sizes down to 1 bp and handle high throughput analysis (128).

Quantitative PCR (qPCR)

Quantitative polymerase chain reaction (qPCR) is a fast, simple, and effective way to detect CRISPR-Cas-induced mutations by amplifying a target locus and sequencing amplified products. The method of using qPCR to differentiate homozygous and heterozygous mutations has been validated in several plant species (129).

Whole Genome Sequencing (WGS)

Whole genome sequencing (WGS) is a highly efficient method for detecting many mutations, such as structural variations, large deletions, insertions, duplications, rearrangements, small indels, SNPs, and on- and off-target mutations induced by CRISPR-Cas in various crops (130). In addition, WGS is effective in detecting low-frequency mutations by utilizing high sequencing depth (123).

Other reported methods include restriction fragment length polymorphism (RFLP) (131), PCR based on two primer pairs (132), tracking of indels by decomposition (TIDE) (133), and CRISPR genome analyzer (CRISPR-GA) (134). Some recently developed methods include PCR coupled with ligation detection reaction (PCR-LDR), annealing at critical temperature PCR (ACT-PCR) (135), indel detection by amplicon analysis (IDAA) (136), cleaved amplified polymorphic sequence (CAPS) (137), and mutation site-based specific primer PCR (MSBSP-PCR) (138). However, most of the developed methods are expensive (PCR and qPCR), sensitive, time-consuming (Sanger sequencing, ACT-PCR, and MSBSP-PCR), less accurate (low detection specificity in CAPS), and unable to detect more significant indel mutations (131, 133). Recently developed methods (WGS and MLPA) can detect natural mutations in addition to -on and off-target mutations (139).

CRISPR-CAS: ITS APPLICATION IN PLANT VIRUS RESISTANCE AND FOOD SECURITY

CRISPR-Cas for the Development of Virus-Resistant Plants

Research efforts on imparting durable resistance to viral infection have recently been reoriented toward genome editing technologies due to their efficiency in creating precise and desired variations in selected loci of plants or viral genomes (118, 120). Among the arrays of genome editing tools, CRISPR-Cas has become more trendy for the development of virus resistance in crops than other tools due to its advantages in terms of targeted genome manipulation and designing (120). The CRISPR-Cas technology has recently been successfully used in many crop species for the engineering of virus-free plants. It is easy to use, has a particular target site of about 20–23 bp, and is easier to predict off-target mutagenesis than RNA-DNA interaction (120, 140). The CRISPR-Cas technique was employed mainly in two ways to develop viral resistance in plants, either by introducing targeted mutations into specific host plants suppressing its susceptibility genes (pro-viral factors) or directly targeting viral genomes (141) (**Figure 3**). Viruses have either DNA or RNA as

genetic material. Around 75% of plant viruses comprise single-stranded RNA (ssRNA) genomes followed by ssDNA and a few double-stranded DNA or RNA viruses (**Figure 3**). A classical study was conducted to engineer the resistance against DNA viruses, beet severe curly top virus (BSCTV), and bean yellow dwarf virus (BeYDV), in *Nicotiana benthamiana* and *Arabidopsis thaliana* through CRISPR-Cas approach (**Table 2**) (146, 147). BSCTV- and BeYDV-resistant plants were developed by the sgRNA and Cas9 constructs targeting the coding replication-associated protein (Rep protein) gene and the non-coding intergenic region (IR). Simultaneously, Ali et al. (145) reported on the engineering of sgRNA molecules targeting the intergenic region (IR), coat protein (CP), and metal-binding site involved in protein conformation and DNA cleavage (RCRII) of the bipartite begomovirus, tomato yellow leaf curl virus (TYLCV), in the model plant *N. benthamiana*. The mutant plants showed a reduction in the viral load along with enhanced viral resistance. The generated TYLCV-resistant plants were also reportedly resistant against a mixed infection of monopartite beet curly top virus (BCTV) and bipartite merremia mosaic virus (MeMV) (145). A tobacco plant expressing Cas9 and dual sgRNA targeting two important crucial regions of monopartite cotton leaf curl Multan virus (CLCuMuV) genome (Rep and IR) resulted in complete resistance to the virus infection. Although most studies on the development of resistant plants against geminivirus were reported in model plants, Kis et al. (144) reported the production of wheat dwarf virus (WDV)-resistant barley plants targeting the coding (MP, CP, and Rep) and a non-coding region (IR). In conformity, Tashkandi et al. (149) also reported the development of TYLCV-resistant tomato plants targeting the Rep and CP regions of the viral genome. Although the targeted coding regions in the reported studies yielded promising results, Mehta et al. (161) reported unsuccessful attempts on gaining resistance against African cassava mosaic virus (ACMV) in cassava where conserved single nucleotide mutation led to CRISPR resistance in the edited plants. Such mutations are potentially harmful, as they can lead to the development of more potent and virulent viruses.

The CRISPR-Cas system has also been effectively employed against several RNA viruses using Cas endonucleases (FNCas9 and Cas13) (**Figure 3**). Zhang et al. (150) reported the first RNA virus-resistant plants of tobacco and *Arabidopsis* targeting various regions of cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) genomes where the mutant plants showed lesser accumulation of the viruses along with reduced symptom expression (**Table 2**). Concurrently, turnip mosaic virus (TuMV)-resistant tobacco plants edited using the Cas13 system targeting various coding regions (HC-Pro and CP) of the viral genome resulted in reduced viral load and symptom expression (151). Similar attempts to develop resistance to potato virus Y (PVY), rice stripe mosaic virus (RSMV), and Southern rice black-streaked dwarf virus (SRBSDV) have been reported on potato and rice. Therefore, Cas endonucleases (FNCas9 and Cas13) have been proven a powerful tool directly targeting viral RNA in engineered virus-resistant plants but following a transgenic-based approach (**Figure 3**). However, studies targeting the *eIF4E* gene, also known as the cap binding protein using the Cas9 system, reported transgene-free resistance

