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Review Article

Enhancing Virus Resistance in Crops through *eIF4E* Knockdown: Prospects and Challenges

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ABSTRACT

Emerging viral diseases pose a significant threat to global food security, impacting crop yield and quality. *eIF4E*, a crucial translation initiation factor, is often exploited by certain viruses for replication within the host. The knockdown mutation of *eIF4E* has demonstrated effectiveness in conferring resistance to a range of viruses, including potyviruses and mycoviruses, with long-lasting effects that limit viral adaptation. Several gene editing approaches such as RNA silencing, TILLING, and CRISPR/Cas9 have been utilized to achieve the *eIF4E* knockdown. While this strategy holds substantial promise, its implementation must consider potential implications for plant growth and productivity. This review explores the potential of *eIF4E* knockdown for enhancing virus resistance in crops, along with recent advancements, existing challenges, and future directions for its application in sustainable agriculture.

Keywords: CRISPR/Cas9, eIF4E, Mycoviruses, RNA silencing, Plant resistance

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INTRODUCTION

Globally viral diseases cause economic losses and impose danger to food security. Virus diseases are becoming a major challenge to worldwide agriculture and causing reduced crop yields and quality. These diseases are causing total failures of food production systems, which are highly devastating (Anderson *et al.*, 2004; Cooper *et al.*, 2007). Studies have shown that the economic impact of virus disease pandemics and epidemics takes more than US$ 30 billion per year (Jones, 2021). These virus diseases affect major food crops and other essential crops. Thereby causing the reduction of food sources and famine (Voinnet, 2005). Both conventional and non-conventional approaches are used to generate plants that are resistant to viruses. According to reports, RNA silencing, also known as RNA interference (RNAi), can also lead to this resistance. RNAi plays a significant role in antiviral and plant-viral defense. RNA interference (RNAi) is often triggered by the presence of double-stranded RNA, resulting in the inhibition or repression of a specific gene (Taliansky *et al.*, 2021). To generate virus-resistant transgenic plants, several approaches have also been devised. For instance, sense/ antisense RNA, RNA interference, hairpin RNA, and miRNA (Pyott *et al.*, 2016). Plant viruses evolve very fast via a process of virus recombination and also mutations, hence the conventional method of controlling these viruses may fail. A solid resistance must continue to be pushed into operation. CRISPR/Cas9 is a useful technique for modifying genetic material. It has more benefits than artificial microRNAs and RNAi for enhancing virus resistance in plants. This is because it disrupts crucial viral or host genes rather than suppressing them at the RNA level. Several researchers have verified the superior effectiveness of this method. Plants modified with this technique have more resistance to DNA and RNA viruses (Chandrasekaran *et al.*, 2016; Macovei *et al.*, 2018; Tahmasebi *et al.*, 2019).

Viruses use Dead-box RNA helicase and the key translational factors *eIF4E*, *eIF4G*, and eIF4A for the translation of their mRNAs, and they are appealing targets for antiviral therapies. *eIF4E* is the primary focus in the investigation of plant-virus interactions. Its cap-binding activity, which hinders cap-independent translation, has been known since its discovery. This activity has significant consequences for plant defense against viral infections. Consequently, it was discovered that it can confer resistance to viral infections through specific sequencing mutations and knockdown *eIF4E* strategies (Sanfaçon, 2015). *eIF4E*, which is a crucial target in translation initiation, contributes to the synthesis of viral and plant proteins. Therefore, this review discusses how *eIF4E* knockdown can enhance virus resistance in different crops. Potyviruses are translated by eIF4F or its isoform eIF(iso)4F. The building of plants that are immune to potyvirus by knock-out of either one isoform of *EIF4G* or *EIF4E* is an engineering approach; it is an important aspect of plant health as well as a potentially significant source of transgenic or non-transgenic resistance (Kawaguchi and Bailey-Serres, 2002). Many Positive strand RNA viruses lack a 5' cap on their mRNA. Instead, they use VPg (viral protein genome-linked) which is covalently linked to the viral RNA, to recruit host translation machinery. VPg of potyviruses and secoviruses are covalently bonded to the RNA, with the genome joined at the 5' end following replication (Fauquet *et al.*, 2005). The VPgs of potyviruses interact with both isoforms of *eIF4E*, and *eIF(iso)4E*, whereas cellular mRNAs only interact with one isoform of *eIF4E*. There is a connection between the VPg of the Turnip mosaic virus and the host *eIF(iso)4E* (Kanyuka *et al.*, 2005). In the past, *eIF4E* and its preliminary *eIF(iso)4E* were found to be rare resistance alleles to all potyviruses from different hosts. The earlier reports demonstrated that the mutation of Arabidopsis *eIF(iso)4E*, generated by either ethyl methane sulfonate or transposon, which is required for viral genome amplification, developed full resistance against multiple potyviruses (Keima *et al.*, 2017).

