Send Orders for Reprints to reprints@benthamscience.net

214 *Current Genomics*, 2021, *22*, 214-231

REVIEW ARTICLE

Engineering Resistance Against Viruses in Field Crops Using CRISPR-Cas9

Vidya R. Hinge[1], Rahul L. Chavhan[1], Sandeep P. Kale[2], Penna Suprasanna[3] and Ulhas S. Kadam[1,4,‡,*]

[1]*Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur; Vasantrao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani 431 402, India;* [2]*USAID-BIRAC International Project, School of Life Sciences, S.R.T.M.U., Nanded, India;* [3]*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, 400 085, India;* [4]*Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, 14476, Germany*

ARTICLE HISTORY

Received: October 06, 2020
Revised: January 29, 2021
Accepted: February 24, 2021

DOI:
10.2174/1389202922666210412102214

Abstract: Food security is threatened by various biotic stresses that affect the growth and production of agricultural crops. Viral diseases have become a serious concern for crop plants as they incur huge yield losses. The enhancement of host resistance against plant viruses is a priority for the effective management of plant viral diseases. However, in the present context of the climate change scenario, plant viruses are rapidly evolving, resulting in the loss of the host resistance mechanism. Advances in genome editing techniques, such as CRISPR-Cas9 [clustered regularly interspaced palindromic repeats-CRISPR-associated 9], have been recognized as promising tools for the development of plant virus resistance. CRISPR-Cas9 genome editing tool is widely preferred due to high target specificity, simplicity, efficiency, and reproducibility. CRISPR-Cas9 based virus resistance in plants has been successfully achieved by gene targeting and cleaving the viral genome or altering the plant genome to enhance plant innate immunity. In this article, we have described the CRISPR-Cas9 system, mechanism of plant immunity against viruses and highlighted the use of the CRISPR-Cas9 system to engineer virus resistance in plants. We also discussed prospects and challenges on the use of CRISPR-Cas9-mediated plant virus resistance in crop improvement.

Keywords: CRISPR, Cas9, plant innate immunity, plant virus, genome editing, crop improvement, food security, RNA.

1. INTRODUCTION

Plant viruses are obligatory intracellular parasites made up of nucleoprotein complexes. Plant virus is the second most notable plant pathogen after fungi, causing 60 billion dollars losses per year worldwide in both agricultural and horticultural crops [1]. Tobacco mosaic virus (TMV) was the first plant virus discovered to cause mosaic disease in Tobacco [2]. As per the classification proposed by the International Committee on Taxonomy of Viruses (ICTV), viruses are categorized into 7 orders, 111 families, 30 subfamilies, 610 genera, and 3705 species. Of these, around 1407 plant virus species are distributed in 73 genera and 49 families [3]. Based on the viral genome and synthesized mRNA, Baltimore has classified these viruses into seven classes [4]:

- *Class I*: dsDNA viruses: mRNA is synthesized normally using -ve strand as a template.
- *Class II*: ssDNA viruses: mRNA is synthesized by double-stranded DNA intermediate.
- *Class III*: dsRNA viruses: mRNA is synthesized by a complementary strand (template strand).
- *Class IV*: ssRNA viruses: RNA directly functions as mRNA.
- *Class V*: sense (-) ssRNA viruses: mRNA is synthesized by the synthesis of +ve strand.
- *Class VI*: (+) strand RNA viruses: virus genome is synthesized by reverse transcription (RT).
- *Class VII*: DNA reverse transcribing viruses with RNA intermediates.

Further classifications of viruses based on the genome types include dsDNA, ssDNA, ssDNA(-), ssDNA(+), ssDNA(+/-), dsDNA-RT, ssRNA-RT, dsRNA, ssRNA(-), ssRNA(+), ssRNA(-/+) and viroid [4]. Economically important plant viruses belong to ssRNA(+), dsDNA, and ssDNA virus groups. Most destructive plant viruses have a single stranded positive sense RNA genome, *i.e.,* ssRNA(+), and theseinclude important families like Bromoviridae [*e.g., Brome mosaic virus* (BMV), *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco streak virus* (TSV)], Closteroviridae [*e.g., Citrus Tristezaclostero virus* (CTV), *Beet yellow virus* (BYV), *Lettuce infectious yellows*

*Address correspondence to this author at the Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, 14476, Germany;
E-mail: kadam@mpimp-golm.mpg.de,
‡Present Address: Division of Life Sciences, Plant Molecular Biology and Biotechnology Research Center, Gyenongsang National University, Jinju-si, Republic of Korea; E-mail: ulhasskadam@gmail.com

virus (LIYV), *Grapevine leafroll associated virus* 3 (GLRaV-3)], Luteoviridae [*e.g., Barley yellow dwarf virus* (BYD), *Potato leaf roll virus* (PLRV), *Pea enations mosaic virus*-1 (PEMV-1)], Potyviridae [*e.g., Plum pox potyvirus* (PPV), *Potato virus Y* (PVY), *Blackberry virus Y* (BVY), *Wheat streak mosaic virus* (WSMV), *Sweet potato mild mottle virus* (SPMMV), *Barley yellow mosaic virus* (BYMV) and Tombusviridae [*e.g., Tomato bushy stunt virus* (TBSV), *Carnation mottle virus Necrovirus* (CarMV), *Tobacco necrosis virus* (TNV), *Maize chlorotic mottle virus* (MCMV)]. In contrast, Bunyaviridae is represented by *Tomato spotted wilt virus* (TSWV), a virus with negative-sense (-) ssRNA. The major plant viruses possessing DNA genome belong to Geminiviridae (ssDNA) [*e.g., Bean golden mosaic virus* (BGMV), *Maize streak virus* (MSV)] and Caulimoviridae (dsDNA) [*e.g., Cauliflower mosaic virus* (CaMV), *African* cassava mosaic virus (ACMV), *Rice tungro bacilliform virus* (RTBV)]. Both these viruses alone are responsible for causing yield losses ranging from $25 million to $100 million annually (Table **1**). Plant viruses constitute almost 50% of plant pathogens that are responsible for the emerging and habitual plant diseases worldwide [5]. This has unpredicted consequences on the natural ecosystems and food security. However, due to the continuous pressure of global climate change and the ever-growing human population, the management of plant viral diseases is becoming a major challenge. Therefore, it is essential to understand the plant-virus interaction, plant innate immunity against plant viruses, and plant virus management practices. In this regard, genomics advances have offered promise with the development of new genetic tools, such as the CRISPR- Cas9 genome editing tool, for improving plant virus resistance.

2. PLANT VIRUS INTERACTION

Virus entry occurs through wounds or through vectors (insects, nematodes, mites, fungi, and plasmodiophoroids) that feed on or infect the plants. The next phase after the viral entry is the genome decapsidation followed by the translation and replication of the viral genome and proceeded by the proliferation of virus particles at the site of infection [16]. The virus particles then spread to non-infected adjacent cells as virions, or viral ribonucleoprotein (vRNP) complexes through plasmodesmata, and the virus particles move to distant tissues through the vascular trafficking mechanism of the host plant [17, 18]. The accumulation of viral nucleic acids or proteins inside the cell disturbs the normal function of the plant and/or induces a symptomatic defense response [19, 20]. The common symptoms of virus-infected plants include yellowing or mottling of the leaves, mosaic patterns, dwarfing and/or developmental abnormalities of the plant, and systemic necrotic symptoms [21].

2.1. Development of Plant Virus Disease Symptoms

Plant viral disease is caused by the outcome of the interaction between a susceptible host plant, a virulent pathogen, and the environment. Such interactions are of two types: consequential virus-host interactions directly contributing to the establishment of systemic infection, while inconsequential virus-host interactions do not contribute to the success of the infection but nevertheless disrupt host physiology. The virus-induced symptoms and disease development are typically explained by two general models [20]. A competitive disease model describes that plant viruses replicate inside the host cell by using a substantial amount of host resources. On the contrary, the *interaction disease model* is based on the specific interactions between plant virus and host components which disrupt host plant physiology, particularly the hormone metabolism, cell cycle control, vesicular transport, protein modifications, allocation of cellular resources, and cell-to-cell communication [22].

2.2. Molecular Basis of Plant Virus-host Interaction

Infection to the host plant and further successful disease development is regulated by virus and host proteins. The infection process includes viral genome disassembly, viral RNA (vRNA) replication, movement, and encapsidation.

Table 1. Global crop yield losses caused by important plant viruses.

Sr. No.	Plant Virus	Crop Infected	Cost of Crop Damage Per Year/Crop Yield Loss Per Year	Location	References
1.	Cassava mosaic begornovirus	Cassava crop	$25 million	Africa, India and Srilanka	[6]
2.	Potato leafroll polerovirus	Potato	$100 million	US	[7]
3.	Potato leafroll polerovirus	Potato	$30 - 50 million	UK	[8]
4.	Barley yellow dwarf luteovirus	Barley, Oats, Rice, Wheat, Maize	$13.93 million	UK	[9]
5.	Rice tungro disease (RTD)	Rice	$1.5 billion	South-East Asia	[10]
6.	Citrus tristezaclosterovirus	Cacoa Trees	$200 million	Worldwide	[11]
7.	Cacoa swollen shoot virus	Citrus trees	unknown	Togo, Ghana, Nigeria	[12]
8.	Maize streak virus (MSV)	Maize	$480 million	Africa	[13]
9.	Banana bunchy top virus (BBTV)	Banana	$50 million	Australia, Africa and Asia	[14]
10.	Papaya ringspot virus (PRSV)	Papaya	$11 million	Hawaii	[15]

The plant infection process is initiated by the formation of virions, a mature infectious virus particle or viral ribonucleoprotein (vRNP) complex inside the host cell and intra-cellular movement through plasmodesmata (PD) to the adjoining non-infected cells. The process is continued until the vRNPs movement through vascular tissues (xylem and phloem) results in systemic infection [23]. Positive sense RNA [(+) RNA] viruses undergo translation and produce viral protein in the cytoplasm of the invaded host cell. This includes the viral proteins like viral RNA dependent RNA polymerase (vRdRp), associated *replication proteins* (*e.g., helicase*), *coat protein* (CP), and *movement protein* (MP) responsible for virus infection, replication, and movement in the host plant. The 'viral factories' are described as virus-induced quasi-organelles associated with cellular membranes where vRNA replication takes place. There are two types of viral factories; *Spherule-Shape* Viral Factories (static, 50 - 400 nm, associated with the membrane of peroxisome, the mitochondrion, and the chloroplast) and *Vesicular-shape* viral factories (motile, 30 to 300 nm, ER-derived and involved in the intracellular movement of the vRNA). The viral factories support viral replication complexes (VRCs) and partitioning of the vRNA replication to specific location [24, 25]. The plant viruses encode a membrane-associated protein that is part of viral replication complexes (VRCs), which triggers membrane rearrangement [25]. The host proteins, *i.e.,* endosomal sorting complexes required for transport (ESCRT) factors, host reticulon homology proteins (RHPs), and the early secretory pathway components are involved in the formation of viral factory and viral replication [26].

DNA viruses, such as Gemini viruses, replicate inside the host cells by hijacking host machinery like DNA polymerase and RNA polymerase II, which accelerate the rate of host cell division [27, 28]. The host-encoded membrane spanning proteins (TOM1 and TOM3), eukaryotic translation initiation factor (iso) 4E [eIF(iso)4E], the translation elongation factor 1A (eEF1A), and the poly(A)-binding protein (PABP) are known to assist the viral replication and their movement in the host plant [25]. A list of various plant viruses, virus proteins, host proteins, and their functions are summarized in Table **2**.

Table 2. Plant virus and host protein regulating the disease development in the host plant.

Sr. No	Plant Virus Protein	Host Plant Protein/Component	Resulting Interaction and Disease Development	References
RNA Replicase Related Proteins				
1	TMV replicase	Aux/IAA proteins	Alterations in auxin response pathways, developmental symptoms	[30]
2	TMV replicase	P58IPK (inhibitor of dsRNA activated PKR)	Regulation of cell death	[31]
3	RDV P2	ent-Kaurene oxidase	Gibberellin synthesis, dwarfing	[32]
4	Gemini virus Rep proteins	Retinoblastoma protein (pRBR)	Cell cycle reprogramming	[33]
5	Nib RNA Replicase (PPV),	-	Virus accumulation and disease development	[34]
6	p126/p183 (TMV and PPMoV),	-	Transport protein and cell to cell movement	[35]
7	2a (CMV)	Protein Kinase	Formation of replicase complex	[36]
8	PSTVd derived siRNA	Host mRNA	Misregulation of host mRNA, induction of disease	[37]
9	TBSV p19	ALY proteins (nuclear shuttle proteins)	Transport	[38, 39]
10	Geminivirus NSP (nuclear shuttle protein)	AtNSI (Acetyltransferase)	Disruption of AtNSI acetylation activity	[40]
11	Geminivirus NSP (nuclear shuttle protein)	NIK kinases	Reduce NIK kinase activity, disrupt defense response	[41]
12	FBNYV 20-kDa protein (F-box protein)	Skp-1 and pRBR	Degradation of pRBR, Cell cycle reprogramming	[42]
Coat Protein Gene				
13	AMV CP	Translation initiation factors eIF4G & eIFiso4G,	Interact with the host translation initiation factors, eIF4G and eIFiso4G, mimicking the function of host PABP	[43]
14	Wheat yellow mosaic virus (WYMV): P2	COPII GTPase Sar1	COPII GTPase Sar1 interacts with the P2 protein of Wheat yellow mosaic virus (WYMV)	[44]
15	TuMV 6K2	COPII coatomer Sec24a	The COPII coatomer Sec24a recognizes the N-terminal cytoplasmic tail of the TuMV 6K2 protein, thus facilitating the incorporation of the viral protein into COPII vesicles	[45]