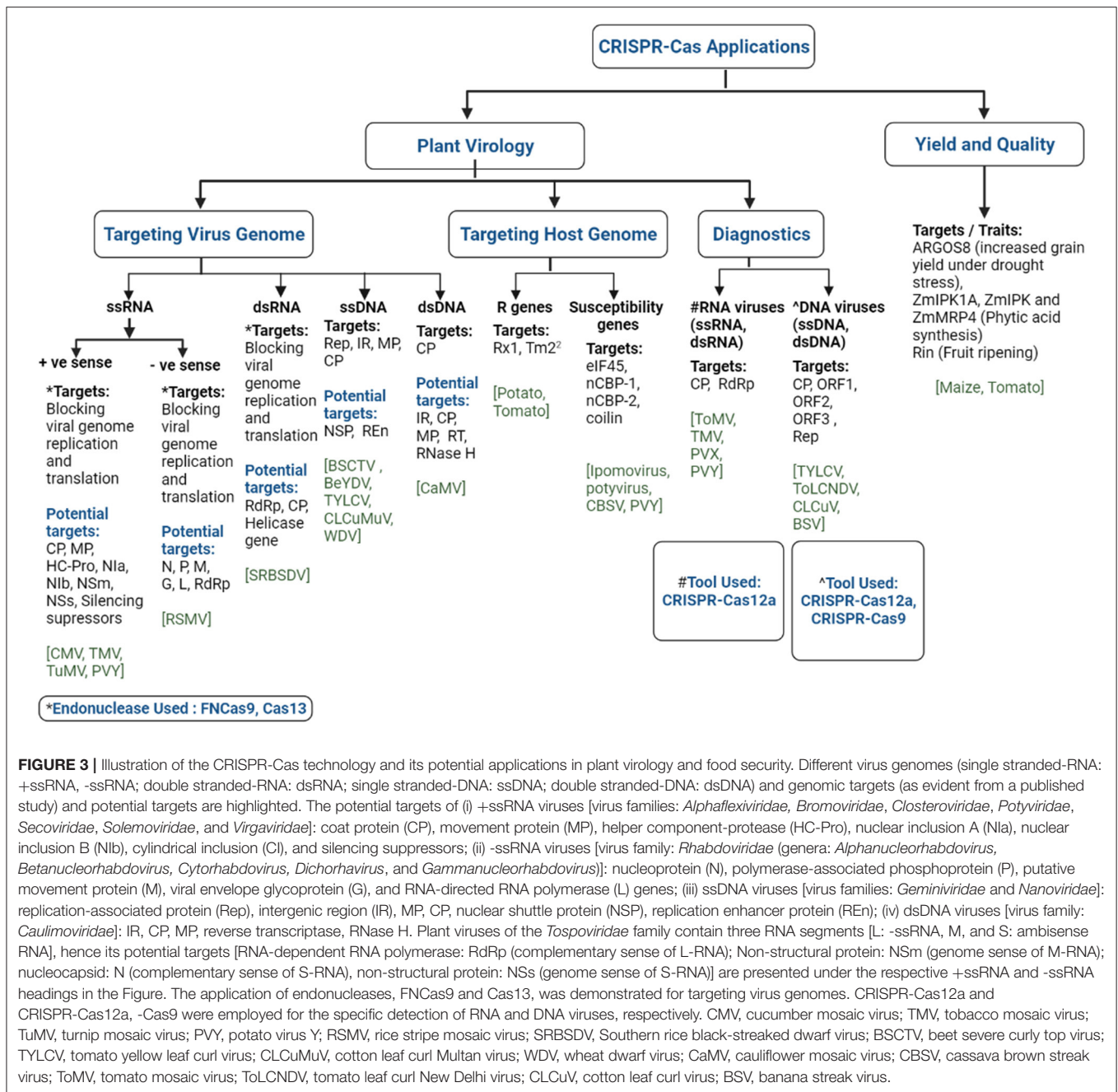


FIGURE 3 | Illustration of the CRISPR-Cas technology and its potential applications in plant virology and food security. Different virus genomes (single stranded-RNA: +ssRNA, -ssRNA; double stranded-RNA: dsRNA; single stranded-DNA: ssDNA; double stranded-DNA: dsDNA) and genomic targets (as evident from a published study) and potential targets are highlighted. The potential targets of (i) +ssRNA viruses [virus families: *Alphaflexiviridae*, *Bromoviridae*, *Closteroviridae*, *Potyviridae*, *Secoviridae*, *Solemoviridae*, and *Virgaviridae*]: coat protein (CP), movement protein (MP), helper component-protease (HC-Pro), nuclear inclusion A (NIa), nuclear inclusion B (NIb), cylindrical inclusion (CI), and silencing suppressors; (ii) -ssRNA viruses [virus family: *Rhabdoviridae* (genera: *Alphanucleorhabdovirus*, *Betanucleorhabdovirus*, *Cytorhabdovirus*, *Dichorhavirus*, and *Gammanucleorhabdovirus*)] nucleoprotein (N), polymerase-associated phosphoprotein (P), putative movement protein (M), viral envelope glycoprotein (G), and RNA-directed RNA polymerase (L) genes; (iii) ssDNA viruses [virus families: *Geminiviridae* and *Nanoviridae*]: replication-associated protein (Rep), intergenic region (IR), MP, CP, nuclear shuttle protein (NSP), replication enhancer protein (REn); (iv) dsDNA viruses [virus family: *Caulimoviridae*]: IR, CP, MP, reverse transcriptase, RNase H. Plant viruses of the *Tospoviridae* family contain three RNA segments [L: -ssRNA, M, and S: ambisense RNA], hence its potential targets [RNA-dependent RNA polymerase: RdRp (complementary sense of L-RNA); Non-structural protein: NSm (genome sense of M-RNA); nucleocapsid: N (complementary sense of S-RNA), non-structural protein: NSs (genome sense of S-RNA)] are presented under the respective +ssRNA and -ssRNA headings in the Figure. The application of endonucleases, FNCas9 and Cas13, was demonstrated for targeting virus genomes. CRISPR-Cas12a and CRISPR-Cas12a, -Cas9 were employed for the specific detection of RNA and DNA viruses, respectively. CMV, cucumber mosaic virus; TMV, tobacco mosaic virus; TuMV, turnip mosaic virus; PVY, potato virus Y; RSMV, rice stripe mosaic virus; SRBSDV, Southern rice black-streaked dwarf virus; BSCTV, beet severe curly top virus; TYLCV, tomato yellow leaf curl virus; CLCuMuV, cotton leaf curl Multan virus; WDV, wheat dwarf virus; CaMV, cauliflower mosaic virus; CBSV, cassava brown streak virus; ToMV, tomato mosaic virus; ToLCNDV, tomato leaf curl New Delhi virus; CLCuV, cotton leaf curl virus; BSV, banana streak virus.

against RNA-based ipomovirus and potyvirus in cucumber and Arabidopsis, respectively (156, 157). Likewise, Gomez et al. (158) reported resistance against cassava brown streak virus (CBSV) attained by targeting cap-binding protein-1 (nCBP-1) and nCBP-2 through CRISPR-Cas9 in cassava. Similarly, a PVY-resistant potato variety was developed targeting the *Coilin* gene using the CRISPR-Cas9 system, and led to virus resistance and enhanced tolerance to salt and osmotic stress (160). Studies have also reported the utilization of the *Dicer-like2* gene to understand its role in plant defense mechanisms. These studies demonstrated the exciting potential of the CRISPR-Cas system as a powerful

tool in developing resistance against plant viruses. The different genomic regions of RNA and DNA viruses that have a role in the infection cycle could be the potential target for CRISPR-Cas-based modification to confer resistance to infection (Figure 3).