ROLE OF *eIF4E* IN TRANSLATION INITIATION

eIF4E creates the eIF4F complex by binding to the 5′ cap structure on mRNA and it also combines with eIF4G. At this juncture, the ribosomal 40S subunit and additional translation initiation components are enlisted to commence mRNA translation. The *eIF4E* protein binds to the 5′ 7-methylguanosine cap on eukaryotic mRNA in order to attract the translation complex for the process of protein production (Chen *et al.*, 2022). *eIF4E* has an important role in protein synthesis at the initial stages. It is stated that a closed-loop structure is formed in the translation of mRNAs, where the 5′- and 3′- ends are brought close together by the interaction of initiation factors (Yang *et al.*, 2021) (Figure 1).

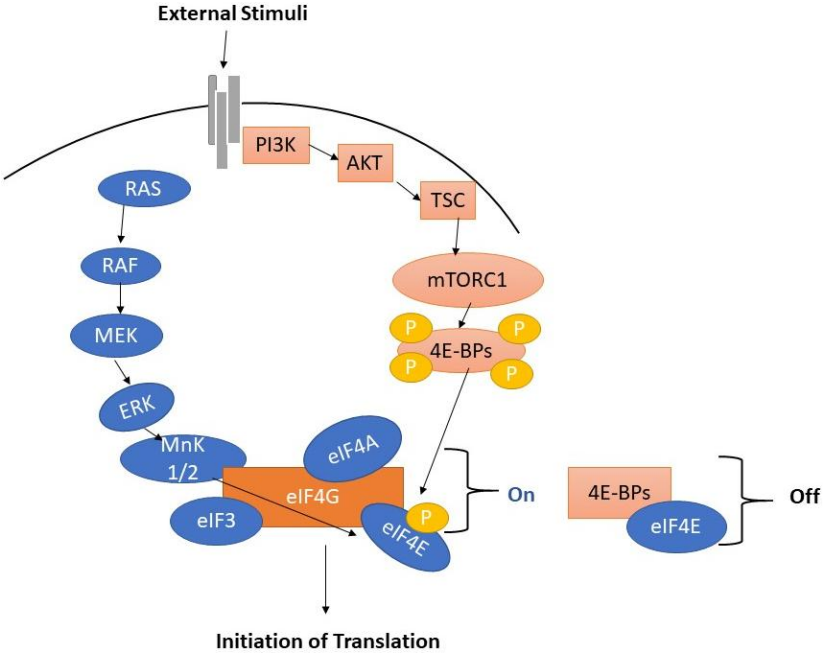

Figure 1. *eIF4E*'s function in translational regulation. An illustration of the main signaling routes that are upstream of *eIF4E*. *eIF4E*-binding proteins and 4E-BPs; Protein kinase B (PKB), also referred to as Akt; eIF3, eukaryotic initiation factor 3; eIF4A, eukaryotic translation initiation factor 4A; *eIF4E*, eukaryotic translation initiation factor 4E; eIF4G, eukaryotic translation initiation factor 4G; GβL, or G protein beta subunit-like; ERK, or extracellular signal-regulated kinase, also called mitogen-activated protein kinase (MAPK); MNK1/2, mitogen-activated protein kinase kinase-interacting serine/threonine-protein kinases 1/2; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; P, phosphorylation site; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; RAPTOR, regulatory-associated protein of mTOR; TSC1/2, tuberculosis proteins 1/2.

KNOCKDOWN OF *eIF4E* AND VIRUS RESISTANCE

Many plant viruses utilize the host's translation machinery for their replication. Plant viruses are parasitic organisms that rely on precise interactions with the host plant's macromolecules, structures, and processes to reproduce. These interactions determine the plant's vulnerability to viral infection. Either a mutation may occur in the susceptibility factor, or the removal of the factor may lead to resistance against the virus. Viruses have developed ways to selectively target and engage with *eIF4E*, facilitating the translation of their own viral RNA instead of host mRNAs (Chen *et al.*, 2022). *eIF4E* has been shown to have a role in the resistance to several viral groups. However, most of the *eIF4E*-mediated resistances are effective against viruses belonging to the Potyvirus group (Moury *et al.*, 2014). Potyvirus is one of the largest genera of plant viruses, which is responsible for significant financial detriment in many species of cultivated crops. According to research, resistance to potyviruses that is mediated by *eIF4E* is caused by a small number of specific mutations in the amino acids of the *eIF4E* protein. In most cases, this resistance is recessive (C. Gauffier *et al.*, 2016). The AIF iso 4G resistance gene in rice provides protection against the RYMV, which belongs to the Sobemovirus genus (Poulicard *et al.*, 2016).

