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Plant protease inhibitors and their antiviral activities - Potent therapeutics for SARS CoV-2

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Abstract

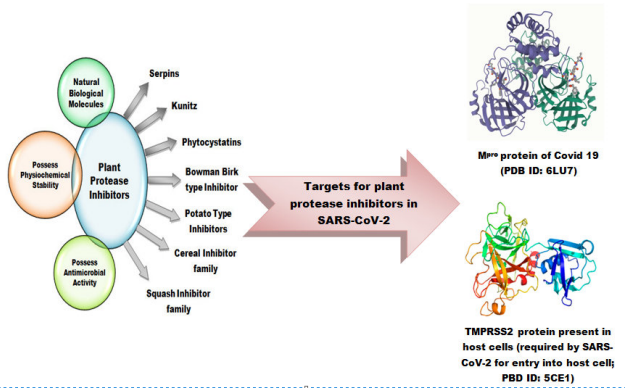
Protease inhibitors are highly active diverse family of poly(peptides) that are generally present in high concentrations in the storage tissues of the plants such as seeds and tubers. They play important roles in the regulation of proteases and the defence mechanism of plants against pathogens and display antimicrobial, antitumor and antiviral properties. Protease inhibitors have proved to be pharmacologically efficient tool in curing infections and systemic diseases via control of proteolysis. Recently, the outbreak of coronavirus (COVID-19) from Wuhan city of China has caused a global pandemic which has put the entire world on a standstill. Although the entire world has diverted all their efforts in finding an appropriate preventive and cure strategy, yet till date no success has been obtained. Since various viral diseases have been successfully cured by inhibition of viral proteases which are necessary for proteolytic processing of polyproteins, the inhibition of the proteases present on the surface of SARS-CoV-2 using protease inhibitors could prove to be fruitful in the treatment of this disease. This review gives a detail about several natural protease inhibitors present in plants and their antiviral potential so as to be used as an effective therapeutic for the ongoing COVID-19 pandemic.

Keywords: Plant protease inhibitors; COVID-19; serpins; antiviral; therapeutics

Key Highlights

- •Plants are one of the most important and natural source of protease inhibitors (PIs).
- •In plants, PIs are known to act in the defence mechanism against pathogens.
- •Plant PIs have been known to possess antiviral activities against several pathogenic viruses such as HIV, Hepatitis C virus and human cytomegalovirus (HCMV).
- •Plant PIs can inhibit the main protease (Mpro or 3CL) of SARS-CoV-2 essential for processing of the polyproteins of the virus into functional proteins.
- •Plant PIs also act as inhibitory molecules against TMPRSS2, a transmembrane protein present on the host cell, required by the virus to enter into the host cell.

Graphical Abstract



Introduction

Proteases (peptidases or proteinases) are one of the most important hydrolytic enzymes with discernable roles in several physiological and biochemical processes. Even though these enzymes are highly essential for maintenance and survival of their host as they are involved in signal transduction, protein-protein interaction, inflammatory response, protein catabolism, blood coagulation and digestion, their regulation is very crucial as they as they can be potentially harmful (Clemente et al., 2019; Fan et al., 2020).

Protease inhibitors (PIs) are highly active compounds which are involved in important physiological reactions related to metabolism, cell physiology and regulation of proteolytic action. In a number of biological pursuits like blood clotting, apoptosis, hormone processing and inflammation, the PIs are now treated as very important signaling molecules (Srikanth and Chen, 2016). They are widely distributed in plants and animals. In plants, PIs are present as small proteins in the storage tissues such as seeds and tubers in high concentrations and in other tissues they exist