CRISPR-Cas for Plant Virus Disease Diagnosis

Timely, accurate, and sensitive detection of viruses causing diseases in plants is key in their mitigation and management. Lately, there has been tremendous growth in viral disease

TABLE 2 | List of virus-resistant plants generated using CRISPR-Cas system.

Virus genome	Plant	Target sequences	System	Mutation	Target pathogen	Result	References
DNA	<i>N. benthamiana</i>	IR and C1(Rep) genes	SpCas9	In-dels	CLCuMuV	Complete resistance to CLCuMuV	(142)
DNA	<i>Musa</i> spp.	eBSV Sequence	SpCas9	In-dels	BSV	Inactivation of eBSV gave asymptomatic plants	(143)
DNA	<i>Hordeum vulgare</i>	MP, CP, Rep/Rep, IR	SpCas9	Insertion	WDV	No disease symptoms and virus presence	(144)
DNA	<i>N. benthamiana</i>	IR, CP, RCR11	SpCas9	In-dels accumulation	TYLCV	No disease symptoms and delayed or reduced virus	(145)
DNA	<i>N. benthamiana</i>	Rep/RepA genes, LIR	SpCas9	In-dels	BeYDV	Reduced virus load and symptoms	(146)
DNA	<i>N. benthamiana</i> and <i>Arabidopsis thaliana</i>	CP, Rep genes, IR	SpCas9	In-dels	BSCTV	Geminivirus-resistant plants of both tobacco and <i>Arabidopsis</i>	(147)
DNA	<i>N. benthamiana</i>	IR, CP, Rep	SpCas9	In-dels	CLCuKoV, TYLCV, MeMV	Durable resistance to virus infection	(148)
DNA	<i>N. benthamiana</i> and <i>Solanum lycopersicum</i>	CP, Rep	SpCas9	In-dels	TYLCV	Disease resistance developed	(149)
RNA	<i>N. benthamiana</i> and <i>Arabidopsis thaliana</i>	ORF1a, ORF2, ORF3, CP and 3'-UTR	Fncas9	No cleavage	CMV	Reduced virus load and symptoms	(150)
RNA	<i>N. benthamiana</i>	GFP, HC-Pro and CP	LshCas13a	N.D.	TuMV	Reduced virus load and appearance	(151)
RNA	<i>Oryza sativa</i>	Various sequences in SRBSDV and RSMV genomes	LshCas13a	Cleavage	SRBSDV, RSMV	Mild symptoms with reduced viral load	(152)
RNA	<i>N. benthamiana</i>	Various sequences in TMV genome	LshCas13a	Cleavage	TMV	Reduced viral load	(152)
RNA	<i>Solanum tuberosum</i>	P3, CI, Nib, CP genes	LshCas13a	N.D.	PVY	Resistance to PVY	(153)
RNA	<i>Solanum lycopersicum</i>	siDCL2	CRISPR-Cas9	In-dels	ToMV, PVX, TMV	Mutants showed enhanced resistance to virus infection	(154, 155)
RNA	<i>Cucumis sativus</i>	<i>elf4E</i> /cfs region	SpCas9	Deletions	CVYV, ZYMV, PRSV-W	Resistance to CVYV, ZYMV and PRSV-W	(156)
RNA	<i>Arabidopsis thaliana</i>	<i>elf(iso)4E</i> /cfs region	SpCas9	In-dels	TuMV	Potyvirus resistant plants	(157)
RNA	<i>Manihot esculenta</i>	<i>nCBP-1</i> and <i>nCBP-2</i> /cfs region	SpCas9	In-dels	CBSV	Reduced virus load and symptoms	(158)
RNA	<i>Arabidopsis thaliana</i>	<i>elf4E</i> /cfs region	SpCas9-cytidine deaminase	Point mutation	CIYV	Prevent virus accumulation	(159)
RNA	<i>Solanum tuberosum</i>	<i>Coilin</i> gene	SpCas9	N.D.	PVY	Resistance to virus infection; tolerance to salt and osmotic stress	(160)

IR, intergenic region; Rep, replicase; LIR, long intergenic region; In-del, insertion and deletion; N.D., not defined; CLCuMuV, cotton leaf curl Multan virus; BSV, banana streak virus; WDV, wheat dwarf virus; TYLCV, tomato yellow leaf curl virus; BeYDV, bean yellow dwarf virus; BSCTV, beet severe curly top virus; CLCuKoV, cotton leaf curl Kokhran virus; CMV, cucumber mosaic virus; TuMV, turnip mosaic virus; SRBSDV, southern rice black-streaked dwarf virus; RSMV, rice stripe mosaic virus; TMV, tobacco mosaic virus; PVY, potato virus Y; ToMV, tomato mosaic virus; PVX, potato virus X; CVYV, cucumber vein yellowing virus; ZYMV, zucchini yellow mosaic virus; PRSV-W, papaya ringspot virus-W; CBSV, cassava brown streak virus; CIYV, clover yellow vein virus.