Sr. No	Plant Virus Protein	Host Plant Protein/Component	Resulting Interaction and Disease Development	References
16	CPMV: 60K helicase	ER localized SNARE-like protein VAP27	Interact with the 60K helicase of CPMV.	[46]
17	TuMV: 6K2	VAP27 protein	Binding VAP27; 6K2 associates with Syp71, which is involved in vesicle fusion.	[47]
Membrane-associated Viral Proteins				
18	TBSV: p33	Peroxisome (switch to ER in the absence of peroxisome)	Upregulates phospholipid biosynthesis, recruits ESCRT factors for VRCs assembly, vRNA recruitment, interacts with the p92pol, binds eEF1A to promote VRCs assembly and (-) vRNA synthesis.	[48]
19	Red clover necrotic mosaic virus (RCNMV): p27)	GTPase, (Arf1)	The GTPase, such as the Arf1, preferentially binds to the C-terminal region of the viral protein p27 of Red clover necrotic mosaic virus (RCNMV).	[49]
20	TBSV: p92pol	Peroxisome	vRdRp, interacts with p33, recruits GAPDH to the VRCs.	[50]
21	BMV: 1a	ER, host reticulon homology proteins (RHPs)	Formation of viral factories, recruits the vRNA to the viral factories, hijacks reticulons for membrane curvature, RHPs involved in viral factory biogenesis.	[51]
22	BMV: 2apol	ER	vRdRp, interacts with the capsid protein, maybe for genome packaging.	[52]
23	TuMV: 6K2	ER	VRCs assembly, virus intracellular, intercellular, and long-distance movement.	[53-55]
24	TuMV: P3	ER	Virus pathogenesis, symptom and avirulence determinant, genome amplification.	[56]
25	BaMV/ PVX: TGBp1	ER	RNA binding, suppresses host gene silencing, virus movement, regulates the size exclusion limit of the PD, induces the formation of X-body.	[57]
26	BaMV/ PVX: TGBp2	ER	Induces VRCs formation, interacts with TGBp3.	[58]
27	BaMV/ PVX: TGBp3	ER	Associates with the virions for virus delivery, interacts with TGBp2.	[59]

3. PLANT INNATE IMMUNITY AGAINST VIRUSES

Plant pathogen resistance mechanism has been explained earlier based on the gene for gene theory. This has successfully demonstrated the resistance mechanism governed by the host plant against diverse plant pathogens. A single resistance gene (R-gene) encoded by the host recognizes the avirulence (Avr) proteins secreted by a pathogen and triggers a hypersensitive response (HR) of resistance leading to rapid cell death [29]. It was elucidated that the R gene (*e.g.,* Kinase protein) from the host plant physically interacts with Avr (*e.g.,* AvrPto or AvrPtoBits) for virulence determination. Several R Genes from different plant species are classified into two types; (a) genes encoding nucleotide-binding leucine-rich repeat (NB-LRR) proteins, (b) genes encoding receptor-like kinase (RLKs)/receptor-like proteins (RLPs) [60]. In recent years, plant disease resistance has been explained by the zig-zag model, which comprises two distinct defense response levels (primary and secondary). The primary defense level is called pathogen or microbe-associated molecular patterns (PAMP/MAMP) triggered immunity (PTI), and the secondary defense level is called effector-triggered immunity (ETI) [61]. The PAMP-triggered immunity (PTI) is activated when cell-surface associated pattern recognition receptors (PRRs) of host plant recognize conserved structural motifs of pathogen, *i.e.,* MAMPs/PAMPs, DAMPs (damage-associated molecular patterns of the plant) (Fig. **1**) [62]. The plant immunity network ensures the biosynthesis of specific defense molecules and enables plants to respond rapidly and efficiently to a wide range of pathogens [63, 64]. The second defense response level is triggered when the R gene product directly or indirectly senses specific effectors (Avr factors) secreted by pathogens into the intracellular host environment and activates effector-triggered immunity (ETI) [67]. Activated ETI leads to hypersensitive response (HR), rapid cell death, production of reactive oxygen species (ROS) and salicylic acid (SA), as well as expression of defense-related genes [68]. The viral proteins, such as replicase (RP), movement proteins (MPs), and coat proteins (CPs), can act as Avr determinants, an essential factor for a successful infection process. Thus, ETI defense is shown to be more robust than PTI (Fig. **1**) [65, 69, 70]. The natural plant viral resistance is classified as R-gene mediated resistance, recessive resistance, antiviral RNA silencing, hormone-mediated antiviral defenses, and proteasome degradation [61, 65, 70].

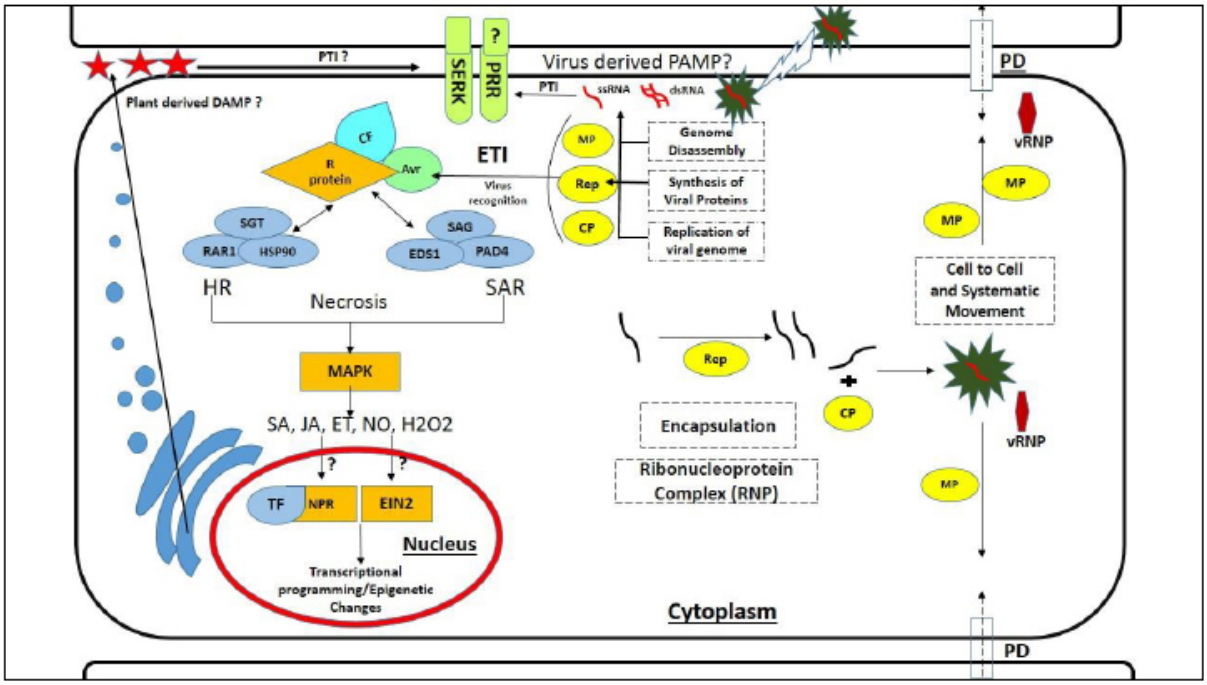


Fig. (1). Plant innate immunity against viruses. In response to the virus infection, viral mRNAs are translated into the cytoplasm, producing viral proteins absolutely required for completion of their life cycle, replication protein (Rep), movement protein (MP) and coat protein (CP). The viral replication proteins interact with plant cellular proteins to produce multiple copies of the virus genome. These newly made genomes interact with CPs to form new virions or viral ribonucleoproteincomplexes (vRNP). The next step is the movement of the virus to neighboring cells, which requires the MP. The intracellular translated viral proteins (Avr) may also provide recognition sites for cytosolic NB-LR receptors (*e.g.,* R proteins), triggering ETI, which results in HR, necrosis, or SAR similarly to non-viral ETI. R proteins, R co-factors (CF), and Avr factors form an interacting complex with the SGT1/RAR1/HSP90, and EDS1/PAD4/SAG101 modules to mediate downstream changes in SA, JA, ET, NO, and H_2O_2 levels or to signal*via* MAP Kinases cascades, culminating in the induction of defense genes. NPR1 complexes with TF to induce defense genes *via* SA signaling, whereas EIN2 is a regulator of ET signaling. Virus infection may also trigger epigenetic changes. At the first line of defense, replication of viral RNA genomes may provide non-self RNA motifs (ssRNA or dsRNA) as virus-derived PAMPs to activate PTI. Alternatively, plant cells may sense viral infection and secrete plant-derived DAMPs, recognized by PRRs. Members of the SERK family also function as coreceptors in viral PTI. Arrows denote unknown or putative paradigms in viral innate immunity [65, 66]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.1. R-gene-mediated Resistance

The HR-mediated resistance causes cell death and eliminates infected cells to prevent the systemic spread of viral infection. The HR response is characterized by the signaling of mitogen-activated protein kinase (MAPK), increase in jasmonic acid (JA), calcium ion influx, high salicylic acid (SA), membrane permeability modification, defense genes activation, accumulation of reactive oxygen species (ROS), nitric oxide (NO) and callose deposition at the plasmodesmata (Fig. **1**) [71]. The majority of plant R genes are nucleotide-binding (NB) and leucine-rich-repeat (LRR) domain-encoding genes, whereas the proteins of Avr have very few common characteristics [65, 72]. The N gene encoding TIRNB-LRR protein from tobacco was the first characterized viral R Gene conferring resistance to TMV [73]. More than 20 viral R genes with the dominant mode of inheritance have been characterized (Table **3**). This class of resistance also comprises an Apaf-1/R protein/CED 4 (ARC) domain, which is involved in the hydrolysis of ATP and intra-molecular interactions [65, 74]. Some non-NB-LRR classes of proteins (JAX1, RTM1, RTM2, Ty-1, Ty-3, and Tm-1) though are not able to induce typical ETI-like defense response, such as HR, but may function as sensors of virus infection (Table **3**). Thus, such a non-NB-LRR class of proteins may act as potential target sites in CRISPR-Cas9 based editing and virus resistance development in the plant.

3.2. Recessive Resistance

The recessive R genes are plant genes that act as essential factors required to the virus to complete their biological cycle. The resistance governed by these genes is often considered as incompatible virus-host interaction wherein the virus infects the plant, but further systematic infection is disrupted by host resistance factors. This includes eukaryotic translation initiation factors, such as eIF4E and eIF4G, and resistance is conferred by functional mutations or modification of these gene products. In many viral diseases caused by *Potyviruses, Bigmoviruses, Cucumoviruses, Ipomoviruses, Sobemoviruses, Carmoviruses,* and *Waikiviruses*, eukaryotic translation initiation factors, eIF4E and eIF4G, play an

Table 3. List of plant resistance genes (D: dominant and R: Resistance) against plant viruses.

Sr. No.	Resistance Genes (D: Dominant and R: Resistance)	Plant Virus	Resistance Factors and Features	Avirulence Factor	References
1.	HRT (D)	Turnip crinkle virus	CC-NBS-LRR (HR)	CP	[75]
2.	JAX1 (D)	Platago asiatica mosaic Virus	Jacalin like lectin (Blocks RNA accumulation)	Unknown	[76]
3.	RCY1 (D)	Cucumber mosaic virus	CC-NBS-LRR (HR)	CP	[77]
4.	RTM1 (D)	Tobacco etch virus	Jacalin family (Blocking systemic Movement)	CP	[78]
5.	RTM2 (D)	Tobacco etch virus	Small heat shock Protein (Blocking Systemic Movement)	CP	[79]
6.	RTM3 (D)	Tobacco etch virus	MATH-containing protein (Blocking Systemic Movement	Unknown	[80]
7.	sp1 (r)	Turnip mosaic virus	eIF(iso)4E (mutagenesis)	VPg	[81]
8.	cum1(r)	Cucumber masaic virus	eIF4E (mutagenesis)	Unknown	[82]
9.	cum2 (r)	Cucumber masaic virus	eIF4E (mutagenesis)	Unknown	[82]
10.	BcTuR3 (D)	Turnip mosaic virus	TIR-NB-LRR (Systemic resistance)	Unknown	[80]
11.	TuRB07 (D)	Turnip mosaic virus	CC-NBS-LRR (ER)	Unknown	[83]
12.	L(multi-alleles) (D)	Tobacco mosaic virus	CC-NBS-LRR (HR)	CP	[84]
13.	pvr1/pvr2(multi- alleles)(r)	Potato virus Y	eIF4E	VPg	[85]
14.	pvr6 (r)	Pepper veinal mottle virus	eIF(iso)4E	VPg	[86]
15.	Nsv (r)	Melon necrotic spot virus	eIF4E	Unknown	[87]
16.	Rsv1 (D)	Soybean mosaic virus	CC-NB-LRR (HR)	P3, HC-Pro	[88]
17.	rym4/5(multi-alleles) (r)	Barley yellow mosaic virus	eIF4E	VPg	[89]
18.	mo1 (multi-alleles) (r)	Lettuce mosaic virus	eIF4E	CI- Cter, VPg	[90]
19.	rymv1 (r)	Rice yellow mottle virus	eIF(iso)	4G, VPg	[91]
20.	rymv2 (r)	Rice yellow mottle virus	CPR5(H)	unknown	[92]
21.	I (D)	Bean common mosaic virus	TIR-NBS-LRR (HR)	Unknown	[93]
22.	RT4-4 (D)	Cucumber mosaic virus	TIR-NBS-LRR (Systemic necrosis)	2a	[94]
23.	bc3 (r)	Bean common mosaic Virus	eIF4E	unknown	[95]
24.	sbm1 (r)	Pea seed-born mosaic Virus	eIF4E	VPg	[96]
25.	Ty1/Ty3 (multi-alleles) (D)	Tomato yellow leaf curl virus	RDR (RNA silencing)	Unknown	[97]
26.	Tm1 (D)	Tomato mosaic virus	TIM-barrel-like domain (Blocking replication)	Replication protein	[98]
27.	pot1 (r)	Potato virus Y	eIF4E	VPg	[87]
28.	Tm2 (multi-alleles) (D)	Tomato mosaic virus	CC-NBS-LRR (HR)	MP	[99]
29.	Sw5b (D)	Tomato spotted wilt virus	CC-NBS-LRR (HR)	MP (NSm)	[100]
30.	Rx (multi-alleles) (D)	Potato virus X	CC-NBS-LRR (Blocking replication)	CP	[101]
31.	Y1 (D)	Potato virus Y	TIR-NBS-LRR (HR)	Unknown	[102]
32.	CYR1 (D)	Mungbean yellow mosaic virus	CC_NB_LRR	CP	[103]

Abbreviations: MATH: meprin and TRAF domain, CP: coat protein, HC Pro: helper component proteinase, MP: movement protein, RDR-RNA: dependent RNA polymerase, ER: extreme resistance without any necrotic local lesion, eIF4E: eukaryotic translation initiation factor 4E, eIF(iso)4E: eukaryotic translation initiation factor iso 4E, Pelo: a messenger RNA surveillance factor, VPg: genome linked viral protein, CPR: constitutive expresser of pathogenesis-related genes, CI-Cter: C terminal of cylindrical inclusion helicase.

essential role in successful infection. The natural variation in eIF4E confers effective resistance to potyvirus infection in multiple crop species. This include pvr1 and pvr2 in pepper [94], sbmlin pea [90], mol in lettuce [104], rym4/5 in barley [87], pot1 in tomato [105] and zym-FL in watermelon [106], and Pelo in resistance ty5 genotype of tomato [107]. Thus,

many of the plant natural resistance genes functioning as essential host factors for virus infection have been mapped and are being exploited in genome editing technologies for the development of plant virus resistance.

