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Review

Cauliflower mosaic virus: Virus-host interactions and its uses in biotechnology and medicine

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ABSTRACT

Cauliflower mosaic virus (CaMV) was the first discovered plant virus with genomic DNA that uses reverse transcriptase for replication. The CaMV 35S promoter is a constitutive promoter and thus, an attractive driver of gene expression in plant biotechnology. It is used in most transgenic crops to activate foreign genes which have been artificially inserted into the host plant. In the last century, producing food for the world's population while preserving the environment and human health is the main topic of agriculture. The damage caused by viral diseases has a significant negative economic impact on agriculture, and disease control is based on two strategies: immunization and prevention to contain virus spread, so correct identification of plant viruses is important for disease management. Here, we discuss CaMV from different aspects: taxonomy, structure and genome, host plants and symptoms, transmission and pathogenicity, prevention, control and application in biotechnology as well as in medicine. Also, we calculated the CAI index for three ORFs IV, V, and VI of the CaMV virus in host plants, the results of which can be used in the discussion of gene transfer or antibody production to identify the CaMV.

1. Background

Many viral diseases affect plants and cause symptoms that reduce the quantity and quality of produce (Jones, 2021). Many pathogenic viruses that reduce crop yields of plants of *Brassicaceae* are distributed worldwide. The most important virus affecting this group is CaMV. This virus is widely distributed, especially in temperate regions such as Africa, Asia, New Zealand, and the United States (Yasaka et al., 2014). CaMV has been reported to infect 60% of plants in the *Brassicaceae* and significantly impacts their growth and development (Spence et al., 2007). CaMV was first discovered by Tompkins in 1937 in *Brassica campestris* and *Brassica oleracea* plants on American farms (Sutic et al., 1999). This virus was formerly called *cabbage mosaic virus*, *broccoli mosaic virus*, *brassica virus3*, and *cabbage virus* (Tompkins, 1937). CaMV belongs to the genus *Caulimovirus* of the *Caulimoviridae,* which are classified together with the animal *hepadnaviruses* and the plant *badnaviruses* as *pararetroviruses*, a group of viruses with DNA or RNA genomes that have a reverse transcription step in their replication cycle. CaMV encodes its own reverse transcriptase, but interestingly, this enzyme does not have an integrase function (Yasaka et al., 2014; Bak and Emerson, 2020; Hoh et al., 2010; King et al., 2012). There are two major differences with *retroviruses*: *Retroviruses* are enveloped RNA viruses, whereas *pararetroviruses* generate encapsidated viral DNA, and the proviral DNA of retroviruses, resulting from reverse transcription of the RNA genome, is integrated into the host DNA, but the DNA of *pararetroviruses* behaves as a free chromosome in the nucleus of the host cell (Haas et al., 2002).

In 2020, the International Virus Classification Committee divided the *Calimoviridae* into eighty-one species and eight genera: *Badnavirus*, *Caulimovirus*, *Cavemovirus*, *Petuvirus*, *Rosadnavirus*, *Solendovirus*, *Soymovirus*, *Tungrovirus* (Teycheney et al., 2020). The CaMV genome is about 8000 bp in size and consists of the alpha or negative strand of DNA (which is used for amplification, as opposed to the complementary strand) and the beta-strand (Hull et al., 1976). The 35S CaMV promoter is generally considered to be a constitutive promoter that facilitates a high level of RNA transcription in a wide variety of plants, including those outside the host range of the virus (Seternes et al., 2016). Since CaMV is one of the many mosaic viruses that infect crops and plants

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around the world, herein we describe those unique characteristics and potential uses in biotechnology and medicine.

2. Structure and genome

CaMV particles are non-enveloped, with a T = 7 structure, and approximately 50 nm in diameter. It has been reported the diameter of virions CaMV determined by TEM (Transmission electron microscopy) can range from 35 to 60 nm, which is a due to deformation during drying and immobilization on the substrate, as well as the type of contrast material used in TEM (Nikitin et al., 2015). CaMV particles are composed of 420 subunits of coat protein (Cheng et al., 1992), which forms a triple-layered structure with a hollow center. Each virion contains a single molecule of circular, double-stranded DNA embedded between the second and third layers of the capsid. The virion DNA contains discontinuities, that are generated as a consequence of the reverse transcription process (Hohn, 2013; Schoelz et al., 2016). CaMV produces three primary RNAs: 35S RNA, 19S RNA, and 8S RNA. The 35S RNA covers the whole genome, is terminally redundant due to a conditional polyadenylation signal, and acts as a pregenomic template for DNA synthesis as well as a polycistronic mRNA for synthesis of the viral proteins. The 19S RNA encodes the gene VI product, P6 (Hohn, 2015), and the 8S RNA likely plays a role in RNA silencing (Hoffmann et al., 2022a; Schoelz and Leisner, 2017).