diagnosis by detecting targeting nucleic acids using CRISPR-Cas based platforms, which are a robust tool compared to other known common diagnostic platforms. The first CRISPR-Cas-based diagnosis was performed using CRISPR-Cas9 endonucleases recognizing the double-stranded DNA (dsDNA) (162) (**Figure 3**). Recent studies reported the use

of CRISPR-associated Cas systems viz., Cas12a, Cas13a, and Cas14, for nucleic acid detection (163–165). Gootenberg et al. (164) developed a rapid and sensitive nucleic acid detection method using the CRISPR effector Cas13a combined with the isothermal amplification method named Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK). The

developed method could detect DNA or RNA at attomolar concentrations and even with single-base mismatch specificity. The first Cas12a endoribonuclease-based detection method, referred to as DNA endonuclease-targeted CRISPR trans reporter (DETECTR), was used to guide dsDNA targets by crRNA triggering collateral cleavage of short ssDNA carrying a quencher and a fluorophore leading to target recognition *via* generation of fluorescent signal upon target recognition and subsequent reporter cleavage (163). One-Hour Low-cost Multipurpose highly Efficient System (HOLMES) utilizes the Cas-12a effector system combined with loop-mediated isothermal amplification (LAMP) capable of fast and highly sensitive detection of target DNA and RNA (166). In other approaches, a sample is amplified to enrich the target DNA using recombinase polymerase amplification (RPA) reactions or reverse-transcription-recombinase polymerase amplification (RT-RPA) reactions when the target is RNA. The RPA product is then transcribed into RNA using a T7 RNA polymerase. The transcripts obtained are subjected to collateral cleavage with Cas12/13 in the presence of a quencherable reporter ssRNA, and fluorescence is quantified. SHERLOCK, DETECTR, and HOLMES are highly specific and provide attomolar sensitivity in detecting viruses, microorganisms, and transgenes (167–170). The CRISPR-based virus detection shows a vast prospective, but its potential is still not fully utilized. Recent years have witnessed several reports on the development and application of CRISPR-based diagnostics for the robust detection of plant viruses (Table 3, Figure 3). Gootenberg et al. (179) developed a rapid, inexpensive, and sensitive lateral flow “paper strip” test method for application in reliable on-site detection of plant viruses. A one-step-RT-RPA-Cas12a assay for the detection of plant viruses was used (175). The study reports on the development of a one-step *in vitro* Specific CRISPR-based Assay for Nucleic acid detection-one pot (iSCAN-OP) for the diagnosis of potato virus X (PVX) and tobacco mosaic virus (TMV). It includes RT-RPA pre-amplification followed by collateral activity using Cas12a endoribonucleases, subsequent cleavage of the ssDNA reporter molecule, and release of fluorescent signals for quantification. The iSCAN-OP detection assay was combined with a commercially available fluorescence viewer device that enabled a fast and affordable in-field diagnostic platform to detect plant RNA viruses.

Similarly, a CRISPR-Cas12a-based visual assay was reported for the field detection of multiple RNA viruses and viroids in apple, i.e., apple stem grooving virus (ASGV), apple necrotic mosaic virus (ApNMV), apple stem pitting virus (ASPV), apple scar skin viroid (ASSVd), and apple chlorotic leaf spot virus (ACLSV) (177). Compared to other detection techniques like RT-qPCR, the CRISPR-Cas12a-RT-RPA platform exhibited higher sensitivity in ASPV and ASGV, detecting 250 copies per reaction to 2,500 copies for ApNMV, ASSVd, and ACLSV, respectively. The CRISPR-Cas12a system was also used for the detection of two begomoviruses, tomato yellow leaf curl virus (TYLCV) and tomato leaf curl New Delhi virus (ToLCNDV) (171). The assay combined LAMP and the CRISPR-Cas12a system to develop a quick and low-cost on-site assay for the diagnosis of TYLCV and ToLCNDV in ~1 h.

Clustered regularly interspaced short palindromic repeat (CRISPR)-based diagnostics within a brief period has evolved from laboratory-based nucleic acid detection tools to the point-of-care or on-site diagnostic tool due to its reliability, cost-effectiveness, and high sensitivity. However, there are still some limitations; one of the major drawbacks is dependence on the pre-amplification step for targets of below femtomolar concentrations. Also, the primers and crRNA designed for the detection assay must be lab tested and standardized (180). Lately, the use of one-step assay combined with easy detection of test subjects has considerably eased the use of CRISPR-based diagnostics. However, using heating devices for higher temperatures and separate steps for sample preparation remains a bottleneck and limits its application for in-field detection of plant viruses. Therefore, overcoming these limitations and further advancement in on-site diagnostic devices linked to the technique will enable additional acceptability of this platform for broader applicability.

CRISPR-Cas for Enhancing Yield and Quality of Crops

In addition to the field of virus diseases, genome editing techniques, specifically CRISPR-Cas, have brought a parallel revolution in crop improvement programs. The ultimate goal of crop improvement programs is to enhance crop yield and nutritional quality while making them resistant to diseases in order to ensure food and nutritional security and ultimately achieve the sustainable development goal of zero hunger (120, 181–184). The otherwise commonly employed molecular breeding and biotechnological approaches are not efficient in case of challenging to improve traits that demand advanced techniques like targeted genome editing (185, 186). Compared to other breeding practices that usually require 8–10 years, genome editing requires less duration (4–6 years) for product development (187) (Figure 1). Over the past few years, the CRISPR-Cas system has been proven as a game-changing technology in crop improvement programs in many ways, such as mutations in the coding region, promoter editing, gene insertion, prime editing, programmed single base editing, and cell type-specific and conditional mutations (188).

Detrimental environmental factors and climate disruptions primarily affect the yield of crops, and, thus, pose a significant threat to food security. Therefore, it is necessary to develop crops that tolerate environmental stresses without any yield penalty. Crop yield is a complex polygenic trait; thus, it is difficult to improve it by targeting a single gene. Alternatively, targeting regulators of yield-related attributes, such as grain size, grain number, and grain weight, could be a practical approach for a targeted increase in yield (187). Crop yield can also be substantially increased by reducing yield losses caused by environmental stresses. Therefore, genome editing to develop resistance/tolerance to various abiotic stresses will ultimately increase the marketable yield of crops. In the recent past, the CRISPR-Cas system has been successfully implemented in several crops for enhancing yields.

TABLE 3 | Application of CRISPR-Cas based diagnostics in detection of plant viruses.

Plant	Plant virus/Pathogen	CRISPR based diagnostics	Targeted genomic region	References
Tobacco	Tomato yellow leaf curl virus (TYLCV), tomato leaf curl New Delhi virus (ToLCNDV)	CRISPR-Cas12a	Coat protein (CP)	(171)
Sugar beet	Beet necrotic yellow vein virus (BNYVV)	CRISPR-Cas12a	RNA-1	(172)
Tomato	Tomato mosaic virus (ToMV)	CRISPR-Cas12a	ORF1	(173)
Tobacco	Cotton leaf curl virus (CLCuV)	CRISPR-Cas9	Rep, β C1	(174)
Tobacco	Tobacco mosaic virus (TMV), potato virus X (PVX), potato virus Y (PVY)	CRISPR-Cas12a	Coat protein (CP)	(175)
Banana	Banana streak virus (BSV)	CRISPR-Cas9	ORF1, ORF2, and ORF3 of BSV	(176)
Apple	Apple necrotic mosaic virus (ApNMV), apple stem pitting virus (ASPV), apple stem grooving virus (ASGV), apple chlorotic leaf spot virus (ACLSV), and apple scar skin viroid (ASSVd)	CRISPR-Cas12a	Coat protein (CP)	(177)
Apple, Pear	Fire Blight (<i>Erwinia amylovora</i>)	CRISPR (CR1-CR2-CR3)	T3SS, T3E	(178)
Rice	Rice blast disease (<i>Magnaporthe oryzae</i>)	CRISPR-Cas12a	Cry1C	(170)