3.3. RNA Interference-mediated Resistance

One of the major mechanisms for plant antiviral immunity is RNA interference (RNAi, also called gene silencing), which is triggered by double-stranded RNAs (dsRNAs) and recognized as an evolutionarily conserved process in most eukaryotes. The dsRNAs are processed by DCL (DICER-like-ribonuclease III-type) enzymes into 21-24 nucleotide small RNAs (sRNAs) and are incorporated into RISC (RNA-induced cytoplasmic silencing complex). RISC complex comprises AGO (Argonaute) and other proteins, whereas sRNAs are base paired to their target-mRNA to induce their cleavage. The key components of the RNA silencing pathways are the existence of multiple copies of AGO (Argonaute), DRB (double-stranded RNA binding), RNA dependent RNA polymerase (RDR) and DCL (Dicer-like) genes, which play an important protective role against invading viral pathogens [108, 109]. However, RNAi-mediated resistance is regularly hindered by several co-evolving viral suppressors (VSRs), which could enhance the viral pathogenicity within susceptible hosts [110].

3.4. Plant Hormone-mediated Resistance

Plant hormones have a significant role in the regulation of intercellular and systemic signaling networks in viral plant defense mechanisms [110]. Plant virus interactions result in the alterations of plant hormone synthesis and signaling in the host plant [20]. The plant hormones which facilitate the natural plant defense against viral disease include Auxin [IAA; Aux/IAA proteins in TMV infection], Gibberellin [GA3 in RDV], Ethylene [CaMV: P6 expression and disruption of the ethylene response pathway], Salicylic acid (SA) [activation of SAR in TMV, CaLCuV, Potato virus Y (PVY) and Tomato ringspot virus (ToRSV)], and Abscisic acid (ABA) [reduces the accumulation of TMV, Bamboo mosaic potyvirus (BaMV) and CMV, and cell to cell movement of the virus] [70, 111-113]. The Jasmonic acid (JA) synthesis genes were found to be suppressed upon the infection of geminivirus [114]. However, Brassinosteroids (BRs) were also observed as a positive regulator for inducing a plant defense against viruses, such as TMV, TCV, and Oilseed rape mosaic virus (ORMV) [115-117]. Thus, genetic variation in the phytohormone levels can restrict virus infection and will offer an opportunity to enhance plant immunity.

3.5. Proteasome Degradation

The ubiquitin-proteasome pathway (UPS) is also an antiviral defense strategy employed by the host plant. This pathway is involved in the fundamental plant processes, including degradation, functional modification of cellular proteins, and signaling in response to abiotic and biotic stimuli. Plant viruses exploit this pathway and inhibit or modify ubiquitin (Ub)-related host proteins, enabling successful host infection. However, UPS also plays a role in the host defense mechanism to eliminate viral components [118]. Several viral proteins have been observed to interact with different subunits of the 20S proteasome or 26S proteasome *viz*., helper component proteases (HcPro) of Lettuce mosaic virus (LMV), PVY, Papaya ringspot virus (PRSV), bC1 protein of geminivirus (TMV), C2 protein of geminivirus (BSCTV), RNA-dependent RNA polymerase of Turnip yellow mosaic virus (TYMV), factor C4 from Beet severe curly top virus (BSCTV), *etc.* [119, 120]. In a nutshell, plant viruses exploit the UPS for regulating the quality of their own proteins and enhancing their efficacy. In parallel, plants also use this pathway for basal resistance and for targeting viral proteins for degradation [70, 121]. Thus the genes involved in this pathway can be explored to build virus resistance in crop plants.

4. PLANT VIRUS MANAGEMENT STRATEGIES

Several strategies are available for the control of plant viruses. The chemical, biological and cultural management practices are mainly based on the control of insect vector(s) for further spread and transmission of the virus in the field. Most of the time, these methods are not found very effective and also associated with environmental hazardous. In this regard, the breeding of virus-resistant varieties is becoming an important, long-term strategy for the control of viral diseases. However, this strategy requires the availability of a resistant gene pool within the germplasm. The overall plant virus management strategies are classified into conventional and advanced methods (Fig. **2**) [2]. While the conventional methods like meristem-tip culture, thermotherapy, cryotherapy and chemotherapy have certain limitations, such as being expensive, time and labor-consuming, and having an issue with acclimatization, variability, production scheduling, and contamination, the advanced approach, namely RNAi silencing and cross-protection are not durable and induced resistance is also hindered by viral suppressor at the field level. The use of the transgenic and gene pyramiding approach is also time-consuming, costly, and has biosafety concerns. In this context, the emerging genome editing technology based upon homing endonucleases (EMNs), zinc finger nucleases (ZFNs), transcription activator-like effector nuclease (TALENs), and clustered regularly interspaced palindromic repeats (CRISPR-Cas9) repair mechanism has several advantages for the management of plant viruses.

5. BIOLOGY OF CRISPR-CAS9

The method based on clustered regularly interspaced palindromic repeats-Cas9 (CRISPR-Cas9) is the most preferred genome editing tool due to its ease, simplicity, specificity, low off-target effects, and precision [122-125]. CRISPR was initially discovered in *E. coli* in the year 1987 [126], and it functions to confer acquired resistance in bacteria and Archea against bacteriophage [127]. The short DNA segments (20 - 50 bp) from invading viruses and plasmids are integrated into their host genomes in between the copies of 20 - 50 bp repeat sequence. The resulting arrangements are hence referred to as clustered regularly interspaced short palindromic repeats [128]. Genes encoding Cas9 endonu-

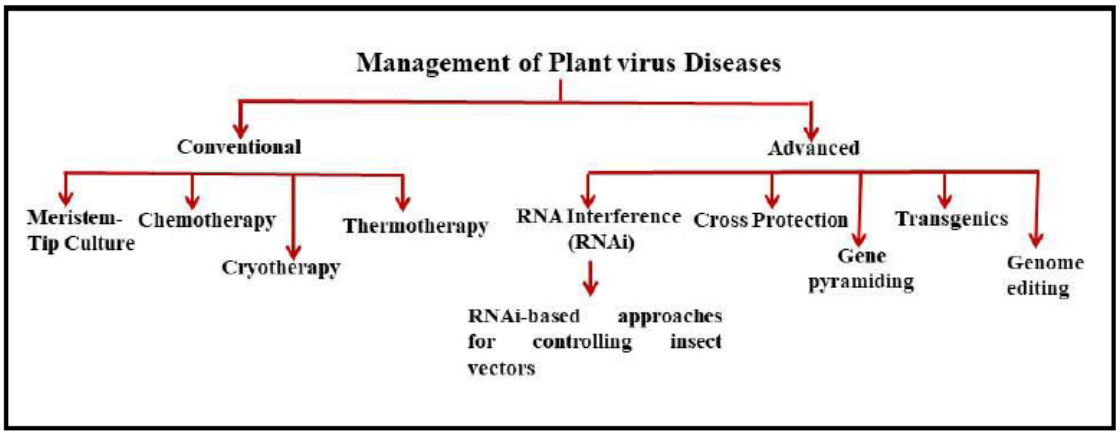


Fig. (2). Plant virus management strategies. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

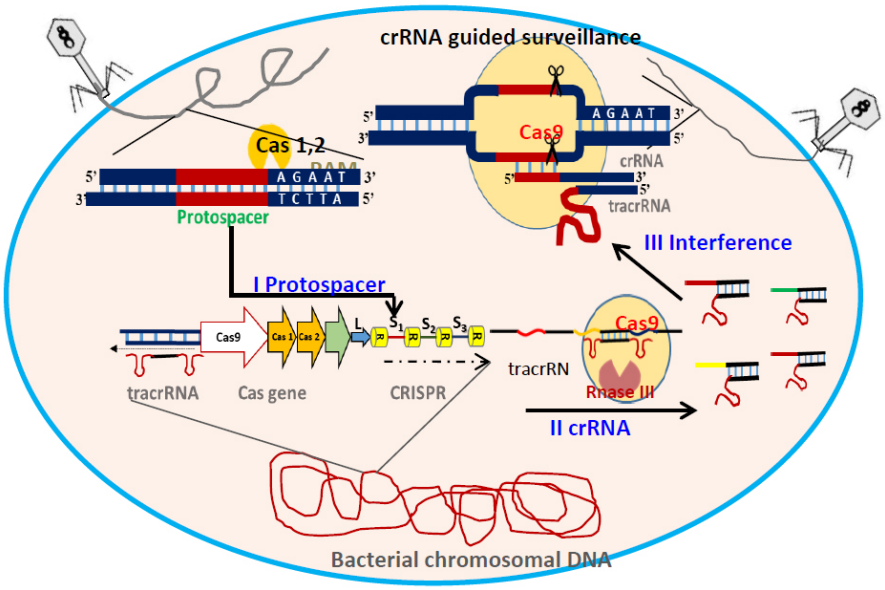


Fig. (3). The biology of CRISPR-Cas9 nuclease system (CRISPR acquisition, biogenesis, interference and surveillance. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

clease and two RNAs, *viz*. CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) are present adjacent to repeat elements (Fig. **3**). The entire CRISPR-Cas9 system drives the bacterial immunity against the invading viruses through acquisition/adaptation, CRISPR RNA (crRNA) biogenesis, and interference (Fig. **3**). In the acquisition process, foreign DNA is selected, processed, and integrated into the CRISPR array for storage as a memory of infection [129].

The transcription of CRISPR array produces a long precursor crRNA (pre-crRNA) which is processed within the repeat sequences to yield mature crRNAs. When a similar virus or plasmid infects, the interference machinery, mainly cas9 protein, is guided by crRNAs to cleave complementary sequences, called protospacers flanked by a protospacer-adjacent motif (PAM), in the incoming foreign nucleic acids (Fig. **3**) [130]. Based on the presence of Cas genes and the nature of the interference complex, CRISPR-Cas9 systems are classified into two classes and subsequently subdivided into six types and several subtypes, each possessing signature *Cas* genes [131]. The Class 1 CRISPR-Cas9 systems (types I, III, and IV) possess multi-Cas protein complexes, whereas, in class 2 systems (types II, V, and VI), single effector protein undertakes interference [132]. The type II CRISPR-Cas9 system with little modification (synthetic single-guided RNA (sgRNA) designed for trRNA and crRNA) was first time used by Doudna and Charpentier in 2012 for

Table 4. Examples of CRISPR/Cas9 mediated for DNA and RNA virus resistance in crop plants.

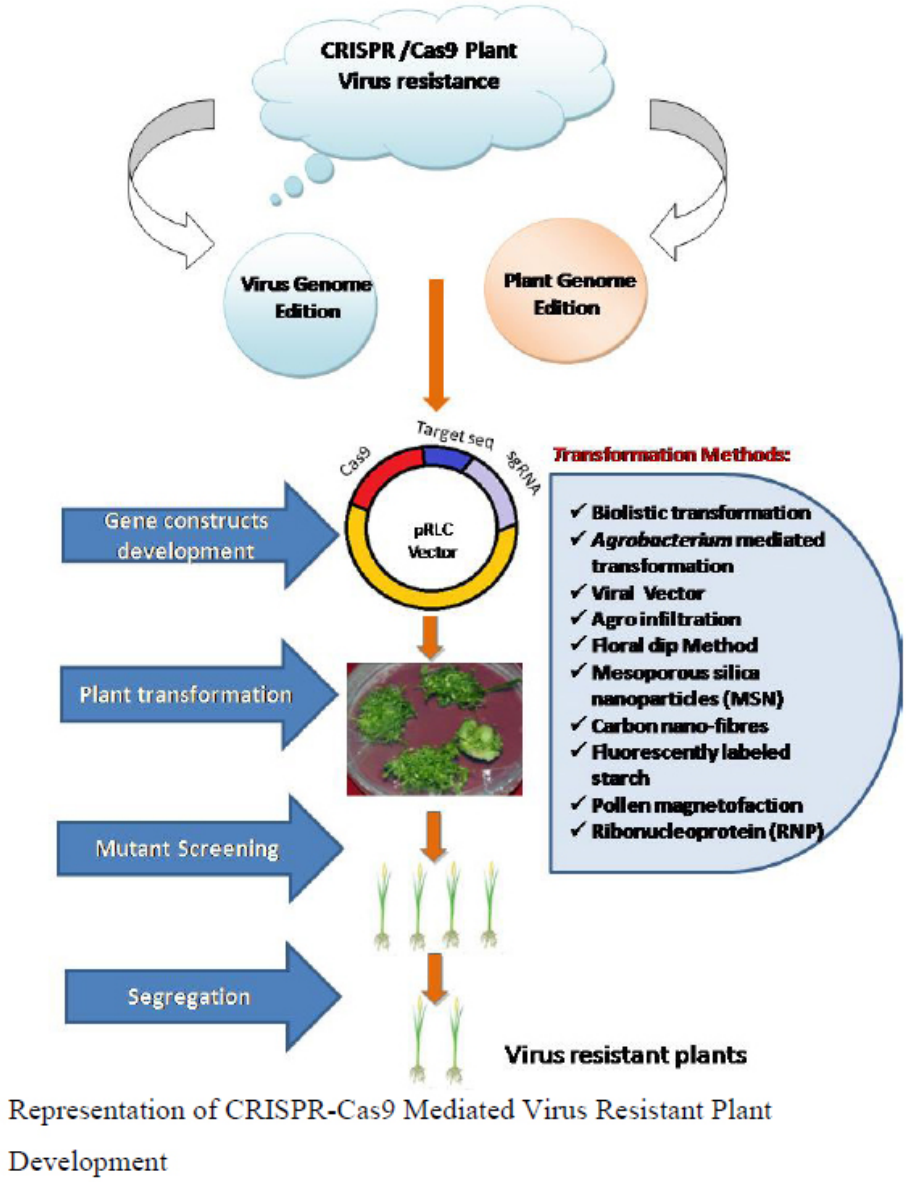
Virus/Viruses Targeted	Host Plant	Target Gene	Gene Function	References
Targeted Viral Genes				
BSCTV	*N. benthamiana* and *A. thaliana*	IR, CP, and Rep	RCA Mechanism	[139]
BeYDL	*N. benthamiana*	LIR and Rep/RepA	RCA Mechanism	[124]
TYLCV, BCTV, MeMV	*N. benthamiana*	IR, CP, and Rep	RCA Mechanism	[140]
CLCuKoV, TYLCV, TYLCSV, MeMV, BCTV-Logan, BCTV	*N. benthamiana*	IR, CP, and Rep	RCA Mechanism	[141]
BeYDV	*N. benthamiana*	LIR and Rep/RepA	Transgene free	[124]
BSCTV	*N. benthamiana* and *A. thaliana*	IR, CP, and Rep	Transgene free	[139]
TuMV	*N. benthamiana*	GFP1,2,3, CP and 3'UTR	Replication mechanism	[142]
CMV, TMV	*N. benthamiana*and *A. thaliana*	ORF,1,2,3, CP and 3'UTR	Replication mechanism	[120]
Targeted Host Genes				
TuMV	*A. thaliana*	eIF(iso)4E	Host factor for RNA viruses translation	[143]
CVYV, ZYMV, and PRSMV	*Cucumis sativus*	eIF4E	Host factor for RNA viruses translation	[144]
RTSV	*Oryza sativa* and *L. japonica*	eIF4G	Host factor for RNA viruses translation	[137]

genome editing [130]. Subsequently, this system has been extensively exploited for genome editing in all the fields of life sciences. Furthermore, different variants of Cas9 nuclease have been developed and adopted for genome editing. The wild-type Cas9 induces site-specific double-stranded break (DSB). The Cas9 mutant, *i.e.,* Cas9D10A, has only nickase activity and cleaves only one DNA strand, whereas dCas9 has lost cleavage activity but possesses DNA binding property used for gene silencing or gene activation [131]. The double-stranded break is repaired by a highly error-prone host repair system which induces genetic mutations. The DNA repair pathways, non-homologous end joining (NHEJ), and homology-directed repair (HDR) are responsible for random indels, which lead to frameshift mutations [132], whereas, in HDR, exogenously supplied homologues DNA sequences are integrated at the targeted site. Hence, these repair pathways are judiciously exploited in CRISPR-Cas9 for precise and targeted genome editing.