Plant viruses are minuscule pathogens with a compact genome, typically containing the genetic information for over a dozen proteins. However, it seems that this is not enough to complete any of the three stages of the viral life cycle in the plant. Viruses employ several host proteins to effectively accomplish these phases. These proteins facilitate a suitable contact between the virus and the plant. Theoretically, if at least one of the components of the virus is absent in the plant cell, it would lead to an incompatible interaction and thus, resistance. This form of resistance is present in various plant-pathogen interactions and is called a loss of susceptibility. The phenomenon of loss of susceptibility is most frequently observed in obligatory plant pathogens, particularly viruses (Konečná *et al.*, 2014). Loss of susceptibility, unlike more well-known forms of resistance, is distinguished by its greater durability and broader range of effectiveness. In this type of resistance, R genes directly activate the plant's defense responses without the need for interaction with pathogen effectors (Nakahara *et al.*, 2010).

Interestingly, there are only a few plant viruses with positive-strand RNA that have genomic and subgenomic mRNAs with a structure similar to that of host mRNAs. These viruses often do not have either the 5′-cap or the poly(A) tail, or sometimes lack both (M. Xu *et al.*, 2017). For instance, members of the family Potyviridae contain VPg, attached to the 5′ end of the viral RNA, instead of a 5′ cap structure. Moreover, potyviruses possess 3′-end poly(A) tailing of the genomic RNA. Thus, the use of these 'VPg' viral protein genome-linked proteins enables VPg to interact with the 5′ UTR of the viral genome as well as with a host *eIF4E* or *eIF(iso)4E*. The potyvirus 'VPg' protein interacts with both the 5′ UTR structure and with the host eIFs.

These types of genetic variations in a single-cell component are responsible for many plant species' inherent recessive resistance to most potyviruses. In some plants, it was shown that many previously identified recessive resistance genes encode the same resistance factor *eIF4E*, despite their distinct origins. Thus, this factor-mediated resistance to many potyviruses has developed frequently and independently in a wide range of systematically distinct plant species. In addition, Bymoviruses, Potexviruses, Tritimoviruses, Ipomoviruses, Carmoviruses, Carlaviruses, and Cucumoviruses also show *eIF4E*-mediated resistance other than potyviruses (V. Nicaise *et al.*, 2003; M. Xu *et al.*, 2017) or artificially obtained (Kan *et al.*, 2023; Miroshnichenko *et al.*, 2020).

Resistance by *EIF4E* is often broad-spectrum and durable and is not easily broken by viruses in the field. It is a consequence of the broad-spectrum nature of this resistance that the presence of a gene for one *eIF4E* allele in a given plant often confers resistance to different strains or viruses of the same species infecting isolates (Ayme *et al.*, 2006; Kühne *et al.*, 2003). Unlike many other resistance mechanisms, which have been mainly identified in cultivated plants in their wild relatives, *eIF4E*-mediated resistance is more frequent in the host plant (Zhao *et al.*, 2020). This implies that these specific resistances are well-suited for cultivated plants and have little negative impact on the development and yield of crops. Knockdown of *eIF4E* and development of virus resistance in different plants: The following are successful cases of *eIF4E* knockdown in different crops, the viruses targeted, and the outcomes observed (Segura *et al.*, 2016).

METHODOLOGIES FOR *eIF4E* KNOCKDOWN

Amino Acid Substitution

The virus adapted to the *eIF4E*-mediated resistances by undergoing amino acid changes in the VPg, leading to the frequent breakdown of resistance (Ito *et al.*, 2015; Lucioli *et al.*, 2022), but some other viral proteins can be targets for this resistance, such as CI (Annadana *et al.*, 2002) and P1 (Frizzi and Huang, 2010).