in low concentrations. Plant PIs act as storage proteins in the form of nitrogen sources, also are involved in modulation of enzymatic processes, regulation of apoptosis and defence mechanism against animals, insects and microorganisms (Volpicella et al., 2011). Plant PIs possess a notable resistance to heat treatment and a high stability against alterations in ionic strength, pH, proteolysis as well as denaturing agents due of the high content of cysteine residues in disulfide bridges (Cotabarren et al., 2019). Several recent investigations report novel biologic activities for plant PIs such as antimicrobial activities, anticoagulant as well as antioxidant action plus inhibition of tumor-cell growth; thus marking their significance in medicine as effective tools for inactivating proteases involved in several human diseases like arthritis, pancreatitis, thrombosis, emphysema, hypertension, cardiovascular morbidities, neurodegenerative diseases (such as Alzheimer's disease) and muscular dystrophy. They have been employed in several fields of biotechnology and agriculture and the spread of several pathogens that cause life threatening diseases like cancer, AIDS, hepatitis, malaria and various others have proved to be prevented by using plant PIs in drug design (Cotabarren et al., 2019). In order to be used in humans, the PIs should be capable of inhibiting each of the major intestinal proteases, such as pancreatic trypsin, α-chymotrypsin, as well as elastase and must be potentially nontoxic. PIs are being commercially used for deterrence of protease-induced perianal dermatitis and several non toxic PIs have been isolated and purified from barley seeds, cabbage leaves, and Streptomyces (Kim et al., 2009).

PIs are found in plants belonging to a variety of systematic groups especially the plants belonging to the Solanaceae family harbour several high levels of PIs. (Kim et al., 2009). In plants PIs were first discovered as chymotrypsin and trypsin inhibitors in tomatoes infected with Phytophthora infestans and were correlated to plant resistance to pathogens (Wolosluk et al., 1991). Later serine PIs of 20-24 kDa were found in potato tubers in response to infection with P. infestans and mechanical wounding (Valueva et al., 1998, 2003).

Classification of Plant Protease Inhibitors

This On the basis of primary and tertiary structure, including the number and position of disulphide bonds and active sites, PIs can be classified in four groups according to the class of proteases they inhibit: serine protease, cysteine protease, metallocarboxy-protease or aspartic protease (Laskowski and Kato, 1980). Based on structural and biochemical properties, plant PIs have also been classified as serpins and Bowman-Birk serine (BBIs), cysteine, potato type I and type II PIs, cereal trypsin/α-amylase, mustard trypsin, squash inhibitors, metallocarboxypeptidase and soybean trypsin (Kunitz) inhibitors (Table 1). On the basis of their amino acid similarities and the structures obtained the 48 identified plant PIs are grouped into 26 related superfamilies' (or clans). According to the MEROPS database, the inhibitors have 82 family members (Fan et al., 2020). Different classes of plant PIs exhibit different mechanisms through which they interact with the target proteases. Some of the PIs utilize an irreversible inhibition of proteolytic activity (e.g. serpins) while most of them exhibit a canonical-competitive inhibition mode via 'substrate-like' binding to the catalytic domain of the targeted protease (e.g., BBIs and Kunitz inhibitors) or they make use of a non-catalytically competitive inhibition (e.g. cystatins or mustard-type PI) else they may act via a mixed mode, where the primary competitive binding to the active site is supported by a secondary binding event (e.g. metalloprotease inhibitors; Hellinger and Gruber, 2019)

Serine PIs or serpins constitute the major class of plant PIs, which have been classified into more than 20 families. Serpins are mainly found in plants belonging to the Solanaceae, Fabaceae, Euphorbiaceae, Poaceae, and Cucurbitaceae families (Haq et al., 2004; Fan and Wu, 2005) with most of these being isolated from barley grain, wheat grain, rye, wild oats, pumpkin, and A. thaliana (Volpicella et al., 2011). In plants they are responsible for controling protein synthesis and turnover, and physiological functions such as fertilization, growth and development, digestion, cell signaling or migration, immune defense, wound healing and disease propagation. They play crucial role in the pathogenesis and/or host tissue penetration of a number of diseases, such as cardiopulmonary disease and emphysema (Mishra et al., 2020). Serpins display a distinctive mechanism of irreversible inhibition termed as "suicide substrate" mechanism rather than the standard reversible inhibition mechanism used by other PIs. They are metastable proteins with a molecular weight usually higher than 40 kDa (Patson and Gettins, 1996).