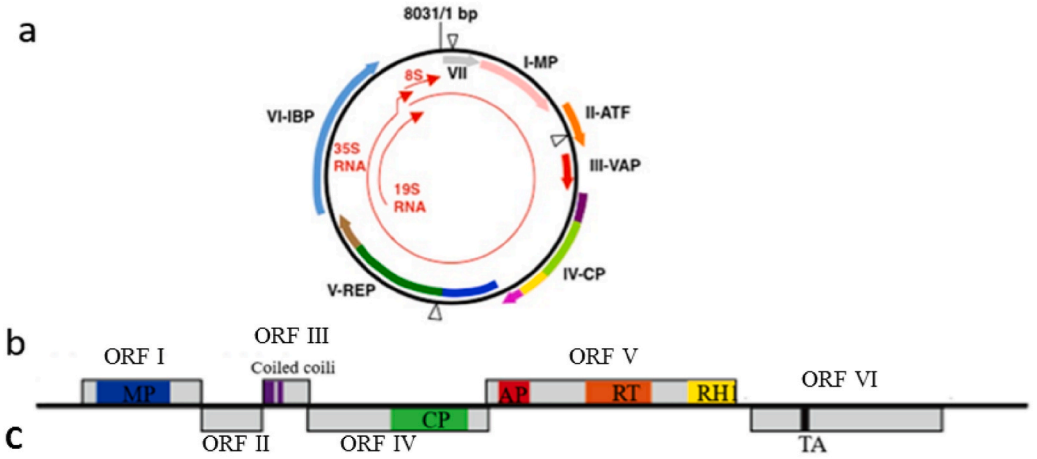
One of the interesting aspects about the CaMV genome is the existence of single-stranded interruptions in the circular structure of the genomic DNA that is packaged into virions (Guilley et al., 1983). Both DNA strands of the duplex DNA contain these interruptions, typically with a single interruption in one strand and two interruptions in another strand. These discontinuities (called Δ) which are remnants of the reverse transcription process, disappear after the virus genomic DNA enters the host nucleus. They have overlapping strands that may have a ribonucleotide sequence at its 5′ end. They are repaired by host enzymes in the nucleus of the host cell to yield a supercoiled DNA molecule. The latter becomes associated with histones to a viral minichromosome covered with nucleosomes (Perbal et al., 1993; Pietrzak and Hohn, 1985; Tang and Leisner, 1998).

The CaMV genome encodes seven proteins, six of which can be detected in virus-infected plants (Haas et al., 2002). P1 (40 kDa) is a cell-to-cell movement protein that forms tubules through the plasmodesmata, allowing CaMV particles to move from one cell to another (Perbal et al., 1993). This protein may also bind cooperatively to RNA to direct 35S and/or 19S RNA to neighboring cells (Citovsky et al., 1991). P2 (18 kDa), or aphid transmission factor (ATF), is required for the aphid transmission of CaMV, but is dispensable for virus replication (Perbal et al., 1993). P2 is the major component of electron-lucent inclusion bodies (Espinoza et al., 1991). These inclusion bodies also contain P3 and some scattered virions (Drucker et al., 2002). P3 (15 kDa), or virion-associated protein (Shivaprasad et al., 2008), is weakly associated with the viral capsid (Dautel et al., 1994). P3 is required for the systemic infection of plants since its deletion from the CaMV genome leads to a loss of virus infectivity (Jacquot et al., 1998; Leh et al., 1999). P3 interacts with P1 and by doing so, permits virus particles to associate with plasmodesmata facilitating virus movement (Stavolone et al., 2005). In addition, P3 was shown to play a pivotal role in the formation of a CaMV complex transmissible by aphids (Leh et al., 1999, 2001). P4 (56 kDa), the coat protein or capsid protein, is the basic building block of the CaMV virion. The CaMV virion is composed of 420 P4 subunits that form three layers (Cheng et al., 1992) after P4 is processed by the viral aspartic proteinase (Torruella et al., 1989). P4 is involved in assembly (Champagne et al., 2007), packaging of the viral RNA (Guerra-Peraza et al., 2000) and delivery of the viral genome to the nucleus (Karsies et al., 2002). There is an interaction between CaMV coat protein and a purine-rich conserved sequence in the middle of the 35S RNA promoter. In caulimoviruses, as in retroviruses, the zinc finger motif of the coat protein is involved in interacting with 35S RNA and is involved in viral infection (Guerra-Peraza et al., 2000). P5 (78 kDa), the reverse transcriptase, is essential for replication of the viral genome (Haas et al., 2002). P5, encoded by the largest ORF in the CaMV genome, is a polyprotein that resembles retrovirus pol proteins. This protein contains protease, reverse transcriptase, and RNase-H domains, the latter two of which, permit copying of the viral 35S RNA into DNA (Raikhy et al., 2006). P6 (62 kDa), also called TAV (TransActivator/Viroplasmin) which is the only CaMV protein encoded by a subgenomic (19S) RNA. This protein has many functions (Harries et al., 2009): translational transactivation (Schoelz et al., 2016; Leclerc et al., 2001), viral genome replication, virion assembly, virus-host interactions, RNA silencing suppressor activity and virus infectivity (Anderson et al., 1991; Bonneville et al., 1989; Geldreich et al., 2017; Love et al., 2007; Lutz, 2014; Wintermantel et al., 1993). P6 is also the major protein comprising electron-dense inclusion bodies (edIBs) (Schoelz and Leisner, 2017). P7 is the first protein encoded by the 35S RNA (Lutz et al., 2012), it is a small, basic protein of unknown function that has not been detected in infected plants (Wurch et al., 1990).