RNA Silencing

This technique uses engineered RNA molecules to specifically target and degrade *eIF4E* mRNA, resulting in reduced levels of the *eIF4E* protein. Up to now, more than 60 plant virus species of economic importance have been successfully controlled using RNAi technology (Zlobin *et al.*, 2023). One of the genetic antiviral trends in modern plant breeding is a disruption of the establishment of a virus in host plants using RNA interference (Kan *et al.*, 2023). Figure 2 explains the RNA silencing pathway in plants. In addition to the absence of inherent resistance in the genetic variation of crop species, biotechnology technologies provide other strategies to control viral infections. Resistance to viruses achieved by transgenic procedures has been among the earliest commercially available biotech properties (Lebaron *et al.*, 2016). Most virus-resistant transgenic plants have been created via pathogen-derived resistance, which is achieved by the use of proteins or nucleic acids via RNA silencing, also known as RNA interference or RNAi (Perez *et al.*, 2012). Specifically, we have successfully created transgenic tobacco plants that are resistant to tobamovirus by using RNA interference to target two host genes that are essential for the virus's replication. These plants were previously known to be sensitive to the virus (Bastet *et al.*, 2018).

Tilling

Another way to generate viral resistance without using costly screens is represented by Targeting Induced Local Lesions in Genomes. A loss of function tilling mutant that confers *potyvirus* resistance was obtained by mutating *eIF4E* genes in tomato plants (Sato *et al.*, 2005).

Table 1. Modification in *eIF4E*, *eIF(iso)4E* led to a loss of susceptibility to potyviruses in several divergent hosts, including Arabidopsis, tomato, pepper, lettuce, pea, peanut, and sugarcane, among others.

Plant	Virus resistant	References
Pea	*potyvirus*	(Segura *et al.*, 2016)
Tomato	*Potyvirus*, PVMV	(Kühne *et al.*, 2003)
Pepper	Pepper yellow mosaic virus	(Kobayashi *et al.*, 2014)
Pea	Clover yellow vein virus	(Frizzi and Huang, 2010)
Sugarcane	Sugarcane mosaic pathogen	(Asano *et al.*, 2005)
Peanut	peanut stripe virus	(Xu *et al.*, 2017)
Lettuce	*potyvirus* Lettuce mosaic virus	(Nicaise *et al.*, 2003)
Arabidopsis	*potyvirus*	(Arora and Narula, 2017)
Potato	PVY	(Gasiunas *et al.*, 2012)
Tobacco	PVY	(Gasiunas *et al.*, 2012)
Melon	*Melon necrotic spot virus*	(Gauffier *et al.*, 2016)
Barley	*Bymovirus*	(Nicaise *et al.*, 2003)
Hordeum vulgare L.	*Bymovirus*	(Moury *et al.*, 2014)
Rice	Rice yellow mottle virus	(Poulicard *et al.*, 2016)
Bean	potyviral infection	(Arora and Narula, 2017)
Wheat	Wheat yellow mosaic virus	(Cai *et al.*, 2015)

CRISPR/Cas9 genome editing

Using this technology, it is possible to induce mutations in the *eIF4E* gene to result in its permanent knockout. The field of site-specific genome editing was recently transformed by the identification and analysis of a new type of DNA endonuclease found in Streptococcus pyrogens. This enzyme, known as Cas9, works together with the CRISPR loci in the bacterial genome to defend against foreign plasmids or DNA viruses in bacteria (Loureiro *et al.*, 2019).

The Cas9 enzyme is directed to the specific DNA sequence by the crRNA, which contains a 20-nucleotide segment that matches the target sequence. Once bound, Cas9 cuts the DNA three base pairs before the matching section, resulting in the creation of double-strand breaks. A second breakthrough in the application of CRISPR was that Cas9 can operate with a designed sgRNA, which is an artificial fusion of segments of crRNA and tracrRNA that retains the targeting and cutting properties of both (Salazar-Díaz *et al.*, 2021). The architecture of the sgRNA is much more adaptable than that of the crRNA since the only requirement is for the 20-nucleotide complement segment shared with the target DNA to occur immediately upstream of a PAM sequence NGG. This breakthrough allowed for designing tens of thousands of sgRNAs for the purpose of targeting genes in any organism.