A report demonstrated that in serine PIs, the 'reactive sites' are mutating faster than amino acids in the rest of the proteins, implying that their roles in defense against microorganisms (and insects) may exert a strong selection pressure on these proteins to conserve the reactive sites and that this selection may be related to plant defense (Ryan, 1990). Serine PIs also called serpins inhibit both serine and cysteine proteases (Cohen et al., 2019). Although several serpins with inhibitory activity against caspases and papain like cysteine proteases have been reported but they predominantly act against trypsin like serine proteinases (Jamal et al., 2013).

Another important class of PIs is the inhibitors of the cysteine proteases (cystatins or phytocustatins) which range in molecular weight from 10 kDa to 23 kDa. They inhibit cysteine proteases in a non-catalytically competent manner (i.e. although they do not bind to proteses in a strictly substrate-like manner but they still block access to the catalytic site; (Hellinger and Gruber, 2019). Cystatins regulate endogenous and heterologous cysteine proteases in a variety of physiological processes such as abiotic stress tolerance, protection against insects and nematodes via inhibition of digestive enzymes in their gut, regulation of peptidase activity during apoptosis, protection of cytosolic metabolism from intracellular peptidases released by incidental rupturing of protein bodies. They have been isolated and characterized from a number of vegetables and crop plants such as cabbage, apple, papaya, avocado, carrot, cowpea, ambrosia, castano, seeds of wheat, maize, sunflower, soybean, sugarcane, rice etc (Cotabarren et al., 2019).

The Kunitz and BBI have been observed in the leguminous family and they generally range in size from 18-24 and 5-16 kDa, respectively. Both of them function via competitive inhibition of

protease using the standard mechanism of substrate like binding to the catalytic site of the protease. Kuntiz inhibitors are known to function in the regulation of physiological homeostasis and in inhibition of pathogenic proteases while the expression of BBIs in plants is strongly induced by pathogenic invasion (Hellinger and Gruger, 2019). Other than this a few aspartate and metalloprotease inhibitors have been reported which are isolated from potato tubers, sunflower flowers, barley and thistle (Cynara cardunculus). Metalloprotease are highly compact and stable proteins in nature because of the high content of disulphide bonds in them (Cotabarren et al., 2019).

Antiviral Potential of Plant PIs

Oral According to various reports, serine PIs in plants provide protection against various pests and pathogens. In most of the pathogenic organisms like bacteria, fungi, viruses, insects and vertebrates, proteases comprise around 1-5% of the genome among which majority of the functions are performed by serine proteases (Mishra et al., 2020). The NS3 protein of Hepatitis C virus (HCV) is a chymotrypsin like protein which contains a serine protease domain that is responsible for processing of the HCV polyprotein as well as the active site of the Human cytomegalovirus (HCMV) contains a Ser-His-His catalytic triad and therefore is a serine protease which is essential for capsid formation during viral replication (Bianchi and Pessi, 2002; Fischmann and Weber, 2002). Therefore, serpins can be effectively utilized to attenuate such serine proteases thereby providing protection against a wide variety of pathogens. Novel antiviral strategies include targeting either host or viral accessory protein to ultimately block viral replication or inhibit cellular proteins necessary for the virus life cycle. Proteolytic cleavage of the precursor hemagglutinin (HA0) into HA1 and HA2 subunits by host proteases is essential for fusion of HA with the endosomal membrane and thus represents an essential step for viral infection (Mishra et al., 2020). The trypsin PIs from the leaf extract of Capsicum baccatum var. pendulum inoculated with Pepper yellow mosaic virus (PepYMV) significantly reduced the yellow mosaic viral infection (Moulin et al., 2014). The Cucumis metuliferus serine PIs (CmSPI) gene when overexpressed and silenced in Nicotiana benthamiana and Cucumis metuliferus displayed potyvirus resistance and synchronous development of potato ring spot viral symptoms respectively (Mishra et al., 2020). The sunflower trypsin inhibitor (TI) from Helianthus annuus is the smallest known BBI which has been explored as a model peptide for drug design (Craik, 2009; Craik et al., 2013; Elliott et al., 2014). Various plant PIs displaying antiviral activity have been previously reported (Table 2).