CaMV is one of the most important viruses in plant pathology (Scholthof et al., 2011). Early in infection it forms inclusion bodies in the host cell cytoplasm that function as virus factories (VFs) (Bak et al., 2013). Virus factories determine where viral protein synthesis, replication, and assembly occur, as well as where newly-formed virions are stored (Bak et al., 2013; Mazzolini et al., 1985). The viral genome of double-stranded circular DNA is approximately 8000 bp in size (Franck et al., 1980). The molecular weight of CaMV particles was calculated 4.5 $\times$ 10^6 (Shepherd et al., 1970). The viral genome exists in two forms: discontinuous linear and cyclic double-stranded molecules. Approximately 90% of the genomes found in extracts derived from CaMV-infected plants are discontinuous cyclic molecules (Cheng et al., 1992; Franck et al., 1980). CaMV uses reverse transcriptase as part of the replicative cycle (Leclerc et al., 1999). The CaMV proliferation cycle consists of two main stages, one in the nucleus and the other in the cytoplasm. After the CaMV enters a plant cell, viral particles move into nucleus throuth nuclear localization signal (NLS)-mediated transport. In this pathway importin alpha and possibly microtubules are contributed. Then the virus disassembles at the nuclear envelope (Karsies et al., 2002), viral dsDNA enters the nucleus, and it associates with histones to form a minichromosome that is used as a template for transcription by the host DNA-dependent RNA polymerase II. Minichromosomes are used as templates to produce 35S RNA, 19S RNA and 8S RNA. P6 is translated from 19S RNA and accumulates in electron-dense inclusion bodies (edIBs) P6 transactivates the expression of P1–P5 from the 35S RNA. P4 and P5 play a role in 35S RNA nuclear export (Kubina et al., 2021). P5 replicates the genome by reverse transcription using the 35S RNA as a template. The packing process is complex, 35S RNA is packaged into virions along with P5 then is reverse transcribed within the capsid, or the 35S RNA may be reverse transcribed before being packaged in edIBs (Hohn et al., 1997). The newly synthesized dsDNA is packaged into virions that can move from cell-to-cell or be transmitted from plant to plant via aphids (Kubina et al., 2021; Hohn et al., 1997; Amack and Antunes, 2020; Haas et al., 2005). CaMV is a pararetrovirus and DNA of the pararetroviruses accumulates within the nucleus as multiple copies of circular minichromosomes (Fig. 1) (Leclerc et al., 1999; Hohn et al., 2008).