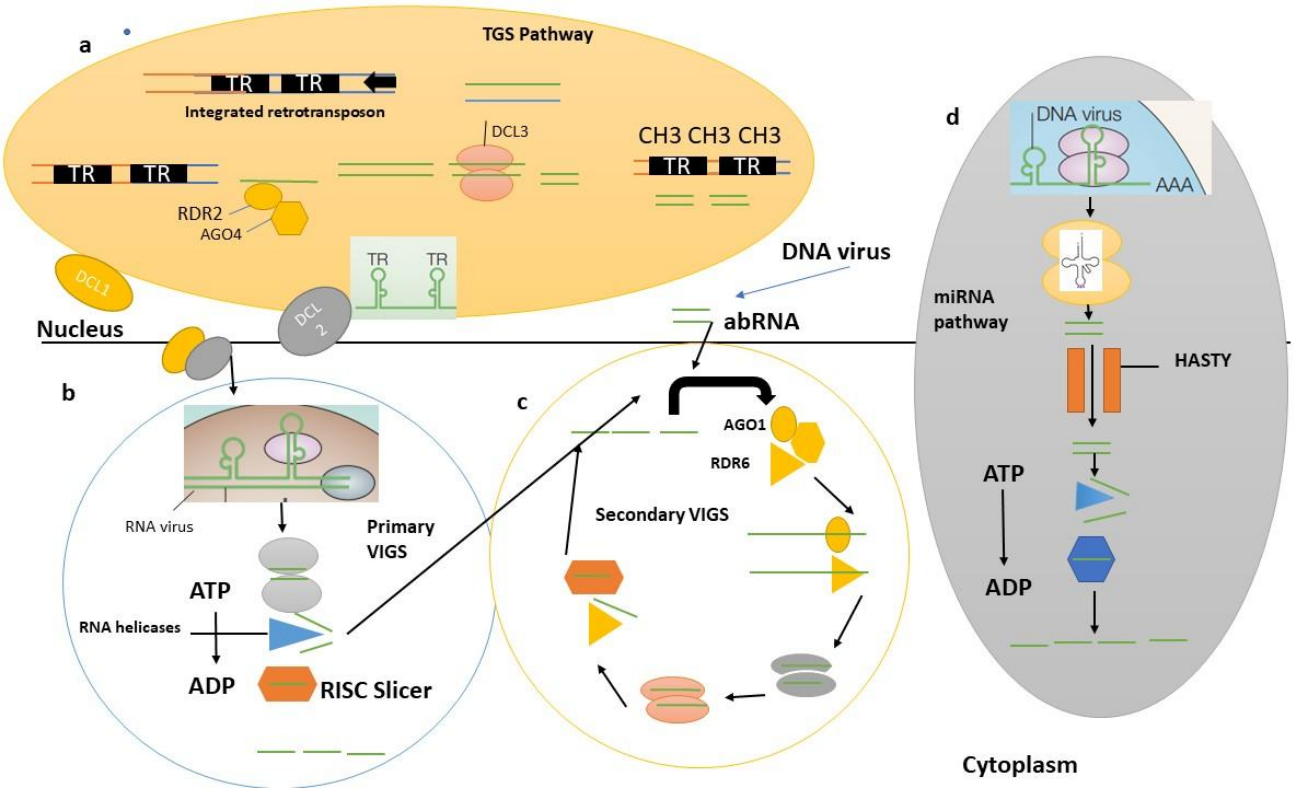

Figure 2. Antiviral RNA silencing pathways in plants. a |Transcriptional gene silencing in the nucleus can be initiated by viruses and sub-viral agents that have been incorporated into the host genome. Double-stranded RNA that is complementary to viral genetic material is produced by read-through transcription. The activity of the argonaute protein AG0 generates dsRNA and the RNA-dependent RNA polymerase RDR2. DCL3 always identifies double-stranded RNA (dsRNA) and produces viral small interfering RNAs (siRNAs). Subsequently, these small interfering RNAs (siRNAs) attach to the corresponding segments in the viral DNA that have become part of the host's genetic material. This attachment directs epigenetic changes in this specific area, as a result, gene activity is suppressed. **b** | Virus- induced gene silencing causes silencing in the cytoplasm. The DCL2 potentially indicates its ability to interact with DCL1, promoting its export and facilitating the processing of defective stem loops present in the RNA virus and viroid genome. The short interfering RNAs of viruses are unraveled by an ATP-dependent RNA helicase and then integrated into the RNA-inducedsilencing complex. The latter is directed towards the matching viral mRNA, which undergoes degradation. **c** | The second VIGS route has the potential to enhance or strengthen the first signal. The tiny RNAs that were produced during the initial VIGS process, such as the abnormal RNA derived from a transgene or a virus, are converted into double-stranded RNA (dsRNA) by the collaborative action of the RNA-dependent RDR6 polymerase, AGO1, and maybe SDE3, which might function as an RNA helicase. Subsequently, these double-stranded RNAs, similar to the initial scenario, undergo processing which leads to the destruction of the matching viral or transgenic mRNA. **d** | VIGS may also implicate the miRNA pathway. Experimental data in human cells suggests that viral double-stranded RNAs (dsRNAs) can undergo processing in the nucleus by DCL1 and then be transported to the cytoplasm, where they participate in the antiviral RNA-silencing pathway.