The Kunitz trypsin inhibitors isolated from B. variegate and G. max seeds termed BvvTI and KBTI respectively, display significant activity against the HIV-1 reverse transcriptase as well as also possesses anti-tumor activity against the human nasopharyngeal cancer cells, human breast cancer cells and hepatoma cells (Fang et al., 2010a; Fang et al., 2010 b) . Another Kunitz trypsin inhibitor, BSKT1 isolated from G. max cv. dull black seeds also possess anti HIV-1 reverse transcriptase activity (Lin and Ng, 2008). According to the invention of Domagala et al., (1996) the derivatives of coumarin which is found in fruits (bilberry and cloudberry), green tea, chicory, soy, higher plants such as Rutaceae and Umbelliferone as well as the stem bark of Calophyllum dispar (Clusiaceae) are inhibitors of aspartyl proteases, especially the aspartyl proteases of retroviruses such as HIV and hence can be expected to be used as an antiviral agent in the treatment of retroviral infections. They also have been found to be potential therapeutics for treatment of malaria, mycoplasmosis, Q fever and mononucleosis (Domagala et al., 1996; Jain and Joshi, 2012). Ye and Ng in 2002 isolated a novel trypsin chymotrypsin inhibitor from Vicia faba (commonly known as bakla in India) seeds which displayed anti HIV-1 reverse transcriptase activity as well as antifungal activity against Mycosphaerella arachidicola and Physalospora piricola (Ye and Ng, 2002). A novel, fairly stable Kunitz trypsin inhibitor of serpin family was isolated from Allium sativum (garlic) by Shamsi and colleagues which could act as a potential nontoxic therapeutic against a number of viral diseases (Shamsi et al., 2016).

Family	Protease Inhibitor	Plant source	Characteristic	Reference
Serpins	At-serpin 1	Arabidopsis thaliana	Acts against metacaspase in vivo and plays role in plant immunity	Vercammen et al., 2006
	OSZa-d	Oat (Avena sativa L.) grain	OSZa and OSZb are	(Hejgaard and Hauge 2002)
			efficient inhibitors of pancreatic elastase.	
			OSZb is an inhibitor of chymotrypsin whereas OSZc is a fast inhibitor of trypsin.	
			Together they display a broader activity against the digestive serine proteinases than the other serpins from rye, barley or wheat.	
	CmPS-1	Cucurbita maxima	Possess anti-elastase activity.	Yoo et al., 2000; Jamal et al., 2013
	(Cucurbita maxima phloem		May impart resistance against bacteria, insects and phytoplasma.	
	serpin-1)			