3. Physico-chemical properties

The density of CaMV virions in CsCl is 1.37 g/cm^3 (Teycheney et al., 2020). The final temperature of inactivation is 75–80 °C. The in vitro stability is 5–7 days. The inactivation dilution point of CaMV is approximately 3^{-10} (Buchen-Osmond, 2006). The virus is precipitated under low-temperature conditions by double-positive magnesium or ethanol at equilibrium salt concentration or by polyethylene glycol, and the amount of virion forming water is estimated to be 1.9 ml/g (Hull et al., 1976). Antigenic characteristics and serological relationships:



	Proposed protein function	Conserved protein domains
ORF I	Movement protein	Transport of virus in host-cell to cell
ORF II	Aphid transmission factor	Aphid transmission protein
ORF III	Virion-associated protein	DNA binding
ORF IV	Coat protein	Nucleic acid binding, Structural molecule activity, Zinc ion binding
ORF V	Enzymatic polyprotein	Aspartic-type endopeptidase activity, Endonuclease activity, RNA-directed DNA polymerase activity, proteolysis
ORF IV	Transactivator/viroplasmin protein	Regulation of translation- Viral translational termination-reinitiation, Host cell cytoplasm

Fig. 1. Cauliflower mosaic virus: (a) Genomic map (Schoelz and Leisner, 2017) (b) Conserved protein domains as listed in the Pfam database for CaMV ORFs (c) Gene Ontology (GO) CaMV (strain CM-1841) in the uniprot database.

CaMV is serologically closely related *to Strawberry vein-banding virus*, *Carnation etched ring virus* and *Dahlia mosaic virus*. It is also closely related to Horseradish latent virus (Martelli and Castellano, 1971).

4. Host plants and symptoms

CaMV exhibits a worldwide distribution and can infect plant species belonging to the *Cruciferae* including *Arabidopsis thaliana* and the *Solanaceae* including *Devil's trumpets* (genus Datura) as well as certain tobacco (*Nicotiana*) species, producing different symptoms in various hosts (Karsies et al., 2002; Chenault and Melcher, 1994; Khelifa et al., 2010; Love et al., 2012; Schoelz and Shepherd, 1988). The virus can induce a range of systemic symptoms, such as chlorosis, mosaic, deformation, mottling, vein banding, vein clearing, stunting (Tompkins, 1937; Bak and Emerson, 2020). CaMV can also be found in mixed infections with other viruses, especially *Turnip mosaic virus*. Mixed infections often show more severe disease symptoms compared to either virus alone. (Spence et al., 2007; Love et al., 2012).

Indicator plants or differential hosts (a plant that respond rapidly and specifically to sap inoculation, and localized lesions appear on inoculated leaves) for this virus include: *Brassica oleracea* var. *Botrytis L.-cauliflower* (Tompkins, 1937), *Brassica campestris* spp. *Rapa cv. Just Right - turnip* is one of the most susceptible *Brassica* species to the virus, and its use as a reference plant is important for virus identification (Covey et al., 1990). Some isolates of this virus, such as D4, are capable of infecting some members of the *Solanaceae*, including Datura (Schoelz et al., 1986). Datura historically was one of the plants commonly used to detect CaMV (Daubert and Routh, 1990).

5. Transmission and pathogenicity

Plant virus transmission is the result of interactions between a particular virus, the host plant and the vector (Martinière et al., 2009). One of the characteristics of members of the *Caulimoviridae* is the formation of cytoplasmic inclusion bodies in infected cells (Rodriguez et al., 1987). In infected plant cells, CaMV proteins are synthesized in the cytoplasm and accumulate in electron-dense (edIBs) and electron-lucent (elIBs) inclusion bodies. The edIBs contain mainly virus particles in a matrix of viral protein P6 and elIBs were described as an electron-lucent matrix made of P2 involved in aphid transmission (Espinoza et al., 1991). CaMV uses the helper strategy for transmission (Palacios et al., 2002). CaMV proteins are made floating around in the cytoplasm and the P3 virion is confined in electron-dense inclusion bodies, whereas P2 is sequestered in electron-lucent inclusion bodies (elIBs) (Khelifa et al., 2007). The edIBs is as the site of viral protein synthesis, replication, and assembly, as well as for storage of newly formed virions role earlier in the infection cycle virus. After the virus stops replicating in the infection cycle, they serve as storage facilities for surplus virions (Bak et al., 2013).

CaMV is transmitted by at least 27 aphid species, in a non-persistent or non-circulative manner (Bak and Emerson, 2020; Kennedy et al., 1962). The virus can be obtained from epidermal and mesophilic cells,