In recent times, CRISPR/Cas9 has effectively been employed to alter the genetic makeup of several plant crops (Arora and Narula, 2017; Jabran *et al.*, 2023). The proven CRISPR/Cas9 technology provides a novel method for creating Potyvirus resistance alleles in important crop plants, eliminating the need for enduring transgenes (Arora and Narula, 2017). CRISPR-Cas9 is used to target the *eIF4E*1gene to extend the PVY resistance spectrum of potatoes. Additionally, reports that the CRISPR/Cas9-guided knockout of *eIF4E* enhances the WYMV resistance (Cai *et al.*, 2015). The mechanism of CRISPR/Cas9 mediated resistance, including how it targets viral genomes and translation factors like eIF4E is illustrated in Figure 3. According to recent studies, plant genes for the eIFs encoding eukaryotic translation initiation factors could serve as key candidates in enhancing the resistance to engineered viruses through New Plant Breeding Techniques (Pechar *et al.*, 2022). However, there are various challenges and limitations associated with the selective knockdown of *eIF4E* to enhance virus resistance.

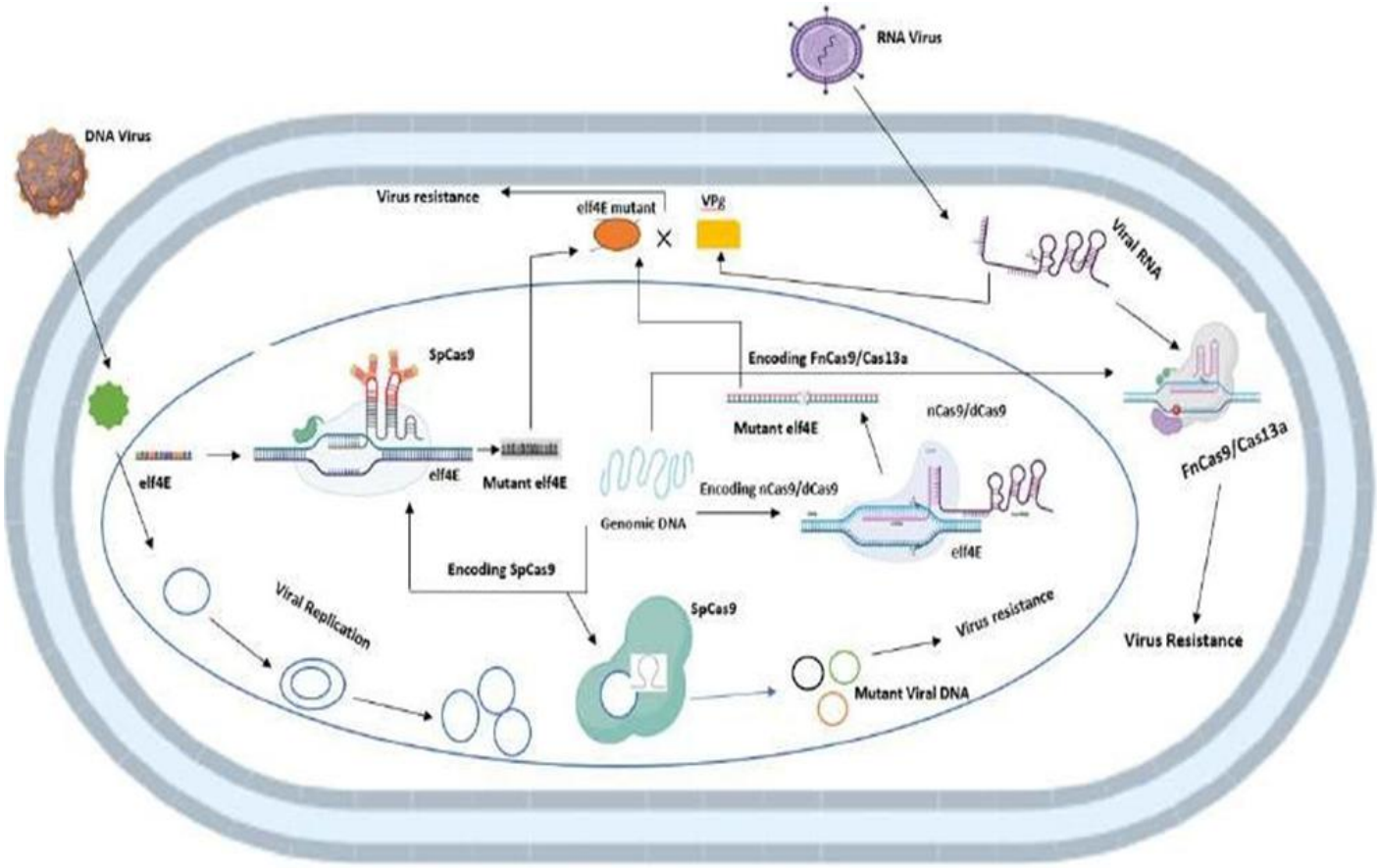

Figure 3. The CRISPR/Cas systems are shown to provide resistance against both DNA and RNA viruses. Upon entering the plant cells, the viral DNA undergoes transcription and translation facilitated by host factors. Afterward, the genetic material of the virus is duplicated and transmitted to more cells. The CRISPR system may specifically target the viral genome by using Cas9/nCas9/dCas9/FnCas9/Cas13a as an endonuclease. The plant genome produces sgRNA and Cas9, which then combine to generate sgRNACas9. During a viral infection, the viral DNA is duplicated and produced in the nucleus of the host cell as a double-stranded DNA replicative form. The sgRNA-Cas9 specifically targets the viral dsDNA and either cleaves the viral genome or induces mutations within it. Similarly, the sgRNA-FnCas9/Cas13a specifically targets the viral RNA, leading to the formation of a mutation in the RNA. *Potyviruses* use the host cellular translation factor *eIF4E* to facilitate the translation of their viral RNA and invade the cells. Therefore, CRISPR/Cas9 may be used to specifically target *eIF4E*, a component of eukaryotic translation initiation factors. This will lead to the creation of a mutant gene form that is recessive and unable to interact with VPg, a protein involved in viral translation machinery. Additionally, the nuclease-defective Cas or nCas9 can be employed in base editing to disrupt viral RNA by including a fused uracil glycosylase inhibitor or UGI.