	WSZI	Triticum aestivum	Inhibits chymotrypsin and Cathepsin G	Hellinger and Gruber, 2019
	HorvuZx (BSZx)	Hordeum vulgare	Inhibits trypsin, chymotrypsin, Factor Xa, thrombin, Factor VIIa, plasma kallikarein and leukocyte elastase.	Hellinger and Gruber, 2019
Kunitz	Kunitz trypsin inhibitor	Artocarpus	Inhibits elastase, trypsin and chymotrypsin.	Bhat
		Integrifolia (Jackfruit)		and Pattabiraman, 1989
			However it displays a very poor action on Streptomyces	
			caespitosus and Aspergillus oryzae proteases	
	Tamarind Kunitz inhibitor	Tamarindus indica	Inhibits trypsin and Factor Xa	Hellinger and Gruber, 2019
		Glycine max	Inhibits plasmin, human Fcator XIIa, plasmin kallikrein, trypsin, chymoreypsin	Hellinger and Gruber, 2019
	SKTI-3			
	PdKI-2	Pithecelobium	Inhibits trypsin as well as papain, a cystein protease. Active against digestive enzyme of larvae from	Oliveira et al., 2007
		dumosum seed	diverse orders and hence can be used as a potent insect	
			antifeedant	
	Kunitz inhibitor CPTI	Cicer arietinum	Show differential inhibitory activity against	Harsulkar et al., 1999
			trypsin, chymotrypsin, H. armigera gut proteases	
			and bacterial protease(s)	
	PFTI	Plathymenia	Inhibits bovine trypsin and bovine chymotrypsin.	Silveira
		foliolosa		et al., 2008
			Exhibits	
			significant inhibitory activity against on larval midgut	
			proteases of A. kuehniella and D. saccharalis	
	PCP1 6.6 and PCPI 8.3	Solanum tuberosum	Possesses inhibitory action against cathepsin B, H and L.	Hellinger and Gruber, 2019
			Also inhibits dipeptidyl peptidase I and Clostripain.	
Bowman birk type inhibitors (BBI)	Soyabean BBI (Isotype 2-II; 8 kDa)	G. max	Inhibits trypsin, chymotrypsin, mast cell chymase, cathepsin G, matriptase, leukocyte elastase, duadenase	Hellinger and Gruber, 2019
	BTCI	Vigna unguiculata (black eyed pea)	Trypsin/ chymotrypsin inhibitor.	Franco et al.,
				2003
			Moderately active against the digestive chymotrypsin of adult insects	
	AsPIs	Acacia Senegal seeds	Highly active against serine proteases.	Babu and Subrahmanyam 2010

			Possess remarkable inhibitory activity towards	
			total gut proteolytic enzymes followed by trypsin and	
			chymotrypsin and retards the growth and development	
			of H. armigera	
	BI-I (seven isotypes I-VII)	Ananas comosus	Possesses inhibitory activity against trypsin, papain, bormelain, cathepsin L and chymotrypsin	Hellinger and Gruber, 2019
Phytocystatin	Oryzacystatin I and Oryzacystatin II	Oryza sativa	Inhibits cathepsin B, Hand L and Legumain	Nagata, 2000; Valadares, 2010
	SQAPI	Cucurbita maxima	Inhibits pepsin proteases	Headey et al., 2010
	Corn cystatin-I	Zea mays	Inhibits Cathepsin H and L	Abe et al., 1994
Potato inhibitor family	CI-1	H. vulgare	Inhibitor of trypsin, chymotrypsin, subtilin and neutrophil elastase	Polya, 2003
	PSI- 1.1	Capsicum annuum	Trypsin and chymotrypsin inhibitor	Hellinger and Gruber, 2019
	TI-II	Solanum lycopersicum	Inhibitor of trypsin, chymotrypsin and subtilisin	Barrette et al., 2003
	PI-2	S. tuberosum	Trypsin and chymotrypsin inhibitor	Hellinger and Gruber, 2019
Cereal inhibitor and squash inhibitor family	Corn Hageman factor inhibitor	Z. mays	Inhibitors of serine proteases and α amylase	Mahoney et al., 1984
	RATI	Eleusine coracana (ragi)	Inhibitors of serine proteases and α amylase	Shivraj and Pattabiraman
				1981
	BTI-CMe	H. vulgare (barley)	Trypsin inhibitor;	Jamal et al., 2013
			Exhibits in vitro inhibition of trypsin-like proteases of the gut extracts of	
			the fall armyworm, Spodoptera frugiperda (Lepidoptera:	
			Noctuidae).	

Table 1: Plant protease inhibitors of different families.