which is characteristic of nonpersistent transmission, as well as from phloem as persistent viruses (Chesnais et al., 2021). The most common vectors include *Myzus persicae*, *Brevicoryne brassicae*, *Rhopalosiphum pseudobrassicae* (Tompkins, 1937), although the most important vector was the green peach aphid (Woolston et al., 1983), which belongs to the order *Aphidoidae* and family *Aphididae* (Kuhar et al., 2005). The vector aphid rapidly takes up the virion from the host plant while feeding and the virion remains in the mouthparts of the aphids for a short period of time. Transmission of the virus is non-circulatory and non-proliferative and CaMV is not transmitted to the next generation (Martín and Elena, 2009). It has been shown that the P2 protein product alone cannot mediate virus transmission by the aphid vector because P2 is unable to bind directly to the virus protein coat (P4). The P3, a 15 kDa protein, does the interaction between P2 and P4. P3 binds the duplicate gene region to the virion, which is a component of the virion vector complex, although the nature of its formation and configuration remains unknown (Hoh et al., 2010). Some isolates of the virus cannot be transmitted by aphids: these isolates contain a deletion within ORF 2. To transmit these isolates, aphids must first feed on a plant contaminated with other caulimoviruses or transmissible isolates of CaMV that contain a stable helper protein (Rodriguez et al., 1987). However, there are isolates of the virus such as Campbell (has a full-length gene II and is very similar to that of CM1841) and CM1841 that cannot be transmitted by aphids. Non-transmission by aphids in these isolates is due to mutations in nucleotides 94 and 105, which change the amino acid glycine, arginine and isoleucine, respectively, to valine (Al-Kaff and Covey, 1994; Al-Kaff et al., 1997). In a study, the mutation was made in P2-94 and heterologous expression with the P3 complex in vitro. It was found that the lack of virus transmission was not due to the low accumulation of the virus, and the reason for this was specifically due to the lack of formation of elIBs in infected plant cells (Khelifa et al., 2007). Caulimoviruses are generally transmitted mechanically, by a vector, by contact between two hosts, and through cuttings (Bak and Emerson, 2020). CaMV can be transmitted by mechanical inoculation and through its aphid vector, However, no evidence exists that CaMV can be transmitted by pollen or via seed (Spence et al., 2007; Blanc et al., 2001).

6. Prevention and control

Throughout the world, plant virus control programs are of great economic importance because plant viruses cause diseases that destroy commercial crops. CaMV control is based on the prevention of contamination, early detection of the disease, destruction of infected crops, avoidance of mixed crops, and control of insect vectors (Spence et al., 2007). The most common method to reduce the number of aphids on leafy vegetables is through the use of insecticides such as organophosphate di-methate. Reduction of aphid numbers results in a concomitant decrease in CaMV incidence (Spence et al., 2007). It should be noted that insecticides are not recommended until 50% of the leaves are infested with aphids and by that stage CaMV has already established a foothold (Kuhar et al., 2005).

In 1992, the resistance of several ecotypes of the Arabidopsis plant for resistance to strains of the CaMV (strains CM1841, W260, and CM4–184) was investigated and it was determined that the ecotypes (Wil-2, En-2, and Sv-0) are resistant to the three virus strain, but ecotype Tsu-0 is susceptible to CaMV strain W260 and resistant to strains CM1841 and CM4–184 (Islam et al., 2019). In the study ecotype En-2 of *Arabidopsis thaliana* it was found that resistant to W260 strains of CaMV but is susceptible to strain NY8153 (Adhab et al., 2018). In other hosts (Solanaceae), P6 showed a hypersensitivity reaction *in Datura stramonium* and *Nicotiana edwardsonii* and a non-necrotic defense response in *N. bigelovii* and *N. glutinosa* (Schoelz and Shepherd, 1988; Schoelz et al., 1986; Cole et al., 2001).

Viral infections depend on the availability of host factors necessary for virus replication and movement and the balance between plant defense and viral suppression of defense responses. Thus identifying these factors could provide targets for editing to engineer resistance to plant viruses (Garcia-Ruiz, 2018). In recent years, comprehension of viral infections, the identity of new resistance genes, and susceptibility factors against viruses have increased. In a recent study examining host genes influencing CaMV infection of *A. thaliana*, the NCED9 (Encodes 9-cis-epoxycarotenoid dioxygenase) abscisic acid biosynthetic gene was identified was among the most important (Hoffmann et al., 2022b). Viral infections can directly or indirectly disrupt phytohormone signaling pathways (Ma and Ma, 2016). In CaMV, selective autophagy receptor NBR1 binds the viral capsid protein and particles of CaMV and mediates their autophagic degradation (Hafrén et al., 2017). Recently has been determined, P6 modified NPR1 and inhibited SA-dependent defense responses (Love et al., 2012). NPR1 acts as a master regulator of the plant hormone salicylic acid signaling, which plays an essential function in plant immunity (Chen et al., 2019). P6 plays a vital role in the movement, replication, and suppression of RNAi of the CaMV. Studies have shown that the expression of P6 results in interference with Ethylene pathway response but the relationship between the components of the ET pathway and P6 has not been identified yet (Islam et al., 2019). In an experiment, it was found that the mutation of the Ethylene signaling pathway insensitive to ethylene 2 (ein2) and ethylene response 1 (etr1) showed more resistance to CaMV infection. Research has also shown that the Ethylene pathway is also related to reactive ROS and was induced systemically after CaMV infection, and the accumulation of ROS was related to Ethylene and NADPH oxidase (Zhao and Li, 2021).