One of the exciting studies involving CRISPR-Cas9-mediated genome editing of multiple genes governing rice yield-related traits resulted in a significant increase in grain yield (189). Four different genes, *Gn1a*, *DEP1*, *GS3*, and *IPA1*, which regulate grain number, panicle architecture, grain size, and plant architecture, respectively, were mutated in this study. *Gn1a* mutant plants showed increased plant height, panicle size, and the number of flowers per panicle. In contrast, *DEP1* gene mutant plants exhibited reduced height and short panicles but increased the number of flowers per panicle. The best results were obtained with *GS3* mutants, with a significant increase in grain weight, grain size, and grain length. Mutations in the *IPA1* gene, which defines plant architecture, could result in enhanced plant height and number of flowers per panicle, and reduced tillers as expected, ultimately resulting in increased grain yield. Several other studies have been conducted by targeting yield-related genes in various crops, such as *OsGRF4* for increased grain size and yield in rice (190), *GW5* (191), *OsAAP3* for increased tiller number in rice (192), *TaGASR7* for high grain weight in wheat (193), *ARGOS8* in maize for enhanced grain yield (194), and several other horticultural crops. Tomato has been intensively subjected to genome editing to improve various traits, such as yield, as a model fruit crop. Rodríguez-Leal et al. (195) engineered a tomato fruit crop for quantitative traits related to fruit size, inflorescence branching, and plant architecture, resulting in increased yield. They targeted the genes involved in the classical CLAVATA-WUSCHEL (CLV-WUS) stem cell circuit. In tomatoes, floral organ number and fruit size are inversely proportional to the expression of the *SICLV3* gene. CRISPR-Cas9-mediated mutations at eight loci in the promoter region of the gene resulted in mutants with increased floral organs and fruit size. Targeting the *COMPOUND INFLORESCENCE (S)* and *SELF PRUNING (SP)* genes that govern inflorescence development and indeterminate growth produced bushy determinate plants with excessively branched inflorescences with hundreds of flowers. These classical studies on the use of the CRISPR-Cas9-mediated genome editing technique in improving yield and

yield-related traits controlled by multiple genetic loci opened new avenues in crop improvement and can be replicated in other crop plants.

In order to fight the issues of hidden hunger on a globe-wide scale, efforts have recently been shifted to focus on enhancing the quality and nutritional content of food grains, vegetables, and fruits, and genome editing technology has served the purpose very effectively (196–198). Among genome-edited foods, the mushroom was the first one to reach the market. The polyphenol oxidase gene in mushrooms was mutated by CRISPR-Cas9 to produce strains with reduced browning traits (199). In rice, starch quality is one of the basic essential quality parameters; thus, CRISPR-Cas9 mediated genome-edited rice with high amylase and low viscosity was developed by knocking out starch-branching enzyme gene *SBEIIb* (200). Besides starch quality, the aroma is a very precious trait determining rice quality due to the presence of 2-acetyl-1-pyrroline in rice grains. Mutations in the *BADH2* gene responsible for the production of γ -aminobutyric acid (GABA) result in more production of 2-acetyl-1-pyrroline (201). Similar results were obtained by knocking out the *BADH2* gene in a Zhonghua 11 rice cultivar using CRISPR-Cas9. Thus, elite and high-yielding rice varieties can be modified for increased content of 2-acetyl-1-pyrroline by knocking out the *BADH2* gene using CRISPR-Cas9. Recently, many other traits, such as low cadmium content (202), high oleic content (203), increased β -carotene (204), and red rice (205), have been targeted by CRISPR-Cas9 editing in rice. Recently, using CRISPR-Cas9, a new allele *BADH2* was created in the non-fragment japonica and indica rice varieties NJ1 and HHZ. This was further utilized for grain aroma improvement in three-line hybrid rice (198). Similarly, in wheat, CRISPR-Cas9-led knockout of α -gliadin genes resulted in low gluten-content seeds, which showed an 85% reduction in immunoreactivity (206). The protein content of wheat grains has also been increased in *GW2* knock-out plants (127). Maize has also been subjected to CRISPR-Cas9-mediated genome editing to target the *IPKA1*, *IPK*, and *MRP4* genes involved in phytic acid synthesis to

reduce the content of phytic acid, which is an anti-nutritional component (207).

Oil content and fatty acid composition are some of the most important quality parameters in oilseed crops. Increased oleic acid and decreased linoleic acid content, increased C18 unsaturated fatty acids, and reduced polyunsaturated fatty acids (PUFAs) are the most desirable traits in this context. The CRISPR-Cas9 technique has been successfully implemented by targeting various fatty acid synthesis pathway genes in rapeseed (increased 18:1 and reduced 18:2 fatty acids), camelina (reduced PUFAs, increased 18:1, altered amino acid profile), soybean (increased 18:1 and reduced 18:2), peanut, and pennycress for enhancing the quality and quantity of oil (208).