6. USE OF CRISPR/CAS9 SYSTEM TO ENGINEER VIRUS RESISTANCE IN PLANTS

The development of virus-resistant plants by using pathogen-derived resistance or RNAi gene technology has shown limited success [133] and has been facing the barriers of regulatory concerns and public acceptance. On the other hand, emerging CRISPR-Cas9 genome editing technology directly disrupts the essential viral genes instead of silencing them at the RNA level [134, 135]. The utility of CRISPR-Cas9 system for plant virus resistance has been reported independently by several researchers using model species like tobacco and Arabidopsis (Table **4**).

Virus resistance through CRISPR-Cas9 based genome editing is achieved either by editing the viral genome or the host plant genome [136]. Both these strategies are commonly employed to develop CRISPR-Cas9 based plant virus resistance. The first strategy aims to develop transgenic plants through engineering and maintenance of Cas9 and sgRNA in the genome of the crop plants, while the second strategy involves the development of non-transgenic mutants during further segregation of CRISPR-Cas9 machinery [137]. The process CRISPR-Cas9 based genome editing methods for the development of virus-resistant plants is depicted in Fig. (**4**). In general, CRISPR-Cas9 based virus-resistant plant developments comprises important steps *viz.* selection of target sites and gRNA synthesis, cloning of gRNA-CRISPR- Cas9 cassette into suitable transformation vector followed by plant transformation, screening of desirable mutant lines, and segregation and selection of desirable mutant line having effective on-target activity and rid of the off-target effect. There are several plant transformation strategies being deployed for the successful delivery of CRISPR-Cas9 gene cassettes. The *Agrobacterium*-mediated and biolistic transformation methods are the most applied transformation methods but have certain limitations. Moreover, several other transformation methods are being adopted for use, for example, viral vector-based transformation method, Agro-infiltration, Floral dip Method, Mesoporous silica nanoparticles (M-SN) method, Carbon nano-fibres, Fluorescently labeled starch method, Pollen magnetofection and DNA free reagent delivery method, *i.e.,* Ribonucleoprotein (RNP), *etc* [138]. Moreover, the commonly available methods for the confirmation of genome-edited crops include screening based on antibiotics (*in vitro* and *in vivo*), PCR amplification and RFLP analysis, restriction enzyme (RE) specific targets digestion or AFLP, probe hybridization and microarrays, sequencing for specific targets, morphological observations, biochemical analysis, *etc*. [139].



Representation of CRISPR-Cas9 Mediated Virus Resistant Plant Development

Fig. (4). Schematic representation of crispr-cas9 mediated virus resistant plant development. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

6.1. CRISPR-Cas9-mediated Resistance for DNA Viruses

The power of the CRISPR-Cas9 system to impart resistance against gemini viruses in plants was efficiently demonstrated in model plants *N. Benthamiana* and *Arabidopsis* [140] against *Tomato yellow leaf curl virus* (TYLCV), *Beet curly top virus* (BCTV), and *Merremia mosaic virus* (MeMV) [124], *Bean yellow dwarf virus* (BeYDV) [139] and *Beet severe curly top virus* (BSCTV). Ji *et al*. transiently expressed *Streptococcus pyogenes Cas9* gene and 43 candidate sgRNA target sites within the coding and non-coding regions of the BSCTV genome into *Nicotiana benthamiana* and *Arabidopsis thaliana* leaves [139]. Furthermore, these Agro-infiltrated leaves were challenged with *Beet severe curly top virus (*BSCTV) after two days of infiltration. The challenged plants after 10 days of post-infection were examined towards symptom development and presence of viral load by qPCR analysis. Control plants exhibited the typical shoot tip leaf curling symptoms, whereas infiltrated plants did not show virus symptoms and qPCR revealed more than 90% reduction in the accumulation of BSCT virus. The results of sequencing of virus DNA showed 1 to10 nucleotide long deletions in the target region. The higher virus resistance was found in the proportion of higher intensity of expression of Cas9 endonuclease gene and *vis-a-vis.*

Transgenic *N. benthamiana* plants conferring resistance to Bean yellow dwarf virus (BeYDV) were generated by

Baltes *et al*. [124]. The researchers exploited 11 sgRNAs and targeted Rep motifs, Rep-binding sites, hairpin, and the nonnucleotide sequence of BeYDV. The constitutively expressed Cas9 and sgRNAs (gBRBS+ or gBM3+) into *N. benthami* showed reduced symptoms and copy number of the BeYDV genome. The transgenic *N. benthamiana* plants showed an 87% reduction in virus load and symptom development due to the induction of mutations in the BeYDV genome. Ali *et al*. expressed the CRISPR-Cas9 machinery into *N. benthamiana* plants which exhibited resistance against tomato yellow leaf curl virus (TYLCV) [140]. The SgRNAs specific to various kinds of tomato yellow leaf curl virus genes, like capsid protein (CP), RCRII motif of the replication protein (Rep) and intergenic region (IR), were used. Among all these tested sgRNAs, the targeted stem-loop invariant IR sequence showed high interference and significantly reduced viral replication. Moreover, they tested this gRNA against *Beetcurly top virus* (BCTV) and *Merremia mosaic virus* (MeMV) and induced mixed infection immunity by single sgRNA developed on conserved sequences of multiple viral strains.

Besides highlighting the efficiencies of the CRISPR-Cas9 tool, Ali *et al*. also described the emergence of mutated viruses and their capability to replicate and move systemically [141]. This study has shown that sgRNA-Cas9 designed in the coding and non-coding sequences of Cotton leaf curl Kokhran virus (CLCuKoV), MeMV, exhibited different severe and mild strains of TYLCV, resulting in the generation of viral variants which were capable of replicating and escaping from genome editing machinery [142-144].

Zaidi *et al*. stated that sgRNA-Cas9 designed on the targets of non-coding intergenic sequences worked efficiently than the coding regions, which produced high levels of virus interference, and provided potential strong resistance in Geminiviruses [145]. Furthermore, this technology was effectively implemented and proved against dsDNA viruses like Cauliflower mosaic virus (CaMV) in the model plant, Arabidopsis. Besides the applications of CRISPR-Cas9 in model plants, recently, the strategy has been successfully demonstrated in cultivated species of barley for the development of plants resistant to the Wheat dwarf virus [146].

6.2. CRISPR-Cas-mediated Resistance for RNA Viruses

Besides the use of the CRISPR-Cas9 system to develop immunity against DNA viruses, the technique has been explored to target plant viruses with RNA genomes [147]. The RNA viruses utilize plant host factors towards maintaining their life cycle. The important host factors of plants being utilized by RNA viruses are eukaryotic translation initiation factors or host susceptibility genes, *i.e.,* eIF4E, eIF(iso)4E, and eIF4G [148], which can be used as potential target sites for inducing mutation through CRISPR-Cas9. Chandrasekaran *et al*. successfully induced mutation within the two different sites of the cucumber (*Cucumis sativus*) translation initiation factor eIF4E gene by CRISPR-Cas9 and developed Cucumis eIF4 Emutant [144]. The testing of homozygous Cucumis mutant plants against a member of the Potyviridae family revealed resistance against Cucumber vein yellowing virus (CVYV), Zucchini yellow mosaic virus (ZYMV), and Papaya ringspot mosaic virus-W (PRSV-W). In another study, the isoform of the eIF4E gene locus was targeted in *Arabidopsis thaliana* to introduce a 1 bp site-specific mutation within the gene [143]. The GE-edited plants showed complete resistance to Potyvirus, Turnip Mosaic Virus (TuMV) without any significant off-target effects.

CRISPR technology has also been applied for the control of RNA viruses. Zhang *et al.* expressed FnCas9 and targeted Cucumber mosaic virus (CMV) and Tobacco mosaic virus (TMV) by using specific sgRNAs in *N. benthamiana* and *Arabidopsis* plants [120]. The authors recorded 40 - 80% reduced accumulation of viruses and obtained resistance stability of sgRNA-FnCas9 against *Cucumber mosaic virus (CMV)* and *Tobacco mosaic virus* (*TMV*) up to T6 generation. This study also showed that instead of the endonuclease activity of FnCas9, its RNA-binding activity was found responsible for making interference with the CMV genome, suggesting that such a mechanism would help restrict the escape mechanism of mutated viral variants. Aman *et al*. exploited RNA-guided ribonuclease Cas13a and manipulated Turnip mosaic virus (TuMV) RNA genome [142]. The authors targeted four different viral genes, namely two targets on green fluorescent protein (GFP) and two on each of the target sites in the helper component, namely proteinase silencing suppressor (HC-Pro) and coat protein (CP) gene. The effective virus interference was obtained in the edited HC-Pro and GFP2 genes, which could result in a decline in the multiplication of Turnip mosaic virus (TuMV) in tobacco, indicating reduced replication and spread of TuMV in tobacco leaves. A recessive trait of RTSV resistance in rice (*Oryza sativa*) is controlled by the translation initiation factor 4 gamma gene (eIF4G). Macovei *et al.*have developed three gRNA's at SNPs nucleotide positions of 4387 and 4390 of eIF4G, depicting reaction to RTSV; they also exploited CRISPR-Cas9 based editing towards the induction of mutation in the eIF4G alleles of rice [137]. Furthermore, *Agrobacterium*-mediated transformation used in IR-64 cultivar and progenies were advanced up to T2 and T3 generation. The study successfully generated RTSV resistant IR-64.

7. OBSTACLES AND POSSIBLE CHALLENGES

The occurrence of off-target effects is a crucial challenge in CRISPR-Cas9 technology. Besides, several other challenges encountered during CRISPR/cas9 based virus resistance development are outlined below:

- Lack of availability of annotated genomic data in crop plants.
- *Frequent occurrence of Off-targets activity:* The mismatches of sgRNA sequence, optimal expression of Cas9 nuclease [148], the location and accessibility of target site inside the host or target genome of an organism, high homology with the desired target in the genome, choice of endonuclease, choice of promoter for sgRNA and Cas9 gene expression [149] influ-

ence the off-target effects. The off-target activity is accelerated due to the presence of non-specific targets, presence of gene paralogs, cultivar to cultivar polymorphism within the same species [150]. The GC content lower than 30% of sgRNA increases the chances of off-target activity. Polyploidy genome and repetitive DNA sequences within organisms make it difficult to induce target-specific gene mutation [151]. The larger size genomes have more PAM sites, and this may increase more possible targets. However, the mismatch far away from the 12 bp seed sequence region also induces unwanted off-target mutations [152]. The unlimited expression of the Cas9 nuclease enzyme could result in the occurrence of off-target activity [153]. The off-target activity could be minimized by reducing sgRNA length from 5'end up to 17 - 18 nucleotides [154].

- *Lack of efficient regeneration system*: The efficient plant regeneration system enables successful delivery of CRISPR-Cas9 cassette into the target genome, but many plants are recalcitrant and lack higher regeneration potential. Besides, regeneration protocols also vary from species to species, are genotype dependant, and are also influenced by media composition factors. Such limitations can be managed through *in-planta* based transient methods, PEG, and electroporation-based protoplast transformation protocols.
- Lack of suitable screening methods and expertise for the identification of edited plants.
- Requirement of a broad range of specific protospacer adjacent motif (PAM) sites for accurate alteration of exact nucleotides in a gene of interest.
- Knocking the host susceptibility genes, a passive target of the viruses, may negatively concern plant vigor and other traits.
- Required knowledge on molecular pathways of plant-virus interaction and the resources to be used in editing.
- A containment facility and proper field evaluation is required for testing the multi-environmental fitness of genome-edited crops with special emphasis on changes in their agronomic features, if any.
- The introduction of true genome editing technology rather than targeted mutagenesis, which induces random mutations leading to the loss of gene function, is quite challenging.
- In the case of developing transgene-free virus-resistant plants through the CRISPR-Cas9 system, knocking out host factors isessential but it may impose a negative influence on plant growth due to their other functions in plant growth and metabolism. Also, the redundancy of such host factors/native genes within the plant may minimize resistance durability against member viruses.
- In case of introduced resistance against DNA viruses through CRISPR-Cas9, there may be frequent chances of evolving mutations among target viruses and getting rid of/escape from cleavage activity by CRISPR-Cas9 [155], which may encourage the evolution of super-viruses.
- Another constraint is in polyploidy crops since it is necessary to study the large numbers of mutants occurring in the multiple alleles [138].

7.1. Strategies to Bypass Off-target Activities

The effect of off-target activities CRISPR-Cas9 can be minimized by the selection and use of virus-induced promoters during the expression of Cas9 protein in place of a constitutive promoter. Under this situation, the antiviral CRISPR-Cas9 system would be expressed only after the presence of the virus in the plant cell. Besides, several other strategies to tackle such off-target activities are also being tested in model plants, which include reducing the concentration of CRISPR-Cas9 reagents leading to an increase in specificity of the targets, selection of target sites possessing high (up to 70%) GC content, sgRNA truncation at 5' or 3' end, reduction of sgRNA length from 5'end up to 17-18 nucleotides, use of paired Cas9 nickases with sgRNA, the addition of additional GG at the 5' end and using *Fok*I nuclease in guiding Ca9 proteins, *etc*. [138]. However, to avoid off-target activities in virus resistance, in addition to using CRISPR-Cas9 derived gene editing, chemical-based base modification could be a promising alternative [156, 157].