In recent years, with the advancement of science and technology, several mechanisms have been identified to create plant virus resistance, including CRISPR/Cas9 and RNA silencing (Zhao et al., 2020). Newly, autophagy has been reported in plants' response to viral infection. During plant-virus interactions autophagy plays the main role in regulating immunity-related cell death, antiviral and promoting viral pathogenesis (Ismayil et al., 2020). The clustered, regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) is a genome editing system consisting of an endonuclease Cas protein and a single-guide RNA (sgRNA) which directs the Cas protein to the DNA or RNA target (Cong et al., 2013). The use of the CRISPR/Cas technology to develop viral-resistant plants has been of interest in recent years (Ali et al., 2016; Chandrasekaran et al., 2016). In general, there are two main approaches to using CRISPR/Cas technology to control plant viruses. The genome of plant viruses can be DNA or RNA and these biomaterials can be targeted, destroyed, or interfered with by CRISPR/Cas9 or CRISPR/Cas13 systems in the nucleus or cytoplasm and ultimately inhibit virals infection. In the second case, by using the CRISPR/Cas9 system the host's susceptibility factors, here genetically factors, that are required for viral infection are mutated or altered which inhibits viral infection (Cao et al., 2020). In one study scientists achieved CaMV resistant plant through Cas9-mediated multiplex targeting of the viral coat protein sequence. In this research they used multiple sgRNAs which were complementary to different regions of ORF IV (encoding coat protein) along with a Cas9 to make CaMV-resistant plant (Liu et al., 2018).

RNA silencing plays an important role in plant resistance to viruses, and both RNA and DNA viruses are suppressed by this mechanism (Wang et al., 2012). This mechanism causes the degradation of viral RNA, and finally, the concentration of the virus and the occurrence of virus symptoms in the infected plant are reduced (Cao et al., 2005). The RNA-induced silencing complex (RISC) is a multiprotein complex, which act as a core element in gene silencing via a variety of pathways at the transcriptional and translational levels (Pratt and MacRae, 2009). This complex functions as a key tool in regulation of gene expression and uses single-stranded RNA (ssRNA) fragments, such as microRNA (miRNA), or double-stranded small interfering RNA (siRNA) to recognize target region. The single strand of RNA acts as a template for RISC to recognize complementary section in messenger RNA (mRNA) and after that, one of the proteins in RISC, Argonaute, is activated and cleaves the mRNA (Redfern et al., 2013). Numerous viruses can disrupt this defense

mechanism in plants. Most plant viruses encode RNA-silencing suppressor proteins that these proteins interact with different components of the RNA-silencing pathway (Qu and Morris, 2005; Zhai et al., 2022). CaMV protein P6 is a suppressor of RNA silencing complex. It has been found that in Arabidopsis infected with CaMV the endogenous tasiRNA (*Trans*-acting siRNA) pathway is suppressed by P6 and leads to the susceptibility of the host to CaMV (Shivaprasad et al., 2008; Love et al., 2007).