CRITICAL ANALYSIS OF CHALLENGES AND LIMITATIONS OF *eIF4E* KNOCKDOWN FOR VIRUS RESISTANCE

Pleiotropic effects, or unexpected results

Knocking down *eIF4E* can have significant physiological and developmental implications for the plant Viruses utilize RNA helicases of the Dead-box family. In the case of Arabidopsis knocking down *eIF4E* resulted in dwarfism, delayed flowering, and reduced seed yield (Figure 4). Surprisingly, the exact knockdown of the entire family of *eIF(iso)4E* was even lethal. We can see from the literature that this form of resistance is well-suited for cultivated plants and is linked to unaffected pleiotropic effects on crop growth and yield (Wan *et al.*, 2015).

Asymptomatic accumulation of Potyviruses

Mutations in the viral genome, particularly in VPg, enables potyviruses to evade *eIF4E*-mediated resistance, allowing limited replication in resistant plants. Studies have demonstrated asymptomatic viral accumulation in Lycopersicon esculentum resistant to Potato Virus Y (PVY) and Capsicum annuum resistant to Pepper veinal mottle virus (PVMV), where low level viral presence was detected despite the absence of symptoms (Gauffier *et al.*, 2016; Piron *et al.*, 2010).

Such latent infection pose a risk for viral transmission via vectors or seed dispersal, emphasizing the need for molecular surveillance and integrated disease management strategies.

Viral adaptation

Plants that have resistant *eIF4E* alleles in the homozygous form may nonetheless develop disease symptoms after being infected with a virus, either naturally or by artificial inoculation (Zlobin *et al.*, 2023), and several plant species have developed resistance to potyviruses through modification in eIF4E and eIF(iso)4E (Table 1). However, viruses can adapt to these mutations and eventually infect the plant. In most cases, the adaptation simply occurred with the VPg protein, which can now interact with the *eIF4E*, which was mutated by the CRISPR technology. This would require the continued development of new synthetic factors and careful monitoring of resistance.

Durability and specificity of resistance

Biological research has demonstrated that these genetic changes enable the reconnection of VPg with the resistant *eIF4E* allele, hence enabling potyviruses to once again utilize the matching *eIF4E* isoform in plants as a vulnerability factor (van Grinsven, 2022). Over time, the same viruses, several strains of Potyvirus, will have to be accounted for, as they continuously adapt to the virus factor. So far, the resistance factor lasted for a year and a half, resulting in the need for the application of new resources.