Therefore, appropriate selection of target site(s) rendering high efficiency of mutagenesis and prediction of fewer chances of off-target effect can be achieved by the use of several online computational tools and servers for designing target gene-specific sgRNAs, gene constructs and data analysis [138]. Also, the use of web tool 'CRISPR-P' for designing sgRNAs [158], careful choice of the sgRNA sequence, and various experimental techniques may help to avoid mismatch and increase on-target genome editing efficiency.

7.2. Multiplex Genome Editing Approach

The multiplex genome editing approach in CRISPR-Cas9 has certain advantages and can simultaneously mutate several different target genes and provides resistance against all types of variants of plant viruses. There are basically two approaches: the first one comprises the expression of individual gRNA with its own promoter, whereas the second one pertains to the expression of multiple gRNAs under a single promoter to produce a single transcript and cleave individual gRNAs [138]. The various multiplex genome editing strategies are being utilized for the improvement of plants against biotic stress tolerance, which are polycistronic tRNA tRNA-gRNA (PTG) based approach, intron based polycistronic transfer RNA guide RNAs (inPTGs), CRISPR system with Yersinia (Csy4) nuclease mediated approach and Drosha based multiple gene-editing approach. The use of a polycistronic tRNA-gRNA (PTG) under a single promoter could also reduce the construct size suitable for easy transformation and minimize silencing risk due to the presence of repeated sequences of various promoters [159]. The use of a multiplex genome editing approach with multiple target regions of viral genomes in one system will minimize the development of escape mutant among viruses.

CONCLUSION AND FUTURE PERSPECTIVES

CRISPR-Cas9 based genome editing is becoming a promising technique to induce resistance against DNA and RNA viruses in crop plants. The technology may also have public acceptability as the developed virus-resistant plants will be non-transgenic plants, which may have fewer regulatory concerns. Besides, this technology can be explored for a variety of agronomic traits in agriculture crops by resolving off-target activity, and accuracy, and increasing the efficiency of the customized enzymes. Moreover, the use of multiplex genome editing approach, development of highly sophisticated bioinformatics programs, modification in endonucleases, gRNA modifications, and sensitive NGS-based detection methods have to be successfully implemented to increase the genome editing efficiency and prevent the interruption of native gene function. The CRISPR/Cas9 and other related genome editing approaches offer great scope for plant molecular breeding aimed at crop improvement.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

The study is supported by Science and Engineering Research Board (DST), India; Grant # YSS/2015/000616 to USK.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Karavina, C.; Gubba, A. Iris yellow spot virus in Zimbabwe: Incidence, severity and characterization of Allium-infecting isolates. *Crop Prot.,* **2017**, *94*, 69-76. http://dx.doi.org/10.1016/j.cropro.2016.12.019

[2] Chauhan, P.; Singla, K.; Rajbhar, M.; Singh, A.; Das, N.; Kumar, K. A systematic review of conventional and advanced approaches for the control of plant viruses. *J. Appl. Biol. Biotechnol.,* **2019**, *7*(4), 89-98. http://dx.doi.org/10.7324/JABB.2019.70414

[3] Matthews, H.R. *Plant Virology,* 4th ed; Academic Press: New York, NY, USA, **2002**.

[4] Baltimore, D. Expression of animal virus genomes. *Bacteriol. Rev.,* **1971**, *35*(3), 235-241. http://dx.doi.org/10.1128/BR.35.3.235-241.1971 PMID: 4329869

[5] Jones, R.A.C.; Naidu, R.A. Global dimensions of plant virus diseases: current status and future perspectives *Annu. Rev. Virol.,* **2019**, *6*(1), 387-409. http://dx.doi.org/10.1146/annurev-virology-092818-015606 PMID: 31283443

[6] Minato, N.; Sok, S.; Chen, S.; Delaquis, E.; Phirun, I.; Le, V.X.; Burra, D.D.; Newby, J.C.; Wyckhuys, K.A.G.; de Haan, S. Surveillance for Sri Lankan cassava mosaic virus (SLCMV) in Cambodia and Vietnam one year after its initial detection in a single plantation in 2015. *PLoS One,* **2019**, *14*(2), e0212780. http://dx.doi.org/10.1371/journal.pone.0212780 PMID: 30794679

[7] Sastry, K.S.; Zitter, T.A. Management of virus and viroid diseases of crops in the tropics. In: *Plant virus and viroid diseases in the tropics*; Springer Netherlands: Dordrecht, The Netherlands, **2014**, pp. 149-480. http://dx.doi.org/10.1007/978-94-007-7820-7_2

[8] Wale, S.; Platt, B.; Cattlin, N.D. *Diseases, pests and disorders of potatoes: a colour handbook*, **2008**. http://dx.doi.org/10.1201/b15127

[9] Ordon, F.; Habekuss, A.; Kastirr, U.; Rabenstein, F.; Kühne, T. Virus resistance in cereals: sources of resistance, genetics and breeding. *J. Phytopathol.,* **2009**, *157*(9), 535-545. http://dx.doi.org/10.1111/j.1439-0434.2009.01540.x

[10] Kumar, G.; Jyothsna, M.; Valarmathi, P.; Roy, S.; Banerjee, A.; Tarafdar, J.; Senapati, BK.; Robin, S.; Manonmani, S.; Rabindran, R.; Dasgupta, I. Assessment of resistance to rice tungro disease in popular rice varieties in India by introgression of a transgene against Rice tungro bacilliform virus. *Arch. Virol.,* **2019**, *164*(4), 1005-1013. http://dx.doi.org/10.1007/s00705-019-04159-3

[11] Harper, S.J. *Citrus tristeza virus*: evolution of complex and varied genotypic groups. *Front. Microbiol.,* **2013**, *4*, 93. http://dx.doi.org/10.3389/fmicb.2013.00093 PMID: 23630519

[12] Dzahini-Obiatey, H.K.; Domfeh, O.; Amoah, F.M. Over seventy years of a viral disease of cocoa in Ghana: From researchers' perspective. *Afr. J. Agric. Res.,* **2010**, *5*(7), 476-485.

[13] Rybicki, E.P. A Top Ten list for economically important plant viruses. *Arch. Virol.,* **2015**, *160*(1), 17-20. http://dx.doi.org/10.1007/s00705-014-2295-9 PMID: 25430908

[14] Zhang, D.; Li, Z.; Li, J.F. Genome editing: new antiviral weapon for plants. *Nat. Plants,* **2015**, *1*(10), 1-2. http://dx.doi.org/10.1038/nplants.2015.146

[15] Kumar, P.L.; Selvarajan, R.; Iskra-Caruana, M.L.; Chabannes, M.; Hanna, R. Biology, etiology, and control of virus diseases of banana and plantain. *Adv. Virus Res.,* **2015**, *91*, 229-269. http://dx.doi.org/10.1016/bs.aivir.2014.10.006 PMID: 25591881

[16] Nicaise, V. Crop immunity against viruses: outcomes and future challenges. *Front. Plant Sci.,* **2014**, *5*, 660. http://dx.doi.org/10.3389/fpls.2014.00660 PMID: 25484888

[17] Dolja, V.V.; McBride, H.J.; Carrington, J.C. Tagging of plant potyvirus replication and movement by insertion of beta-glucuronidase into the viral polyprotein. *Proc. Natl. Acad. Sci. USA,* **1992**, *89*(21), 10208-10212. http://dx.doi.org/10.1073/pnas.89.21.10208 PMID: 1438210

[18] Zwart, M.P.; Daròs, J.A.; Elena, S.F. Effects of potyvirus effective population size in inoculated leaves on viral accumulation and the onset of symptoms. *J. Virol.,* **2012**, *86*(18), 9737-9747. http://dx.doi.org/10.1128/JVI.00909-12 PMID: 22740417

[19] García, J.A.; Pallás, V. Viral factors involved in plant pathogenesis. *Curr. Opin. Virol.,* **2015**, *11*, 21-30. http://dx.doi.org/10.1016/j.coviro.2015.01.001 PMID: 25618249

[20] Culver, J.N.; Padmanabhan, M.S. Virus-induced disease: altering host physiology one interaction at a time. *Annu. Rev. Phytopathol.,* **2007**, *45*, 221-243. http://dx.doi.org/10.1146/annurev.phyto.45.062806.094422 PMID: 17417941

[21] Roossinck, M.J. Plants, viruses and the environment: Ecology and mutualism. *Virology,* **2015**, *479-480*, 271-277. http://dx.doi.org/10.1016/j.virol.2015.03.041 PMID: 25858141

[22] Jameson, P.C.S. Hormone-virus interaction in plants. *Crit. Rev. Plant Sci.,* **2002**, *21*, 205-228. http://dx.doi.org/10.1080/0735-260291044241

[23] Laliberté, J.F.; Sanfaçon, H. Cellular remodeling during plant virus infection. *Annu. Rev. Phytopathol.,* **2010**, *48*(48), 69-91. http://dx.doi.org/10.1146/annurev-phyto-073009-114239 PMID: 20337516

[24] den Boon, J.A.; Ahlquist, P. Organelle-like membrane compartmentalization of positive-strand RNA virus replication factories. *Annu. Rev. Microbiol.,* **2010**, *64*, 241-256. http://dx.doi.org/10.1146/annurev.micro.112408.134012 PMID: 20825348

[25] Laliberte, J.J. Membrane Association for Plant Virus Replication and Movement. *Current Research Topics in Plant Virology*; Wang, X.Z.A., Ed., **2016**, pp. 1-335. http://dx.doi.org/10.1007/978-3-319-32919-2

[26] Diaz, A.; Zhang, J.; Ollwerther, A.; Wang, X.; Ahlquist, P. Host

ESCRT proteins are required for bromovirus RNA replication compartment assembly and function. *PLoS Pathog.,* **2015**, *11*(3), e1004742.
http://dx.doi.org/10.1371/journal.ppat.1004742 PMID: 25748299

[27] Hanley-Bowdoin, L.; Settlage, S.B.; Robertson, D.; Robertson, B. Reprogramming plant gene expression: a prerequisite to geminivirus DNA replication. *Mol. Plant Pathol.,* **2004**, *5*(2), 149-156.
http://dx.doi.org/10.1111/j.1364-3703.2004.00214.x PMID: 20565592

[28] Sanfaçon, H. Plant translation factors and virus resistance. *Viruses,* **2015**, *7*(7), 3392-3419.
http://dx.doi.org/10.3390/v7072778 PMID: 26114476

[29] Dangl, J.L.; Jones, J.D. Plant pathogens and integrated defence responses to infection. *Nature,* **2001**, *411*(6839), 826-833.
http://dx.doi.org/10.1038/35081161 PMID: 11459065

[30] Padmanabhan, M.S.; Shiferaw, H.; Culver, J.N. The Tobacco mosaic virus replicase protein disrupts the localization and function of interacting Aux/IAA proteins. *Mol. Plant Microbe Interact.,* **2006**, *19*(8), 864-873.
http://dx.doi.org/10.1094/MPMI-19-0864 PMID: 16903352

[31] Bilgin, D.D.; Liu, Y.; Schiff, M.; Dinesh-Kumar, S.P. P58(IPK), a plant ortholog of double-stranded RNA-dependent protein kinase PKR inhibitor, functions in viral pathogenesis. *Dev. Cell,* **2003**, *4*(5), 651-661.
http://dx.doi.org/10.1016/S1534-5807(03)00125-4 PMID: 12737801

[32] Zhu, S.; Gao, F.; Cao, X.; Chen, M.; Ye, G.; Wei, C.; Li, Y. The rice dwarf virus P2 protein interacts with ent-kaurene oxidases *in vivo*, leading to reduced biosynthesis of gibberellins and rice dwarf symptoms. *Plant Physiol.,* **2005**, *139*(4), 1935-1945.
http://dx.doi.org/10.1104/pp.105.072306 PMID: 16299167

[33] Kong, L.J.; Orozco, B.M.; Roe, J.L.; Nagar, S.; Ou, S.; Feiler, H.S.; Durfee, T.; Miller, A.B.; Gruissem, W.; Robertson, D.; Hanley-Bowdoin, L. A geminivirus replication protein interacts with the retinoblastoma protein through a novel domain to determine symptoms and tissue specificity of infection in plants. *EMBO J.,* **2000**, *19*(13), 3485-3495.
http://dx.doi.org/10.1093/emboj/19.13.3485 PMID: 10880461

[34] Guo, H.S.; García, J.A. Delayed resistance to plum pox potyvirus mediated by a mutated RNA replicase gene: involvement of a gene-silencing mechanism. *Mol. Plant Microbe Interact.,* **1997**, *10*(2), 160-170.
http://dx.doi.org/10.1094/MPMI.1997.10.2.160

[35] Citovsky, V.; Zambryski, P. Transport of protein-nucleic acid complexes within and between plant cells. *Membrane Protein Transport,* **1995**, *1*, 39-57.