It is also assumed that plants and plant viruses coevolve (Fraile and García-Arenal, 2010). Here, we explore the relationship between the codon adaptation index (CAI) of CaMV genes based on its hosts to identify any clues to CaMV-host co-evolution. The codon adaptation index (CAI) is the most widespread technique for analyzing codon usage bias. This index is used as a quantitative method to predict the expression level of a gene based on its codon sequence (Jansen et al., 2003). This index assesses the extent to which natural selection has been effective in molding the pattern of codon usage that indicates how frequently a favored codon is used amongst highly expressed genes. CAI calculating can be a useful procedure for predicting the expression level of foreign genes in heterologous hosts (Lee et al., 2010; Nambou and Anakpa, 2020), such as recombinant vaccine production, as well as an indicator to determine the co-evolution of host and pathogen (Gustafsson et al., 2012; Khandia et al., 2019). The ideal range for CAI is 0.8–1.0, the lower the number is, the higher the chance that the gene will be expressed poorly and higher values indicate a higher gene expression potential (Fu et al., 2020). Various reports show that the CAI is frequently used to assess the adaptation of viral genes to their hosts (Carmi et al., 2021; He et al., 2022; Qin et al., 2022; Xu et al., 2008). We calculated the CAI index of some important ORFs of the CaMV virus in host plants and some common heterologous hosts for recombinant protein production by web site http://www.kazusa.or.jp/codon/and http://genomes.urv.es/CAIcal/. The results of this study demonstrated that ORFIV, ORFV, and ORFVI have a CAI index higher than 0.8 in host plants in comparison to the non-hosts (Table 1). It is inferred from these results that CaMV has co-evolved with its hosts such that both host and virus use the same synonymous codons for expressing special amino acids. As these genes displayed an upper value higher 0.8 CAI in host plants. One of the applications of these results can be to express CaMV coat protein (ORF IV), CaMV reverse transcriptase (ORF V), and Transactivator/viroplasmin protein (ORF VI) genes in a safe and easy-to-scale-up heterologous host-plant platform.

7. Applications of CaMV in biotechnology

Several years ago, the 35S promoter, derived from the CaMV, was first identified and used to drive expression of transgenes in transgenic plants (Odell et al., 1985a; Somssich, 2019). The CaMV 35S promoter or a variation of it is present in more than 60% of all transgenic crops currently produced worldwide and is of great importance in agricultural biotechnology (Amack and Antunes, 2020). This element is used so frequently in plant biotechnology because it is a constitutive promoter that is active in most plant species, including many that are not hosts (Bak and Emerson, 2020; Seternes et al., 2016; Dutt et al., 2014; Kay et al., 1987; Odell et al., 1985b). Sequence analysis of the 35S promoter shows the presence of several regulatory elements that are located along the entire length of the promoter. It also has two domains A and B, which are mostly divided into several subdomains (Benfey and Chua, 1990). An analysis of regulatory elements in the 35S promoter and the study of the role of *Cis*-regulatory elements in gene transcription increases our knowledge of producing chimeric versions of the 35S promoter that contain *cis*-elements or duplicate enhancers (Mitsuhara et al., 1996).

As of 2013, about 336 species of transgenic crops comprising 27 different species have been commercially introduced worldwide, and this number is increasing as more transgenic crops enter field trials during the research phase (Wu et al., 2014). Different countries have different regulations for the cultivation and consumption of genetically modified (Liao et al., 2022) crops and consumers want the labeling of GM crops. Another problem with transgenic plants is their biosafety and sometimes they may cause problems if biosafety regulations are missed, so it is necessary to identify GM crops and label them. Most recently, a fluorescent construct termed PE-MC/SDA-CRISPR/Cpf1 (Multiple cascade strand displacement amplification connected with CRISPR/Cpf1) (Liu et al., 2022a) was proposed as a biosensor for detecting the CaMV 35S promoter in genetically modified organisms GMOs), Therefore, the method has great application potential for detection transgenic crops (Liu et al., 2022b). Among the applications of the 35S promoter, we can mention its use in horticultural products, which has led to tolerance to biotic and abiotic stress and improved the quality traits desired in crop plants. For example, the 35S promoter has been used for transgene expression in transgenic papaya, which is more resistant to *Papaya ring spot virus* (Fitch et al., 1990) and showed an increase in host plant resistance to the plum pox virus, the cause of Sharka disease of plum (Scorza et al., 2001). Similarly, the 35S promoter was used to drive expression of factors permitting the generation of transgenic plants resistant to a variety of abiotic stress such as cold, drought, and salinity. For example, a variety of transgenic crop plants generated with the 35S promoter driving the expression of *A. thaliana* CBF (C-repeat binding factor) transcription factor exhibit increased cold and drought tolerance (Dhekney et al., 2007; Wisniewski et al., 2011). Baicalein is a flavonoid typically extracted from the root of *Scutellaria baicalensis* Georgi and is effective against several cancers. Recently, its heterologous biosynthesis using the 35S promoter in *Lycopersicon esculentum* is reported (Liao et al., 2022). In addition to the use of the 35S promoter in plants, 35S CaMV promoter has been shown to be functional in animal and human cell lines, such as the human enterocyte-like cells (Myhre et al., 2006), Chinese hamster ovary cells (Tepfer et al., 2004), human embryonic kidney cells (Tepfer et al., 2004), *Xenopus oocytes* (Ballas et al., 1989), The CaMV 35S promoter is not as strong in those systems as it is in plants but it still can drive the expression of genes in animal and human cell lines (Vlasák et al., 2003).