Protease inhibitor	Plant source	Antiviral activity	Reference
Capsicum baccatum trypsin protease inhibitor	Capsicum baccatum var. Pendulum	Reduction in the yellow mosaic virus infection of Capsicum baccatum	Moulin et al., 2014
BSKTI (Kunitz trypsin protease inhibitor)	G. max cv.Dull Black seeds	Anti HIV-1 reverse transcriptase activity	Lin and Ng, 2008
BvvTI (Kunitz trypsin protease inhibitor)	B. variegate seeds	Anti HIV-1 reverse transcriptase activity as well as antitumor activity against human nasopharyngeal cells	Fang et al., 2010 (a)
KBTI (Kunitz trypsin protease inhibitor)	G. max seeds	Anti HIV-1 reverse transcriptase activity as well as antitumor activity against human nasopharyngeal cells, breast cancer cells and hepatoma cells	Fang et al., 2010 (b)
Coumarin derivatives (aspartyl protease inhibitor)	Fruits such as (bilberry, cloudberry), green tea, chicory, soy, higher plants such as Rutaceae and Umbelliferone, stem bark of Calophyllum dispar (Clusiaceae)	Act against aspartyl proteases of reteroviruses such as HIV. Potential therapeutic for malaria, Q fever, mycoplasmosis, nucleoplasmosi	Domagala et al., 1996; Jain and Joshi, 2012
CmSPI (Cucumis metuliferus serine protease inhibitor)	Cucumis metuliferus	Overexpression of the gene provides resistance to potyvirus in Nicotiana benthamiana;	Mishra et al., 2020

		Silencing of the CmSPI gene in Cucumis metuliferus results in development of potato ring spot viral symptoms	
Novel trypsin-chymotrypsin inhibtor	Vicia faba (bakla) seeds	Anti HIV-1 reverse transcriptase activity as well as antifungal activity against Mycosphaerella arachidicola and Physalospora piricola.	Ye and Ng, 2002
Chymotrypsin inhibitor	Acacia confusa seeds	Anti HIV-1 reverse transcriptase activity	Lam and Ng, 2010

Table 2: Plant protease inhibitors with antiviral activity.

The COVID-19 pandemic

In December 2019, the city of Wuhan, the capital of Hubei province in China, reported the outbreak of a pulmonary disease caused by a novel strain of coronavirus and since then the virus spread globally (Wang et al., 2020). The spread of 2019-nCoV, now officially known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still progressing despite of the severe containment measures being taken (Devaux et al., 2020). The virus consists of an RNA genome which is 82% identical to the SARS coronavirus (SARS-CoV) and both viruses belong to clade b of the genus Betacoronavirus and hence it has been named as SARS-CoV-2 and the disease caused by SARS-CoV-2 is called COVID-19 (Zhou et al., 2020; Wu et al., 2020). Although less is known about the origin of the virus but on the basis of the sequence of the viral genome and the evolutionary analysis, bats have been suspected as their natural hosts and it has been supposed that in humans SARS-CoV-2 might have been transmitted from bats via some unknown intermediate host (Guo et al., 2020). Within humans, the disease is transmitted by inhalation or contact with infected droplets released by an infected person and the incubation period ranges from 2 to 14 d. The symptoms usually consist of fever, cough, sore throat, breathlessness, fatigue, malaise etc. Although the disease is mild in most people; but in some (usually the elderly and those with comorbidities), it may advance to pneumonia, acute respiratory distress syndrome (ARDS) and multi organ dysfunction. Many people are asymptomatic. The case fatality rate is estimated to range from 2 to 3%. It was listed as a potential global health emergency by WHO due to high mortality, high basic reproduction number and lack of clinically approved drugs and vaccines for COVID-19. India too has reported more than 75,000 of coronavirus cases along with 2,440 deaths all over the country till 14th of May, 2020.

The replication cycle of the SARS-CoV-2 virus has been illustrated (Figure 1) to focus on therapeutics for efficient neutralization of virus or inhibition of some intervening virus adsorption or replication step(s). For entry into the host cell the viral S protein binds to the host cellular receptor angiotensin converting enzyme 2 (ACE2). The binding requires the host cell surface associated trans-membrane protease serine 2 (TMPRSS) for cleavage of the trimeric S protein (Guo et al., 2020). After binding of the S protein and ACE2 there occurs a conformational change in the S protein which facilitates the fusion of the viral envelope with the host cell's membrane through endosomal pathway. After entry into the host cell, the virus un-coats itself and releases its RNA, which is replicated and translates into viral replicase polyproteins. The polyproteins are then processed into functional proteins by the main protease of SARS-CoV-2, Mpro also called as 3CL protease. The viral proteins and the genomic RNA subsequently assemble into virions in the endoplasmic reticulum and Golgi and subsequently released out of the cell (Shereen et al., 2020).