[36] Kim, S.H.; Palukaitis, P.; Park, Y.I. Phosphorylation of cucumber mosaic virus RNA polymerase 2a protein inhibits formation of replicase complex. *EMBO J.,* **2002**, *21*(9), 2292-2300.
http://dx.doi.org/10.1093/emboj/21.9.2292 PMID: 11980726

[37] Wang, M.B.; Bian, X.Y.; Wu, L.M.; Liu, L.X.; Smith, N.A.; Isenegger, D.; Wu, R.M.; Masuta, C.; Vance, V.B.; Watson, J.M.; Rezaian, A.; Dennis, E.S.; Waterhouse, P.M. On the role of RNA silencing in the pathogenicity and evolution of viroids and viral satellites. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(9), 3275-3280.
http://dx.doi.org/10.1073/pnas.0400104101 PMID: 14978267

[38] Canto, T.; Uhrig, J.F.; Swanson, M.; Wright, K.M.; MacFarlane, S.A. Translocation of Tomato bushy stunt virus P19 protein into the nucleus by ALY proteins compromises its silencing suppressor activity. *J. Virol.,* **2006**, *80*(18), 9064-9072.
http://dx.doi.org/10.1128/JVI.00953-06 PMID: 16940518

[39] Uhrig, J.F.; Canto, T.; Marshall, D.; MacFarlane, S.A. Relocalization of nuclear ALY proteins to the cytoplasm by the tomato bushy stunt virus P19 pathogenicity protein. *Plant Physiol.,* **2004**, *135*(4), 2411-2423.
http://dx.doi.org/10.1104/pp.104.046086 PMID: 15299117

[40] Carvalho, M.F.; Turgeon, R.; Lazarowitz, S.G. The geminivirus nuclear shuttle protein NSP inhibits the activity of AtNSI, a vascular-expressed Arabidopsis acetyltransferase regulated with the sink-to-source transition. *Plant Physiol.,* **2006**, *140*(4), 1317-1330.
http://dx.doi.org/10.1104/pp.105.075556 PMID: 16461385

[41] Fontes, E.P.; Santos, A.A.; Luz, D.F.; Waclawovsky, A.J.; Chory, J. The geminivirus nuclear shuttle protein is a virulence factor that suppresses transmembrane receptor kinase activity. *Genes Dev.,* **2004**, *18*(20), 2545-2556.
http://dx.doi.org/10.1101/gad.1245904 PMID: 15489295

[42] Aronson, M.N.; Meyer, A.D.; Györgyey, J.; Katul, L.; Vetten, H.J.; Gronenborn, B.; Timchenko, T. Clink, a nanovirus-encoded protein, binds both pRB and SKP1. *J. Virol.,* **2000**, *74*(7), 2967-2972.
http://dx.doi.org/10.1128/JVI.74.7.2967-2972.2000 PMID: 10708410

[43] Krab, I.M.C.; Caldwell, C.; Gallie, D.R.; Bol, J.F. Coat protein enhances translational efficiency of Alfalfa mosaic virus RNAs and interacts with the eIF4G component of initiation factor eIF4F. *J. Gen. Virol.,* **2005**, *86*(Pt 6), 1841-1849.
http://dx.doi.org/10.1099/vir.0.80796-0 PMID: 15914864

[44] Sun, L.; Andika, I.B.; Shen, J.; Yang, D.; Chen, J. The P2 of Wheat yellow mosaic virus rearranges the endoplasmic reticulum and recruits other viral proteins into replication-associated inclusion bodies. *Mol. Plant Pathol.,* **2014**, *15*(5), 466-478.
http://dx.doi.org/10.1111/mpp.12109 PMID: 24304930

[45] Jiang, J.; Patarroyo, C.; Garcia Cabanillas, D.; Zheng, H.; Laliberté, ´.J.F. The vesicle-forming 6K2protein of turnip mosaic virus interacts with the COPII coatomer Sec24a for viral systemic infection. *J. Virol.,* **2015**, *89*(13), 6695-6710.
http://dx.doi.org/10.1128/JVI.00503-15 PMID: 25878114

[46] Carette, J.E.; Verver, J.; Martens, J.; van Kampen, T.; Wellink, J.; van Kammen, A. Characterization of plant proteins that interact with cowpea mosaic virus '60K' protein in the yeast two-hybrid system. *J. Gen. Virol.,* **2002**, *83*(Pt 4), 885-893.
http://dx.doi.org/10.1099/0022-1317-83-4-885 PMID: 11907339

[47] Wei, T.; Zhang, C.; Hou, X.; Sanfaçon, H.; Wang, A. The SNARE protein Syp71 is essential for turnip mosaic virus infection by mediating fusion of virus-induced vesicles with chloroplasts. *PLoS Pathog.,* **2013**, *9*(5), e1003378.
http://dx.doi.org/10.1371/journal.ppat.1003378 PMID: 23696741

[48] Barajas, D.; Martín, I.F.; Pogany, J.; Risco, C.; Nagy, P.D. Noncanonical role for the host Vps4 AAA+ ATPase ESCRT protein in the formation of Tomato bushy stunt virus replicase. *PLoS Pathog.,* **2014**, *10*(4), e1004087.
http://dx.doi.org/10.1371/journal.ppat.1004087 PMID: 24763736

[49] Hyodo, K.; Mine, A.; Taniguchi, T.; Kaido, M.; Mise, K.; Taniguchi, H.; Okuno, T. ADP ribosylation factor 1 plays an essential role in the replication of a plant RNA virus. *J. Virol.,* **2013**, *87*(1), 163-176.
http://dx.doi.org/10.1128/JVI.02383-12 PMID: 23097452

[50] Huang, T.S.; Nagy, P.D. Direct inhibition of tombusvirus plus-strand RNA synthesis by a dominant negative mutant of a host metabolic enzyme, glyceraldehyde-3-phosphate dehydrogenase, in yeast and plants. *J. Virol.,* **2011**, *85*(17), 9090-9102.
http://dx.doi.org/10.1128/JVI.00666-11 PMID: 21697488

[51] Diaz, A.; Wang, X.; Ahlquist, P. Membrane-shaping host reticulon proteins play crucial roles in viral RNA replication compartment formation and function. *Proc. Natl. Acad. Sci. USA,* **2010**, *107*(37), 16291-16296.
http://dx.doi.org/10.1073/pnas.1011105107 PMID: 20805477

[52] Chaturvedi, S.; Rao, A.L.N. Live cell imaging of interactions between replicase and capsid protein of Brome mosaic virus using Bimolecular Fluorescence Complementation: implications for replication and genome packaging. *Virology,* **2014**, *464-465*, 67-75.
http://dx.doi.org/10.1016/j.virol.2014.06.030 PMID: 25046269

[53] Agbeci, M.; Grangeon, R.; Nelson, R.S.; Zheng, H.; Laliberté, J.F. Contribution of host intracellular transport machineries to intercellular movement of turnip mosaic virus. *PLoS Pathog.,* **2013**, *9*(10), e1003683.
http://dx.doi.org/10.1371/journal.ppat.1003683 PMID: 24098128

[54] Grangeon, R.; Agbeci, M.; Chen, J.; Grondin, G.; Zheng, H.; Laliberté, J.F. Impact on the endoplasmic reticulum and Golgi apparatus of turnip mosaic virus infection. *J. Virol.,* **2012**, *86*(17), 9255-9265.
http://dx.doi.org/10.1128/JVI.01146-12 PMID: 22718813

[55] Wan, J.; Cabanillas, D.G.; Zheng, H.; Laliberté, J.F. Turnip mosaic virus moves systemically through both phloem and xylem as membrane-associated complexes. *Plant Physiol.,* **2015**, *167*(4), 1374-1388.

http://dx.doi.org/10.1104/pp.15.00097 PMID: 25717035

[56] Cui, X.; Wei, T.; Chowda-Reddy, R.V.; Sun, G.; Wang, A. The Tobacco etch virus P3 protein forms mobile inclusions *via* the early secretory pathway and traffics along actin microfilaments. *Virology,* **2010**, *397*(1), 56-63. http://dx.doi.org/10.1016/j.virol.2009.11.015 PMID: 19945728

[57] Tilsner, J.; Linnik, O.; Wright, K.M.; Bell, K.; Roberts, A.G.; Lacomme, C.; Santa Cruz, S.; Oparka, K.J. The TGB1 movement protein of Potato virus X reorganizes actin and endomembranes into the X-body, a viral replication factory. *Plant Physiol.,* **2012**, *158*(3), 1359-1370. http://dx.doi.org/10.1104/pp.111.189605 PMID: 22253256

[58] Samuels, T.D.; Ju, H.J.; Ye, C.M.; Motes, C.M.; Blancaflor, E.B.; Verchot-Lubicz, J. Subcellular targeting and interactions among the Potato virus X TGB proteins. *Virology,* **2007**, *367*(2), 375-389. http://dx.doi.org/10.1016/j.virol.2007.05.022 PMID: 17610926

[59] Chou, Y.L.; Hung, Y.J.; Tseng, Y.H.; Hsu, H.T.; Yang, J.Y.; Wung, C.H.; Lin, N.S.; Meng, M.; Hsu, Y.H.; Chang, B.Y. The stable association of virion with the triple-gene-block protein 3-based complex of Bamboo mosaic virus. *PLoS Pathog.,* **2013**, *9*(6), e1003405. http://dx.doi.org/10.1371/journal.ppat.1003405 PMID: 23754943

[60] Rathjen, J.P.; Moffett, P. Early signal transduction events in specific plant disease resistance. *Curr. Opin. Plant Biol.,* **2003**, *6*(4), 300-306. http://dx.doi.org/10.1016/S1369-5266(03)00057-8 PMID: 12873522

[61] Islam, W.; Zaynab, M.; Qasim, W.; Wu, Z. Plant-virus interactions: Disease resistance in focus. *Hosts Viruses,* **2017**, *4*(1), 5-20. http://dx.doi.org/10.17582/journal.bjv/2017/4.1.5.20

[62] Macho, A.P.; Zipfel, C. Plant PRRs and the activation of innate immune signaling. *Mol. Cell,* **2014**, *54*(2), 263-272. http://dx.doi.org/10.1016/j.molcel.2014.03.028

[63] Bigeard, J.; Colcombet, J.; Hirt, H. Signaling mechanisms in pattern-triggered immunity (PTI). *Mol. Plant,* **2015**, *8*, 521-539.

[64] Roux, F.; Voisin, D.; Badet, T.; Balagué, C.; Barlet, X.; Huard-Chauveau, C.; Roby, D.; Raffaele, S. Resistance to phytopathogens e tutti quanti: placing plant quantitative disease resistance on the map. *Mol. Plant Pathol.,* **2014**, *15*(5), 427-432. http://dx.doi.org/10.1111/mpp.12138 PMID: 24796392

[65] Gouveia, B.C.; Calil, I.P.; Machado, J.P.B.; Santos, A.A.; Fontes, E.P. Immune receptors and co-receptors in antiviral innate immunity in plants. *Front. Microbiol.,* **2017**, *7*, 2139. http://dx.doi.org/10.3389/fmicb.2016.02139 PMID: 28105028

[66] Mandadi, K.K.; Scholthof, K.B.G. Plant immune responses against viruses: how does a virus cause disease? *Plant Cell,* **2013**, *25*(5), 1489-1505. http://dx.doi.org/10.1105/tpc.113.111658 PMID: 23709626

[67] Howden, A.J.; Huitema, E. Effector-triggered post-translational modifications and their role in suppression of plant immunity. *Front. Plant Sci.,* **2012**, *3*, 160. http://dx.doi.org/10.3389/fpls.2012.00160 PMID: 22811685

[68] Calil, I.P.; Fontes, E.P.B. Plant immunity against viruses: antiviral immune receptors in focus. *Ann. Bot.,* **2017**, *119*(5), 711-723. PMID: 27780814

[69] Coll, N.S.; Epple, P.; Dangl, J.L. Programmed cell death in the plant immune system. *Cell Death Differ.,* **2011**, *18*(8), 1247-1256. http://dx.doi.org/10.1038/cdd.2011.37 PMID: 21475301

[70] Reimer-Michalski, E.M.; Conrath, U. Innate immune memory in plants. *Seminars in immunology*; Academic Press, **2016**. Vol. 28, No. 4, pp. 319-327. http://dx.doi.org/10.1016/j.smim.2016.05.006

[71] Yang, K.Y.; Liu, Y.; Zhang, S. Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*(2), 741-746. http://dx.doi.org/10.1073/pnas.98.2.741 PMID: 11209069

[72] Jones, J.D.; Dangl, J.L. The plant immune system. *Nature,* **2006**, *444*(7117), 323-329. http://dx.doi.org/10.1038/nature05286 PMID: 17108957

[73] Whitham, S.; Dinesh-Kumar, S.P.; Choi, D.; Hehl, R.; Corr, C.; Baker, B. The product of the tobacco mosaic virus resistance gene N: similarity to toll and the interleukin-1 receptor. *Cell,* **1994**, *78*(6), 1101-1115. http://dx.doi.org/10.1016/0092-8674(94)90283-6 PMID: 7923359

[74] Rairdan, G.J.; Collier, S.M.; Sacco, M.A.; Baldwin, T.T.; Boettrich, T.; Moffett, P. The coiled-coil and nucleotide binding domains of the Potato Rx disease resistance protein function in pathogen recognition and signaling. *Plant Cell,* **2008**, *20*(3), 739-751. http://dx.doi.org/10.1105/tpc.107.056036 PMID: 18344282

[75] Ren, T.; Qu, F.; Morris, T.J. HRT gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to turnip crinkle virus. *Plant Cell,* **2000**, *12*(10), 1917-1926. http://dx.doi.org/10.1105/tpc.12.10.1917 PMID: 11041886

[76] Yamaji, Y.; Maejima, K.; Ozeki, J.; Komatsu, K.; Shiraishi, T.; Okano, Y.; Himeno, M.; Sugawara, K.; Neriya, Y.; Minato, N.; Miura, C.; Hashimoto, M.; Namba, S. Lectin-mediated resistance impairs plant virus infection at the cellular level. *Plant Cell,* **2012**, *24*(2), 778-793. http://dx.doi.org/10.1105/tpc.111.093658 PMID: 22307853

[77] Takahashi, H.; Miller, J.; Nozaki, Y.; Takeda, M.; Shah, J.; Hase, S.; Ikegami, M.; Ehara, Y.; Dinesh, Kumar; S.P., RCY *Arabidopsis thaliana* RPP8/HRT family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. *Plant J.,* **2002**, *32*(5), 655-667. http://dx.doi.org/10.1046/j.1365-313X.2002.01453.x PMID: 12472683

[78] Chisholm, S.T.; Mahajan, S.K.; Whitham, S.A.; Yamamoto, M.L.; Carrington, J.C. Cloning of the Arabidopsis RTM1 gene, which controls restriction of long-distance movement of tobacco etch virus. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*(1), 489-494. http://dx.doi.org/10.1073/pnas.97.1.489 PMID: 10618445

[79] Whitham, S.A.; Anderberg, R.J.; Chisholm, S.T.; Carrington, J.C. Arabidopsis RTM2 gene is necessary for specific restriction of tobacco etch virus and encodes an unusual small heat shock-like protein. *Plant Cell,* **2000**, *12*(4), 569-582. PMID: 10760245

[80] Cosson, P.; Sofer, L.; Le, Q.H.; Léger, V.; Schurdi-Levraud, V.; Whitham, S.A.; Yamamoto, M.L.; Gopalan, S.; Le Gall, O.; Candresse, T.; Carrington, J.C.; Revers, F. RTM3, which controls long-distance movement of potyviruses, is a member of a new plant gene family encoding a meprin and TRAF homology domain-containing protein. *Plant Physiol.,* **2010**, *154*(1), 222-232. http://dx.doi.org/10.1104/pp.110.155754 PMID: 20584941