Limited specificity

While *eIF4E*, as stated above, can apply to a wide spectrum, it can be equally fatal for the plant since its knockdown affects other desirable mRNA. Hence, more developed measuring tools will be required in selective knockdown.

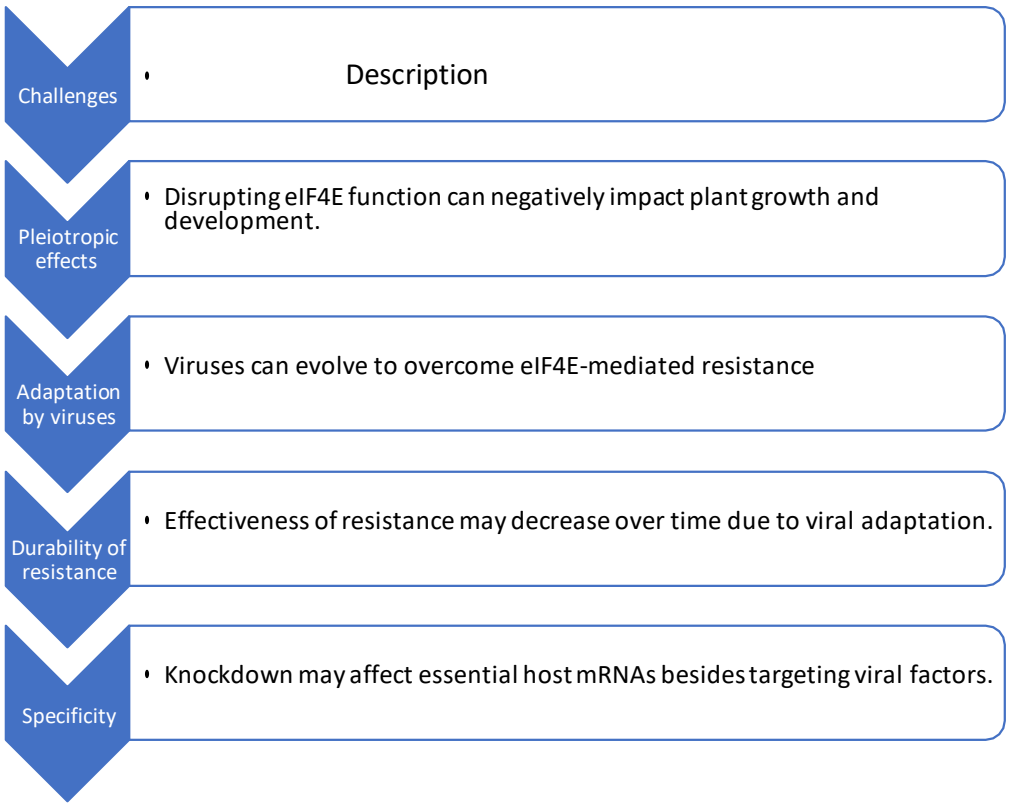

Figure 4. Under laboratory conditions, the deactivation of *eIF4E*1 in Arabidopsis thaliana was linked to minor pleiotropic consequences. These plants exhibited reduced size, a 7-day delay in flowering, and slightly lower seed production (Salazar-Díaz *et al.*, 2021). At the same time, double knockout *eIF4E*1 and eIF iso 4E in Arabidopsis had many negative effects. Inactivation of *eIF4E*1 or eIF is o 4E in Arabidopsis also decreased tolerance to low temperatures.

CONCLUSION

eIF4E inactivation is a promising strategy for broad-spectrum virus resistance in crops, facilitated by RNA silencing, TILLING, and CRISPR/Cas9. However, optimizing these approaches is critical to minimize adverse effects on plant development and mitigate viral adaptation. Future research should focus on precision editing strategies, such as allele-specific modifications and domain-targeted mutagenesis, to retain essential *eIF4E* functions while disrupting viral interaction. Integrating *eIF4E* knockdown with complementary resistance mechanisms could further enhance durability.

Additionally, addressing regulatory challenges is essential for the effective implementation of this approach in commercial agriculture. With continued advancement, *eIF4E* based resistance holds significant potential for sustainable plant virus management and global food security.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Hammad Ahmed Abbasi conceived the study, performed the research, and wrote the initial manuscript draft. Muhammad Naveed Anjum contributed to data analysis and manuscript review. Muhammad Atif assisted with data interpretation and provided critical revisions. All authors read and approved the final manuscript.

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