The CRISPR-Cas system was employed to enhance the quality, shelf life, and functional metabolites of fruits and vegetable crops. Using the CRISPR-Cas9 technology, tomato fruits with high lycopene content were developed, leading to their enhanced quality (209). This was achieved by inhibiting the conversion of lycopene to β - and α -carotene by mutating the *SGR1*, *LCY-E*, *BLC*, *LCY-B1*, and *LCY-B2* genes. Besides lycopene, breeders aim to increase the content of γ -aminobutyric acid (GABA), a neuro-suppressant that acts in blood pressure regulation. CRISPR-Cas9-mediated knock out of the *SIGAD2* and *SIGAD3* genes resulted in 7–15 times increased accumulation of GABA (210). To delay the ripening of tomato fruits to ultimately enhance the shelf life, a ripening inhibitor (*RIN*) gene was knocked out in different tomato cultivars (211). Alternatively, the targeted mutation in long non-coding RNA1459 (*lncRNA1459*) through the CRISPR-Cas system resulted in delayed ripening along with reduced ethylene and higher accumulation of lycopene (212). Similarly, starch quality in potatoes was altered to produce tubers with increased amylopectin by editing the granule-bound starch synthase gene (213). CRISPR-Cas9-led knocking out of the polyphenol oxidase gene reduced enzymatic browning in potatoes (214). In potatoes, steroidal alkaloid α -solanine is considered an anti-nutritional compound; therefore, CRISPR-Cas9-edited potato plants free of α -solanine were produced by targeting the *St16DOX* gene (215). In the same way, tartaric acid, an anti-nutritional factor in grapes, was efficiently reduced by targeting the *IdnDH* gene by CRISPR-Cas9 (112). Bioactive compounds or other nutritionally essential compounds in most crops are synthesized by complex pathways involving many genes. Therefore, manipulation of such pathways for the production of desired compounds needs simultaneous alteration of more than one gene. Recent advancements in multiplexing in the CRISPR-Cas system help to target multiple genes for editing at a time by CRISPR-Cas9, which has made it possible to manipulate such metabolic or biosynthetic pathways. These advancements have opened new avenues not only to target and improve yield and quality-related multigenic traits but also simultaneously develop resistance to biotic and abiotic stresses particularly to combat the most difficult viral pathogens. The CRISPR-Cas technology, with a wide range of applications, therefore, has ample potential in paving ways to achieve food and nutritional security for the benefit of the entire globe.

DISCUSSION

In the era of climate disruptions, decreasing cultivable lands, and burgeoning human population, which is expected to touch 10 billion within the next few decades, food security remains a major challenge at the global level. To meet food and nutritional demands, various programs targeting nutritional enhancement and increase in crop productivity are being undertaken on a war footing. While achieving higher yields per unit of cultivated land and quality standards of agricultural produce have remained generalized targets globally, emerging concerns raised by climatic disruptions and various biotic and abiotic factors pose a serious hurdle in achieving the targeted growth rate in food production. Plant diseases alone cause around 40% of global crop losses, and their emergence and re-emergence have always remained an alarming concern (216). Recently, a series of new emerging viral diseases in crops, along with rapidly emerging or re-emerging viral pathogens, has caused a global concern, the reason for which ranges from dynamic cropping practices, free global trade, to the introduction of infected germplasm coupled with the ability of viruses to evolve and adapt rapidly (6). The American Phytopathological Society (APS) classified these pathogens into four categories: new, emerging, re-emerging, and threatening, respectively. In the past, geminiviruses, potyviruses, and some new groups of viruses were witnessed as major viral pathogens associated with diseases in tomatoes, cotton, melons, lettuce, beans, and other crops. The emergence or re-emergence of pathogens is a concern because these newly evolved pathogens can infect new crops in the vicinity and cause epiphytotic. Sharma et al. (217) reported the infection of a new virus (Large cardamom chirke virus: macluravirus) in chili, which is the result of its natural host shift from large cardamom plantations (218). Such events of host shift cause major impediments to crop productivity and affect food security. Therefore, to tackle these, newer and more efficient techniques are utilized across the world. However, conventional breeding along with molecular techniques have been used for increasing crop production in the past. Recently, the advent of gene editing techniques has opened new avenues for a better outcome. The CRISPR-Cas system, among gene-editing techniques, is the most powerful tool to achieve the targeted editing in genomes through faster and precise means.

The CRISPR-Cas system was first used to detect the Zika virus in humans (164). This tool has evolved over the past years for the precise and cost-effective detection of various plant viruses. The technique has been used to detect a single pathogen and multiplexed to diagnose multiple viruses at a time (179). The recent development of tools like SHERLOCK, DETECTR, and HOLMES, which are highly specific and sensitive in detecting viruses, has opened new horizons in virus diagnostics (167–170) (Figure 3). The CRISPR-Cas system has also been used for in-field and on-site detection of plant viruses (179) along with the development of a one-step assay (iSCAN-OP) (175). A wide range of wheat, tomato, cassava, potato, rice, and cucumber plants was developed through CRISPR-Cas engineering, with the target of imparting disease resistance mainly to plant viruses (144, 149, 156–158).

Due to its simplicity and applicability, CRISPR-Cas tools have been widely used in crop improvement programs *via* mutations in codon region, promoter editing, gene insertion, prime editing, programmed single base editing, and cell type-specific and conditional mutations (188). In order to diversify the nutritional and functional traits of crops controlled by polygenes, genes and their controlled traits associated with the metabolic pathway need to be investigated for successful trait improvement without undesirable pleiotropic defects. For instance, targeting multiple genes governing yield-related traits in rice regulating grain number, panicle architecture, grain size, and plant architecture through the CRISPR-Cas system resulted in a significant increase in grain yield, thereby improving yield and yield-related traits (189). Similar studies on genes related to the yield trait in wheat (219) and maize for enhanced grain yield (194) have also been successfully reported. Concurrently, this technique has also been utilized to enhance the quality of starch in rice along with enhancement of aroma (200, 201), low cadmium content (202), increased β -carotene (204), etc., thereby producing elite and high-yielding rice varieties. Apart from enhancing the nutritional aspect, CRISPR-Cas has also been utilized to reduce the impact of anti-nutritional factors in certain crops like maize and grapes (112, 207). Recent advancements in the development of base editors and primer editors offer more exciting options and robustness in the efficiency of the CRISPR-Cas system (118, 119).

The futuristic and potential use of the CRISPR-Cas technique lies in targeted editing of pro-viral (susceptibility) genes having a positive role in the virus infection cycle. The multiplexing in the CRISPR-Cas system has now enabled to target of multiple genes in a single approach and develop end products encompassing more efficient desirable traits like yield, quality, and resistance to disease. In a recent study, a transient assay was performed by multiplexing of gRNA-Cas9 modules in the model plant *N. benthamiana*, and showed a high degree of resistance to chili leaf curl virus (ChiLCV) and restriction of the generation of escape mutants as compared to single sgRNA (220). A similar study on targeting multiple overlapping viral genes of cotton leaf curl virus (CLCuV) resulted in better interference with virus proliferation (221). A schematic representation of different usage of the CRISPR-Cas tool indicates its versatile applications right from precise genome editing to diagnosis within a short period (Figures 2, 3). However, efficient multiplexing, reduced off-targets, and designing on-site diagnostic devices remain the main bottlenecks that need to be addressed with advancement in the field thereby garnering its broader applicability. The coming years are expected to witness the exciting widespread applications of CRISPR-Cas-based multiplexing and specific targeting of genomes up to a single-base-resolution using base editors and prime editors to fight the menace of viral infections in plants and achieve the ultimate goal of food and nutritional security. The merits of this technique far outweigh the demerits. However, the differential regulatory guidelines and general acceptability of CRISPR-Cas-engineered crops are a hurdle that has to be dealt with comprehensive, collaborative plans between policymakers and researchers for the integration of CRISPR-Cas-based products to achieve global food and nutritional security. These points are discussed in detail in the subsequent section of the review.