[81] Lellis, A.D.; Kasschau, K.D.; Whitham, S.A.; Carrington, J.C. Loss-of-susceptibility mutants of *Arabidopsis thaliana* reveal an essential role for eIF(iso)4E during potyvirus infection. *Curr. Biol.,* **2002**, *12*(12), 1046-1051. http://dx.doi.org/10.1016/S0960-9822(02)00898-9 PMID: 12123581

[82] Yoshii, M.; Nishikiori, M.; Tomita, K.; Yoshioka, N.; Kozuka, R.; Naito, S.; Ishikawa, M. The *Arabidopsis cucumovirus* multiplication 1 and 2 loci encode translation initiation factors 4E and 4G. *J. Virol.,* **2004**, *78*(12), 6102-6111. http://dx.doi.org/10.1128/JVI.78.12.6102-6111.2004 PMID: 15163703

[83] Ma, J.F.; Hou, X.L.; Xiao, D.; Qi, L.; Wang, F.; Sun, F.F.; Wang, Q. Cloning and characterization of the BcTuR3 gene related to resistance to Turnip mosaic virus (TuMV) from non-heading chinese cabbage. *Plant Mol. Biol. Report.,* **2010**, *28*, 588-596. http://dx.doi.org/10.1007/s11105-010-0183-3

[84] Tomita, R.; Sekine, K.T.; Mizumoto, H.; Sakamoto, M.; Murai, J.; Kiba, A.; Hikichi, Y.; Suzuki, K.; Kobayashi, K. Genetic basis for the hierarchical interaction between Tobamovirus spp. and L resistance gene alleles from different pepper species. *Mol. Plant Microbe Interact.,* **2011**, *24*(1), 108-117. http://dx.doi.org/10.1094/MPMI-06-10-0127 PMID: 20879841

[85] Nieto, C.; Morales, M.; Orjeda, G.; Clepet, C.; Monfort, A.; Sturbois, B.; Puigdomènech, P.; Pitrat, M.; Caboche, M.; Dogimont, C.; Garcia-Mas, J.; Aranda, M.A.; Bendahmane, A. An eIF4E allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. *Plant J.,* **2006**, *48*(3), 452-462. http://dx.doi.org/10.1111/j.1365-313X.2006.02885.x PMID: 17026540

[86] Ruffel, S.; Dussault, M.H.; Palloix, A.; Moury, B.; Bendahmane, A.; Robaglia, C.; Caranta, C. A natural recessive resistance gene against potato virus Y in pepper corresponds to the eukaryotic initiation factor 4E (eIF4E). *Plant J.,* **2002**, *32*(6), 1067-1075. http://dx.doi.org/10.1046/j.1365-313X.2002.01499.x PMID: 12492847

[87] Ruffel, S.; Gallois, J.L.; Lesage, M.L.; Caranta, C. The recessive potyvirus resistance gene pot-1 is the tomato orthologue of the pepper pvr2-eIF4E gene. *Mol. Genet. Genomics,* **2005**, *274*(4), 346-353. http://dx.doi.org/10.1007/s00438-005-0003-x PMID: 15971038

[88] Hayes, A.J.; Jeong, S.C.; Gore, M.A.; Yu, Y.G.; Buss, G.R.; Tolin, S.A.; Maroof, M.A. Recombination within a nucleotide-binding-site/leucine-rich-repeat gene cluster produces new variants conditioning resistance to soybean mosaic virus in soybeans. *Genetics,* **2004**, *166*(1), 493-503. http://dx.doi.org/10.1534/genetics.166.1.493 PMID: 15020438

[89] Stein, N.; Perovic, D.; Kumlehn, J.; Pellio, B.; Stracke, S.; Streng, S.; Ordon, F.; Graner, A. The eukaryotic translation initiation factor 4E confers multiallelic recessive Bymovirus resistance in Hordeum vulgare (L.). *Plant J.,* **2005**, *42*(6), 912-922. http://dx.doi.org/10.1111/j.1365-313X.2005.02424.x PMID: 15941403

[90] Nicaise, V.; German-Retana, S.; Sanjuán, R.; Dubrana, M.P.; Mazier, M.; Maisonneuve, B.; Candresse, T.; Caranta, C.; LeGall, O. The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the Potyvirus Lettuce mosaic virus. *Plant Physiol.,* **2003**, *132*(3), 1272-1282. http://dx.doi.org/10.1104/pp.102.017855 PMID: 12857809

[91] Albar, L.; Bangratz-Reyser, M.; Hébrard, E.; Ndjiondjop, M.N.; Jones, M.; Ghesquière, A. Mutations in the eIF(iso)4G translation initiation factor confer high resistance of rice to Rice yellow mottle virus. *Plant J.,* **2006**, *47*(3), 417-426. http://dx.doi.org/10.1111/j.1365-313X.2006.02792.x PMID: 16774645

[92] Orjuela, J.; Deless, E.F.; Kolade, O.; Chéron, S.; Ghesquière, A.; Albar, L. A recessive resistance to rice yellow mottle virus is associated with a rice homolog of the CPR5 gene, a regulator of active defense mechanisms. *Mol. Plant Microbe Interact.,* **2013**, *26*(12), 1455-1463. http://dx.doi.org/10.1094/MPMI-05-13-0127-R PMID: 23944999

[93] Vallejos, C.E.; Astua-Monge, G.; Jones, V.; Plyler, T.R.; Sakiyama, N.S.; Mackenzie, S.A. Genetic and molecular characterization of the I locus of *Phaseolus vulgaris*. *Genetics,* **2006**, *172*(2), 1229-1242. http://dx.doi.org/10.1534/genetics.105.050815 PMID: 16322513

[94] Seo, Y.S.; Rojas, M.R.; Lee, J.Y.; Lee, S.W.; Jeon, J.S.; Ronald, P.; Lucas, W.J.; Gilbertson, R.L. A viral resistance gene from common bean functions across plant families and is up-regulated in a non-virus-specific manner. *Proc. Natl. Acad. Sci. USA,* **2006**, *103*(32), 11856-11861. http://dx.doi.org/10.1073/pnas.0604815103 PMID: 16880399

[95] Naderpour, M.; Lund, O.S.; Larsen, R.; Johansen, E. Potyviral resistance derived from cultivars of *Phaseolus vulgaris* carrying bc-3 is associated with the homozygotic presence of a mutated eIF4E allele. *Mol. Plant Pathol.,* **2010**, *11*(2), 255-263. http://dx.doi.org/10.1111/j.1364-3703.2009.00602.x PMID: 20447274

[96] Gao, Z.; Johansen, E.; Eyers, S.; Thomas, C.L.; Noel Ellis, T.H.; Maule, A.J. The potyvirus recessive resistance gene, sbm1, identifies a novel role for translation initiation factor eIF4E in cell-to-cell trafficking. *Plant J.,* **2004**, *40*(3), 376-385. http://dx.doi.org/10.1111/j.1365-313X.2004.02215.x PMID: 15469495

[97] Butterbach, P.; Verlaan, M.G.; Dullemans, A.; Lohuis, D.; Visser, R.G.; Bai, Y.; Kormelink, R. Tomato yellow leaf curl virus resistance by Ty-1 involves increased cytosine methylation of viral genomes and is compromised by cucumber mosaic virus infection. *Proc. Natl. Acad. Sci. USA,* **2014**, *111*(35), 12942-12947. http://dx.doi.org/10.1073/pnas.1400894111 PMID: 25136118

[98] Ishibashi, K.; Masuda, K.; Naito, S.; Meshi, T.; Ishikawa, M. An inhibitor of viral RNA replication is encoded by a plant resistance gene. *Proc. Natl. Acad. Sci. USA,* **2007**, *104*(34), 13833-13838. http://dx.doi.org/10.1073/pnas.0703203104 PMID: 17699618

[99] Lanfermeijer, F.C.; Dijkhuis, J.; Sturre, M.J.; de Haan, P.; Hille, J. Cloning and characterization of the durable tomato mosaic virus resistance gene Tm-2(2) from *Lycopersicon esculentum*. *Plant Mol. Biol.,* **2003**, *52*(5), 1037-1049. http://dx.doi.org/10.1023/A:1025434519282 PMID: 14558663

[100] Brommonschenkel, S.H.; Frary, A.; Frary, A.; Tanksley, S.D. The broad-spectrum tospovirus resistance gene Sw-5 of tomato is a homolog of the root-knot nematode resistance gene Mi. *Mol. Plant Microbe Interact.,* **2000**, *13*(10), 1130-1138. http://dx.doi.org/10.1094/MPMI.2000.13.10.1130 PMID: 11043474

[101] Bendahmane, A.; Farnham, G.; Moffett, P.; Baulcombe, D.C. Constitutive gain-of-function mutants in a nucleotide binding site-leucine rich repeat protein encoded at the Rx locus of potato. *Plant J.,* **2002**, *32*(2), 195-204. http://dx.doi.org/10.1046/j.1365-313X.2002.01413.x PMID: 12383085

[102] Vidal, S.; Cabrera, H.; Andersson, R.A.; Fredriksson, A.; Valkonen, J.P. Potato gene Y-1 is an N gene homolog that confers cell death upon infection with potato virus Y. *Mol. Plant Microbe Interact.,* **2002**, *15*(7), 717-727. http://dx.doi.org/10.1094/MPMI.2002.15.7.717 PMID: 12118888

[103] Maiti, S.; Paul, S.; Pal, A. Isolation, characterization, and structure analysis of a non-TIR-NBS-LRR encoding candidate gene from MYMIV-resistant *Vigna mungo*. *Mol. Biotechnol.,* **2012**, *52*(3), 217-233. http://dx.doi.org/10.1007/s12033-011-9488-1 PMID: 22205242

[104] Ling, K.S.; Harris, K.R.; Meyer, J.D.; Levi, A.; Guner, N.; Wehner, T.C.; Bendahmane, A.; Havey, M.J. Non-synonymous single nucleotide polymorphisms in the watermelon eIF4E gene are closely associated with resistance to zucchini yellow mosaic virus. *Theor. Appl. Genet.,* **2009**, *120*(1), 191-200. http://dx.doi.org/10.1007/s00122-009-1169-0 PMID: 19820912

[105] Kang, B.C.; Yeam, I.; Frantz, J.D.; Murphy, J.F.; Jahn, M.M. The pvr1 locus in Capsicum encodes a translation initiation factor eIF4E that interacts with Tobacco etch virus VPg. *Plant J.,* **2005**, *42*(3), 392-405. http://dx.doi.org/10.1111/j.1365-313X.2005.02381.x PMID: 15842624

[106] Wicker, T.; Zimmermann, W.; Perovic, D.; Paterson, A.H.; Ganal, M.; Graner, A.; Stein, N. A detailed look at 7 million years of genome evolution in a 439 kb contiguous sequence at the barley Hv-eIF4E locus: recombination, rearrangements and repeats. *Plant J.,* **2005**, *41*(2), 184-194. http://dx.doi.org/10.1111/j.1365-313X.2004.02285.x PMID: 15634196

[107] Lapidot, M.; Karniel, U.; Gelbart, D.; Fogel, D.; Evenor, D.; Kutsher, Y.; Makhbash, Z.; Nahon, S.; Shlomo, H.; Chen, L.; Reuveni, M.; Levin, I. A novel route controlling begomovirus resistance by the messenger RNA surveillance factor Pelota. *PLoS Genet.,* **2015**, *11*(10), e1005538. http://dx.doi.org/10.1371/journal.pgen.1005538 PMID: 26448569

[108] Deleris, A.; Gallego-Bartolome, J.; Bao, J.; Kasschau, K.D.; Carrington, J.C.; Voinnet, O. Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. *Science,* **2006**, *313*(5783), 68-71. http://dx.doi.org/10.1126/science.1128214 PMID: 16741077

[109] Bologna, N.G.; Voinnet, O. The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. *Annu. Rev. Plant Biol.,* **2014**, *65*, 473-503. http://dx.doi.org/10.1146/annurev-arplant-050213-035728 PMID: 24579988

[110] Lewsey, M.; Palukaitis, P.; Carr, J. P. Plant-virus interactions: defence and counter-defence. *Ann. Plant Rev.,* **2018**, 134-176. http://dx.doi.org/10.1002/9781119312994.apr0366

[111] Jovel, J.; Walker, M.; Sanfaçon, H. Salicylic acid-dependent restriction of Tomato ringspot virus spread in tobacco is accompanied by a hypersensitive response, local RNA silencing, and moderate systemic resistance. *Mol. Plant Microbe Interact.,* **2011**, *24*(6), 706-718. http://dx.doi.org/10.1094/MPMI-09-10-0224 PMID: 21281112

[112] Baebler, Š.; Witek, K.; Petek, M.; Stare, K.; Tušek-Žnidarič, M.;

Pompe-Novak, M.; Renaut, J.; Szajko, K.; Strzelczyk-Żyta, D.; Marczewski, W.; Morgiewicz, K.; Gruden, K.; Hennig, J. Salicylic acid is an indispensable component of the Ny-1 resistance-gene-mediated response against Potato virus Y infection in potato. *J. Exp. Bot.,* **2014**, *65*(4), 1095-1109. http://dx.doi.org/10.1093/jxb/ert447 PMID: 24420577

[113] Alazem, M.; Lin, N.S. Roles of plant hormones in the regulation of host-virus interactions. *Mol. Plant Pathol.,* **2015**, *16*(5), 529-540. http://dx.doi.org/10.1111/mpp.12204 PMID: 25220680

[114] Ascencio-Ibáñez, J.T.; Sozzani, R.; Lee, T.J.; Chu, T.M.; Wolfinger, R.D.; Cella, R.; Hanley-Bowdoin, L. Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. *Plant Physiol.,* **2008**, *148*(1), 436-454. http://dx.doi.org/10.1104/pp.108.121038 PMID: 18650403

[115] Nakashita, H.; Yasuda, M.; Nitta, T.; Asami, T.; Fujioka, S.; Arai, Y.; Sekimata, K.; Takatsuto, S.; Yamaguchi, I.; Yoshida, S. Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *Plant J.,* **2003**, *33*(5), 887-898. http://dx.doi.org/10.1046/j.1365-313X.2003.01675.x PMID: 12609030

[116] Piroux, N.; Saunders, K.; Page, A.; Stanley, J. Geminivirus pathogenicity protein C4 interacts with *Arabidopsis thaliana* shaggy-related protein kinase AtSKeta, a component of the brassinosteroid signalling pathway. *Virology,* **2007**, *362*(2), 428-440. http://dx.doi.org/10.1016/j.virol.2006.12.034 PMID: 17280695