One of the uses of plant viruses in biotechnology is their use as vectors for the transient expression of heterologous proteins including vaccine antigens and antibodies (Gleba et al., 2007). In addition, virus-based vectors are used for the production of human or animal therapeutic proteins, as well as for the specific study of plant biochemical processes (Abrahamian et al., 2020). In one study, replacement of CaMV ORF II by the bacterial dihydrofolate reductase (dhfr) gene resulted in chimeric viral DNA capable of producing functional DHFR in infected turnip plants (Brisson et al., 1984).

Table 1
A comparison of the CAI index of CaMV ORFs IV, V, and VI in different genetic backgrounds.

	ORF IV/CAI	ORF V/CAI	ORF VI/CAI
Brassica oleracea[a]	0.834	0.830	0.821
Brassica napus[a]	0.810	0.803	0.803
Brassica rapa[a]	0.820	0.816	0.816
Arabidopsis thaliana[a]	0.804	0.799	0.772
Triticum aestivum[b]	0.624	0.617	0.648
Oryza sativa[b]	0.741	0.728	0.751
Pichia pastoris[b]	0.758	0.768	0.721
Saccharomyces cerevisiae[b]	0.729	0.751	0.710
Escherichia coli[b]	0.700	0.696	0.693

[a] Host organisms.
[b] Non-hosts organisms.

8. Applications in medicine

It has been accepted that there are some differences between plant viruses and vertebrate viruses in terms of host range as well as pathogenicity. Plant viruses only infect plants and they have not been shown to be pathogenic to humans and other vertebrates (Balique et al., 2015;

Colson et al., 2010). Recently, viral nanoparticles have been considered for medical uses (Singh et al., 2006). Applications of plant virus nanoparticles in medicine include the production of a vaccine based on viral nanoparticles (Yildiz et al., 2011), targeted drug delivery (Ruoslahti et al., 2010; Ma et al., 2012), epitope carriers for vaccines to agents in cancer immunotherapy (Venkataraman et al., 2021), gene delivery carriers (Acosta-Ramírez et al., 2008) and viral nanoparticles in imaging (Leong et al., 2010). Recently, the efficacy and safety of recombinant plant-based adjuvanted Covid-19 vaccine has also been reported (Hager et al., 2022). In 2020, a study of the use of plant viruses for the clinical therapy of human autoimmune diseases revealed the design and synthesis of plant virus recombinant nanoparticles that could inhibit autoimmune diabetes and improve rheumatoid arthritis. This is precisely the basis of a peptide-related mechanism in which the virus nanoparticles perhaps by stabilizing the peptide and concentrating multiple peptides in a small area act as both a peptide scaffold and an adjuvant (Zampieri et al., 2020). Recent studies have shown that adjuvants based on plant viruses can be a useful tool in further vaccine development (Lebel et al., 2015). In an experiment in mice, CaMV was a potential adjuvant, and OVA (Ovalbumin) was a model antigen. Although OVA is a relatively weak immunogen but demonstrated that CaMV increased the anti-OVA IgG titers compared to using the OVA alone. It was suggested that the DNA-genome of CaMV or its capsid structure plays a role both in the stimulation of the target antigen and in the stimulation of self-immunity (Evtushenko et al., 2020).

The use of CaMV in medicine was reported in 2020 by Turri et al. They showed that CaMV has sequence similarity with and behaves like the human ribonuclease H1 (RNase H1) in reducing DNA/RNA hybrids (Turri et al., 2020). These data could pave the way for the use of plant virus proteins in the treatment of human diseases and be used in gene therapy in the future. There are some speculations regarding how viral proteins could be useful in human medicine.

9. Conclusions

CaMV was the first plant virus to be discovered with a DNA genome that uses the reverse transcriptase enzyme to replicate its genome. This virus is the source of the 35S promoter, which is dominantly utilized in cassettes for stable and transient expression of heterologous genes in plants and is therefore, a key element in the production of transgenic plants. Despite extensive studies on CaMV, many unknowns remain, including the role of ORFVII in viral infection as well as the details underlying the exciting splicing process. In this review, we shed light on the different aspects and characteristics of this virus. This information can help to better understand this virus which can lead to new strategies for controlling infection in the field as well as new applications of this virus in different areas of science such as enhancing vaccine efficacy. We believe more applications of this virus in medicine will be discovered in the future.

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Not applicable.

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Declaration of competing interest

The authors declare that they have no competing interests.

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