Future Prospective and Challenges for CRISPR-Edited Crops

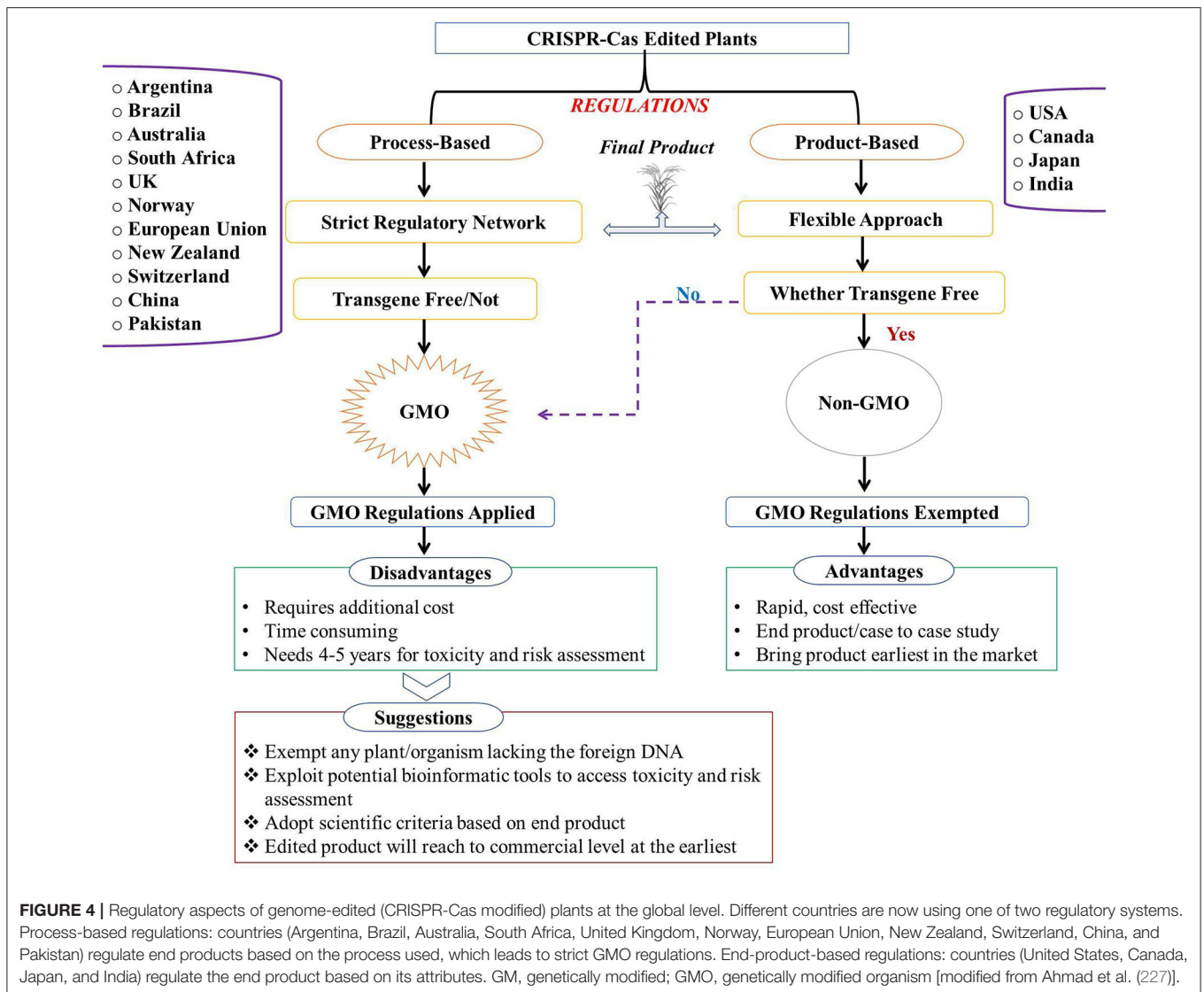
Regulations for CRISPR-Edited Plants: A Global and Indian Scenario

During the CRISPR-based genome editing process, several bases with large segments are removed or interchanged (222). There are three classes of genome editing employing site-directed nuclease SDNs: (i) induction of single point mutations or InDels (SDN-1), (ii) editing of a few base-pairs with an external DNA template sequence (SDN-2), and (iii) insertion of longer strands (SDN-3) of transgenes or cisgenes. The most recent advancement in genome editing is base editors, which require the combination of non-cutting Cas9 with deaminase nucleotide, leading to the point mutation of A/T base pairs into C/G vice-versa without cleaving the genome (166, 219, 223), which comes under the SDN-1 class.

The Cartagena Protocol on Biosafety created the regulation norms for international trading in living genetically modified organisms (LMOs). However, there are some differing viewpoints on the production, consumption, and regulation of CRISPR-edited plants that have been taken by several countries (224). Essentially, two frameworks are being followed by many nations to regulate CRISPR-edited plants, i.e., (i) regulation of the procedure to generate genome-edited plants and (ii) regulation based on final product attributes (225, 226) (Figure 4). Policies vary among nations, wherein a few countries have exceeded procedures to deal with genome-edited plants or deregulate them, while many have passed the guidelines (228). The United States regulatory policies are mainly based on technical characteristics of modified qualities and their eventual use as an end product (risk-based) (227–229). The Canadian Food Inspection Agency (CFIA) attributed more importance to the product developed rather than the process employed in its development (novelty and risk-based) concerning CRISPR applications (230). Furthermore, Germany and the Netherlands are nearing the completion of rules requiring genome editing crops to be labeled as non-GMO (231). All European Union (EU) member states are working on their national regulatory guidelines, and their regulatory trigger is process-based. Chinese authorities have taken steps to ensure that CRISPR-edited food is managed and risk assessed on a case-by-case basis.