[117] Kørner, C.J.; Klauser, D.; Niehl, A.; Domínguez-Ferreras, A.; Chinchilla, D.; Boller, T.; Heinlein, M.; Hann, D.R. The immunity regulator BAK1 contributes to resistance against diverse RNA viruses. *Mol. Plant Microbe Interact.,* **2013**, *26*(11), 1271-1280. http://dx.doi.org/10.1094/MPMI-06-13-0179-R PMID: 23902263

[118] Alcaide-Loridan, C.; Jupin, I. Ubiquitin and plant viruses, let's play together! *Plant Physiol.,* **2012**, *160*(1), 72-82. http://dx.doi.org/10.1104/pp.112.201905 PMID: 22802610

[119] Sahana, N.; Kaur, H.; Basavaraj, F.T.; Tena, F.; Jain, R.K.; Palukaitis, P.; Canto, T.; Praveen, S. Inhibition of the host proteasome facilitates papaya ringspot virus accumulation and proteosomal catalytic activity is modulated by viral factor HcPro. *PLoS One,* **2012**, *7*(12), e52546. http://dx.doi.org/10.1371/journal.pone.0052546 PMID: 23300704

[120] Zhang, T.; Zheng, Q.; Yi, X.; An, H.; Zhao, Y.; Ma, S.; Zhou, G. Establishing RNA virus resistance in plants by harnessing CRISPR immune system. *Plant Biotechnol. J.,* **2018**, *16*(8), 1415-1423. http://dx.doi.org/10.1111/pbi.12881 PMID: 29327438

[121] Dielen, A.S.; Sassaki, F.T.; Walter, J.; Michon, T.; Ménard, G.; Pagny, G.; Krause-Sakate, R.; Maia, Ide.G.; Badaoui, S.; Le Gall, O.; Candresse, T.; German-Retana, S. The 20S proteasome α5 subunit of *Arabidopsis thaliana* carries an RNase activity and interacts in planta with the lettuce mosaic potyvirus HcPro protein. *Mol. Plant Pathol.,* **2011**, *12*(2), 137-150. http://dx.doi.org/10.1111/j.1364-3703.2010.00654.x PMID: 21199564

[122] Shan, Q.; Wang, Y.; Li, J.; Zhang, Y.; Chen, K.; Liang, Z.; Zhang, K.; Liu, J.; Xi, J.J.; Qiu, J.L.; Gao, C. Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat. Biotechnol.,* **2013**, *31*(8), 686-688. http://dx.doi.org/10.1038/nbt.2650 PMID: 23929338

[123] Araki, M.; Ishii, T. Towards social acceptance of plant breeding by genome editing. *Trends Plant Sci.,* **2015**, *20*(3), 145-149. http://dx.doi.org/10.1016/j.tplants.2015.01.010

[124] Baltes, N.J.; Hummel, A.W.; Konecna, E.; Cegan, R.; Bruns, A.N.; Bisaro, D.M. Conferring resistance to geminiviruses with the CRISPR–Cas prokaryotic immune system. *Nat. Plants,* **2015**, *1*, 1-4. http://dx.doi.org/10.1038/nplants.2015.145

[125] Kanchiswamy, C. N.; Malnoy, M.; Maffei, M.E. Chemical diversity of microbial volatiles and their potential for plant growth and productivity. *Science,* **2015**, *315*, 1709-1712. http://dx.doi.org/10.3389/fpls.2015.00151

[126] Ishino, Y.; Shinagawa, H.; Makino, K.; Amemura, M.; Nakata, A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. *J. Bacteriol.,* **1987**, *169*(12), 5429-5433. http://dx.doi.org/10.1128/JB.169.12.5429-5433.1987 PMID: 3316184

[127] Barrangou, R.; Fremaux, C.; Deveau, H.; Richards, M.; Boyaval, P.; Moineau, S.; Romero, D.A.; Horvath, P. CRISPR provides acquired resistance against viruses in prokaryotes. *Science,* **2007**, *315*(5819), 1709-1712. http://dx.doi.org/10.1126/science.1138140 PMID: 17379808

[128] Jansen, R.; Embden, J.D.A.V.; Gaastra, W.; Schouls, L.M. Identification of genes that are associated with DNA repeats in prokaryotes. *Mol. Microbiol.,* **2002**, *43*(6), 1565-1575. http://dx.doi.org/10.1046/j.1365-2958.2002.02839.x PMID: 11952905

[129] Sorek, R.; Lawrence, C.M.; Wiedenheft, B. CRISPR-mediated adaptive immune systems in bacteria and archaea. *Annu. Rev. Biochem.,* **2013**, *82*, 237-266. http://dx.doi.org/10.1146/annurev-biochem-072911-172315 PMID: 23495939

[130] Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science,* **2012**, *337*(6096), 816-821. http://dx.doi.org/10.1126/science.1225829 PMID: 22745249

[131] Butler, N.M.; Jiang, J.; Stupar, R.M. Crop improvement using genome editing. *Plant Breed. Rev.,* **2018**, 55-101.

[132] Hille, F.; Richter, H.; Wong, S.P.; Bratovič, M.; Ressel, S.; Charpentier, E. The Biology of CRISPR-Cas: Backward and Forward. *Cell,* **2018**, *172*(6), 1239-1259. http://dx.doi.org/10.1016/j.cell.2017.11.032 PMID: 29522745

[133] Cristea, S.; Freyvert, Y.; Santiago, Y.; Holmes, M.C.; Urnov, F.D.; Gregory, P.D.; Cost, G.J. *In vivo* cleavage of transgene donors promotes nuclease-mediated targeted integration. *Biotechnol. Bioeng.,* **2013**, *110*(3), 871-880. http://dx.doi.org/10.1002/bit.24733 PMID: 23042119

[134] Makarova, K.S.; Wolf, Y.I.; Alkhnbashi, O.S.; Costa, F.; Shah, S.A.; Saunders, S.J.; Barrangou, R.; Brouns, S.J.; Charpentier, E.; Haft, D.H.; Horvath, P.; Moineau, S.; Mojica, F.J.; Terns, R.M.; Terns, M.P.; White, M.F.; Yakunin, A.F.; Garrett, R.A.; van der Oost, J.; Backofen, R.; Koonin, E.V. An updated evolutionary classification of CRISPR-Cas systems. *Nat. Rev. Microbiol.,* **2015**, *13*(11), 722-736. http://dx.doi.org/10.1038/nrmicro3569 PMID: 26411297

[135] Unniyampurath, U.; Pilankatta, R.; Krishnan, M. N. RNA interference in the age of CRISPR: will CRISPR interfere with RNAi?. *Int. J. Mol. Sci.,* **2016**, *17*(3), 291. http://dx.doi.org/10.3390/ijms17030291

[136] Khatodia, S.; Bhatotia, K.; Tuteja, N. Development of CRISPR/-Cas9 mediated virus resistance in agriculturally important crops. *Bioengineered,* **2017**, *8*(3), 274-279. http://dx.doi.org/10.1080/21655979.2017.1297347 PMID: 28581909

[137] Macovei, A.; Sevilla, N.R.; Cantos, C.; Jonson, G.B.; Slamet-Loedin, I.; Čermák, T.; Voytas, D.F.; Choi, I.R.; Chadha-Mohanty, P. Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. *Plant Biotechnol. J.,* **2018**, *16*(11), 1918-1927. http://dx.doi.org/10.1111/pbi.12927 PMID: 29604159

[138] Vats, S.; Kumawat, S.; Kumar, V.; Patil, G.B.; Joshi, T.; Sonah, H.; Sharma, T.R.; Deshmukh, R. Genome editing in plants: exploration of technological advancements and challenges. *Cells,* **2019**, *8*, 1386. http://dx.doi.org/10.3390/cells8111386

[139] Ji, X.; Zhang, H.; Zhang, Y.; Wang, Y.; Gao, C. Establishing a CRISPR-Cas-like immune system conferring DNA virus resistance in plants. *Nat. Plants,* **2015**, *1*(10), 15144. http://dx.doi.org/10.1038/nplants.2015.144 PMID: 27251395

[140] Ali, Z.; Abulfaraj, A.; Idris, A.; Ali, S.; Tashkandi, M.; Mahfouz, M.M. CRISPR/Cas9-mediated viral interference in plants. *Genome Biol.,* **2015**, *16*, 238. http://dx.doi.org/10.1186/s13059-015-0799-6 PMID: 26556628

[141] Ali, Z.; Ali, S.; Tashkandi, M.; Zaidi, S.S.; Mahfouz, M.M. CRISPR/Cas9-mediated immunity to geminiviruses: differential interference and evasion. *Sci. Rep.,* **2016**, *6*(26), 9-12.

[142] Aman, R.; Ali, Z.; Butt, H.; Mahas, A.; Aljedaani, F.; Khan, M.Z.; Ding, S.; Mahfouz, M. RNA virus interference *via* CRISPR/-Cas13a system in plants. *Genome Biol.,* **2018**, *19*(1), 1. http://dx.doi.org/10.1186/s13059-017-1381-1 PMID: 29301551

[143] Pyott, D.E.; Sheehan, E.; Molnar, A. Engineering of CRISPR/-Cas9-mediated potyvirus resistance in transgene-free *Arabidopsis* plants. *Mol. Plant Pathol.,* **2016**, *17*(8), 1276-1288. http://dx.doi.org/10.1111/mpp.12417 PMID: 27103354

[144] Chandrasekaran, J.; Brumin, M.; Wolf, D.; Leibman, D.; Klap, C.; Pearlsman, M.; Sherman, A.; Arazi, T.; Gal-On, A. Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Mol. Plant Pathol.,* **2016**, *17*(7), 1140-1153. http://dx.doi.org/10.1111/mpp.12375 PMID: 26808139

[145] Zaidi, S.S.E.A.; Tashkandi, M.; Mahfouz, M.M. Engineering molecular immunity against plant viruses. *Prog. Mol. Biol. Transl. Sci.,* **2017**, *149*, 167-186. http://dx.doi.org/10.1016/bs.pmbts.2017.03.009 PMID: 28712496

[146] Kis, A.; Hamar, É.; Tholt, G.; Bán, R.; Havelda, Z. Creating highly efficient resistance against wheat dwarf virus in barley by employing CRISPR/Cas9 system. *Plant Biotechnol. J.,* **2019**, *17*(6), 1004-1006. http://dx.doi.org/10.1111/pbi.13077 PMID: 30633425

[147] Price, A.A.; Sampson, T.R.; Ratner, H.K.; Grakoui, A.; Weiss, D.S. Cas9-mediated targeting of viral RNA in eukaryotic cells. *Proc. Natl. Acad. Sci. USA,* **2015**, *112*(19), 6164-6169. http://dx.doi.org/10.1073/pnas.1422340112 PMID: 25918406

[148] Tsai, S.Q.; Joung, J.K. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nat. Rev. Genet.,* **2016**, *17*(5), 300-312. http://dx.doi.org/10.1038/nrg.2016.28 PMID: 27087594

[149] Pattanayak, V.; Lin, S.; Guilinger, J.P.; Ma, E.; Doudna, J.A.; Liu, D.R. High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity. *Nat. Biotechnol.,* **2013**, *31*(9), 839-843. http://dx.doi.org/10.1038/nbt.2673 PMID: 23934178

[150] Kadam, U.S.; Shelake, R.M.; Chavhan, R.L.; Suprasanna, P. Concerns regarding 'off-target' activity of genome editing endonucleases. *Plant Physiol. Biochem.,* **2018**, *131*, 22-30. http://dx.doi.org/10.1016/j.plaphy.2018.03.027 PMID: 29653762

[151] Bortesi, L.; Zhu, C.; Zischewski, J.; Perez, L.; Bassié, L.; Nadi, R.; Forni, G.; Lade, S.B.; Soto, E.; Jin, X.; Medina, V.; Villorbina, G.; Muñoz, P.; Farré, G.; Fischer, R.; Twyman, R.M.; Capell, T.; Christou, P.; Schillberg, S. Patterns of CRISPR/Cas9 activity in plants, animals and microbes. *Plant Biotechnol. J.,* **2016**, *14*(12), 2203-2216. http://dx.doi.org/10.1111/pbi.12634 PMID: 27614091

[152] Zhang, H.; Zhang, J.; Wei, P.; Zhang, B.; Gou, F.; Feng, Z.; Mao, Y.; Yang, L.; Zhang, H.; Xu, N.; Zhu, J.K. The CRISPR/C as9 system produces specific and homozygous targeted gene editing in rice in one generation. *Plant Biotechnol. J.,* **2014**, *12*(6), 797-807. http://dx.doi.org/10.1111/pbi.12200 PMID: 24854982

[153] Zhao, X.; Zhang, W.; Qiu, X.; Mei, Q.; Luo, Y.; Fu, W. Rapid and sensitive exosome detection with CRISPR/Cas12a. *Anal. Bioanal. Chem.,* **2020**, *412*(3), 601-609. http://dx.doi.org/10.1007/s00216-019-02211-4 PMID: 31897558

[154] Fu, Y.; Sander, J.D.; Reyon, D.; Cascio, V.M.; Joung, J.K. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. *Nat. Biotechnol.,* **2014**, *32*(3), 279-284. http://dx.doi.org/10.1038/nbt.2808 PMID: 24463574

[155] Mehta, D.; Stürchler, A.; Anjanappa, R.B.; Zaidi, S.S.E.A.; Hirsch-Hoffmann, M.; Gruissem, W.; Vanderschuren, H. Linking CRISPR-Cas9 interference in cassava to the evolution of editing-resistant geminiviruses. *Genome Biol.,* **2019**, *20*(1), 80. http://dx.doi.org/10.1186/s13059-019-1678-3 PMID: 31018865

[156] Kim, D.; Alptekin, B.; Budak, H. CRISPR/Cas9 genome editing in wheat. *Funct. Integr. Genomics,* **2018**, *18*(1), 31-41. http://dx.doi.org/10.1007/s10142-017-0572-x PMID: 28918562

[157] Rees, H.A.; Liu, D.R. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat. Rev. Genet.,* **2018**, *19*(12), 770-788. http://dx.doi.org/10.1038/s41576-018-0059-1 PMID: 30323312

[158] Lei, Y.; Lu, L.; Liu, H.Y.; Li, S.; Xing, F.; Chen, L.L. CRISPR-P: a web tool for synthetic single-guide RNA design of CRISPR-system in plants. *Mol. Plant,* **2014**, *7*(9), 1494-1496. http://dx.doi.org/10.1093/mp/ssu044 PMID: 24719468

[159] Borrelli, V.M.G.; Brambilla, V.; Rogowsky, P.; Marocco, A.; Lanubile, A. The enhancement of plant disease resistance using CRISPR/Cas9 technology. *Front. Plant Sci.,* **2018**, *9*, 1245. http://dx.doi.org/10.3389/fpls.2018.01245 PMID: 30197654