Possible inhibitory effects of plant PIs on SARS CoV-2

Most of the nation's world-wide have been diverting their best efforts for the implementation of appropriate preventive and control strategies. Neither vaccines nor direct-acting antiviral drugs are available for the treatment of human and animal coronavirus infections (Dhamam et al., 2020). The inhibition of viral proteases necessary for proteolytic processing of polyproteins has been a successful strategy in the pharmacological treatment of HIV and HCV respectively, proving the potential of PIs for the treatment of viral infections. Similarly, the main protease of SARS-CoV-2, Mpro or 3CL is thought to be essential for viral replication and therefore, is regarded as promising target for plant PIs and antiviral pharmacotherapy (Figure 1; Fischer et al., 2020). Inhibiting the activity of this enzyme would block viral replication. Since no human proteases with similar cleavage specificity are known, inhibitors are unlikely to be toxic. Approved PIs including disulfiram, lopinavir and ritonavir have been reported to be active against SARS and MERS. Disulfiram, an approved drug to treat alcohol dependence, has been reported to inhibit the papain-like protease of MERS and SARS in cell cultures, but clinical evidence is lacking. According to the observation of Baden and colleagues lopinavir–ritonavir combination does not seem to be highly effective in patients with COVID-19 (Baden et al., 2020) and adverse gastrointestinal effects were seen in approximately 13% of the patients (Cao et al., 2020). Since better effective therapies for COVID-19 is the demand of the moment and plant PIs may prove to be potential therapeutic agents by inhibiting this main protease of the virus.

As described before, TMPRSSs plays a major role in 2019-nCoV infection as it is the main protease and allow the fusion of the virus particle with human cells. Hence, because TMPRSSs is required by the COVID-19 virus to enter into the human cells, the inhibition of this protease by nontoxic plant serine PIs may prove to be potential treatment options in 2019-nCoV infection (Figure 1; Meng et al., 2020).

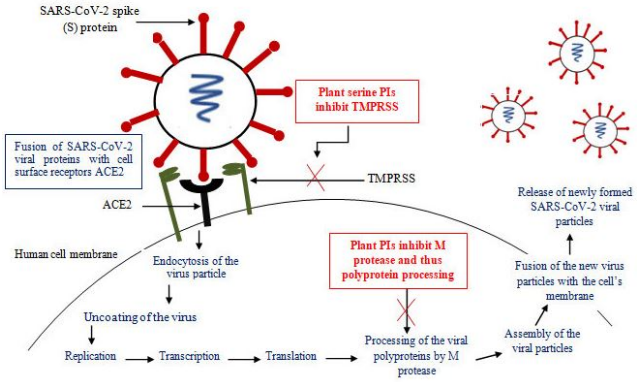


Figure 1: Schematic representation of replication cycle of the SARS-CoV-2 and the possible inhibitory effects of plant PIs on its replication in human cells.

Conclusion

In conclusion, the review suggests that PIs are widely distributed in several plants where they play an important role in providing defense against pathogenic diseases. The plant PIs have been classified into different families on the basis of their structural similarity and protease inhibited. Because of their non toxic nature and fairly good stability, they have been employed in several biotechnological and pharmaceutical applications. The PIs are effective tools in inhibiting proteases associated with a number of diseases and are also highly efficient in inhibiting viral proteases, they can be employed as a potential therapeutic in the treatment of the ongoing COVID-19 pandemic which has been declared by the WHO as a global emergency. Further docking and in vivo studies are required for finding the possible use of these plant PIs in the treatment of COVID-19.

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