In Australia, the organisms manipulated through SDN-1 are not regulated under GMO regulations, whereas SDN-2 and SDN-3 are regulated under strict GMO regimes (232, 233). In New Zealand, the Hazardous Substances and New Organisms (HSNO) involves a process-based regulatory framework for GMOs (227). As a result, this allows products produced by any genome-editing technology to be regulated within the GMO framework. Additionally, any product developed through classical/induced mutagenesis by chemical/physical mutagen or transgene-free will be evaluated case by case (234). In India, the Department of Biotechnology (DBT) issued draft guidelines for gene editing regulation in 2020, which required additional safety and efficacy testing for genome-edited crops. The guidelines regulate the process used to generate genetically manipulated plants rather than final product attributes. There are no established timelines for the regulatory approval of gene-edited products at present.

From the above discussion, it is clear that regulations governing gene editing are constantly changing worldwide.



Therefore, under the Genetic Literacy Project (GLP), an Agriculture Gene Editing Index has been formed to compare the regulatory restrictions from country to country. Ratings on the gene-editing index (**Supplementary Table 1**) reflect the current state of gene editing regulations and can be accessed from the Global Gene Editing Regulation Tracker [<https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/>].

Risk assessment and biosafety issues may be addressed by utilizing the advanced bioinformatics tool to detect the potential off-target effects by comparing the gRNA and dsRNA sequences with the reference genome. The activity of Cas9 in subsequent generations also needs to be addressed (227) (**Figure 4**). Plasmid-free transformation events are now available to tackle this issue. Virus-based vector systems can overcome these issues but have some limitations of poor editing efficiencies and poor inheritability in the subsequent generations of desired edits. For the future development of Cas9-free plants, these constraints must be addressed. In this case, protoplast-based gRNA and Cas9 complex insertions can be performed. In all crops, efficient

chloroplast-based transformation systems are still lacking. These issues will need to be addressed properly if the latest gene-editing tools are to be fully utilized.

Advantages, Limitations, and Challenges of CRISPR-Based Gene Editing

Clustered regularly interspaced short palindromic repeat (CRISPR)-administered genome editing is a powerful technique leading to a precisely targeted mutation in the genome of crop plants. Several CRISPR-Cas-derived editors that can execute precise genome alterations have been devised in addition to the indel mutations caused by the CRISPR-Cas nuclease. Allelic variants could also be produced, which serve as a potential genomic resource in crop variety development programs and developing resistance to plant viruses. Since the advent of next-generation sequencing (NGS), sufficient information on genome sequences and gene annotation of most crop plants has been available in the public domain. Such information could be best utilized to develop genome-edited crops with desired biotic

and abiotic stress tolerance, nutritional quality, and higher yield. CRISPR can create null alleles by acting on exons or coding regions, and it can boost expression by acting on regulatory regions and ORFs. It can produce single or multiple mutations in homologous and non-homologous regions. Additionally, transgenes get eliminated in late generations due to segregation, leading to the development of transgene-free plants, which could be utilized without any regulatory issues in the near future.

Genome editing was used to successfully imitate tomato domestication, revealing the power of genome editing technologies (219). Several independent studies on CRISPR-engineered broad-spectrum disease resistance have demonstrated its versatile applications in various crop species. Moreover, several recent developments have been made to generate CRISPR-engineered dicotyledonous plants by *de novo* meristem induction (220), large DNA insertion (up to 2 kb) in rice (221), enhanced gene targeting efficiency through a heat-inducible CRISPR system in maize (222), and reconstructing the plant genome through genome engineering and somatic chromosome engineering, enabling genetic linkage (223). The CRISPR technique has been practically utilized to impart resistance to several plant RNA and DNA viruses. Geminiviruses are responsible for most economically important plant diseases, and to date, direct virus DNA targeting has been utilized to impart CRISPR-administered geminivirus resistance. However, this approach has limitations due to the eventual emergence of resistance-blocking strains and virus escape.

The off-target effect is the major challenge in CRISPR application, in which Cas9 nucleases cleave the wrong sites at the target genome. The SgRNA of 20 nucleotide sequences mainly controls Cas9 target specificity. Next to the target sequence, an adjacent PAM has the target specificity, but still, off-target cleavage occurs with 3-5 base pair mismatches. Additionally, the transformation of CRISPR-Cas-edited plants as a regeneration process is time-consuming, labor-intensive, and genotype-dependent. It could also cause somatic mutations during the process of regeneration. Low innate HDR efficiency is also a significant hurdle in CRISPR-based applications and causes unintended gene replacement and deletions. New developments for improving HDR efficiency could excel the applications of the CRISPR-Cas technique in crop plants. The future of CRISPR-Cas editing will lie in its simplified and robust use for the simultaneous targeting of multiple genetic loci in the same plant species, called multiplexing. This approach is emerging to the forefront in developing broad-spectrum and durable plant resistance against different viruses. These approaches, coupled with base and prime editor-based high-resolution modifications, are expected to make the target of zero hunger a reality in the coming years.

The current legal framework of genome-edited crops still regulates CRISPR-engineered crops as a process or product-based. Since regulatory guidelines vary from country to country, many countries consider genome-edited crops under strict GMO regulatory guidelines. Variety development takes a long time and costs more money because of these regulatory frameworks. Therefore, scientists and policymakers

must collaborate to develop comprehensive plans for the integration of CRISPR-Cas edited crops to achieve food and nutritional security.

CONCLUSIONS

Usually, crop losses are considered only in terms of reduction in yield, but it is equally important that reduction in the market value of crops due to the reduced quality and nutritional contents are taken into account. Understanding the concept and mechanism of plant-pathogen interactions and evolution, and additional analyses are essential to generate a holistic view of the combined effects of these factors on food and nutrition security worldwide. Using new emerging techniques and technologies like genome editing tools (CRISPR-Cas system), we can cope with some emerging issues on food production and increase nutritional quality in a short span of time in a more robust way. The current coronavirus disease-2019 (COVID-19) pandemic, in addition to the currently visible effects, might also put a negative effect on food security and nutrition in the long run. Thus, potential futuristic applications of the CRISPR tool will be to tackle the emerging and re-emerging viruses through targeted gene editing (singleplex and multiplex) to impart resistance to dreadful viral infections while simultaneously enhancing the yield and quality of end products. The scope of the application of CRISPR technologies and their rapidly evolving field will make global food and nutritional security realistic in years to come.

AUTHOR CONTRIBUTIONS

SKS, OPG, NP, AM, DS, and PS conceived the idea and designed the outline. SKS, NP, DS, AM, PS, JS, and SGK drafted the manuscript. AM, JS, PS, and DS prepared the illustrations. SKS, OPG, SK, and BB reviewed and improved the draft. All authors collected, compiled, analyzed, and interpreted the literature, contributed significantly to the article, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2021.751512/full#supplementary-material>

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