Physiological and Molecular Plant Pathology 113 (2021) 101597

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp

Plant-virus infection inhibitors: The great potential of Caryophyllales species

Lígia Maria Lembo Duarte [a,*], Maria Amélia Vaz Alexandre [a], Alexandre Levi Rodrigues Chaves [a], Déborah Yara Alves Cursino dos Santos [b], Ana Claudia Oliveira de Souza [c], Luis Carlos Bernacci [c]

[a] Laboratório de Fitovirologia e Fisiopatologia, Instituto Biológico, São Paulo, Brazil
[b] Laboratório de Fitoquímica, Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil
[c] Centro de Recursos Genéticos Vegetais, Instituto Agronômico de Campinas, São Paulo, Brazil

ARTICLE INFO

Keywords:
Caryophyllales
Antiviral protein
Defense inducer
Inhibitor mode of action
Signaling plant defense

ABSTRACT

Caryophyllales is one of the largest orders in eudicots and comprises 39 families with approximately 12,500 species. Although extracts from species of this order have been considered potential inhibitors of plant virus infection since the early 20th century, few species have actually been investigated. In this review, we present an exhaustive analysis of published papers that investigate this inhibitory effect, organized into one table with more than 100 species. In addition, the main hypotheses regarding the mode of action by which the compounds inhibit viral infection are discussed, providing several examples. The proteinaceous nature of antiviral proteins (AVP) produced by Caryophyllales, as well as the role of ribosome-inactivating proteins (RIPs) and pathogenesis-related proteins (PRs) as plant-defense inducers have received considerable attention. It is worth mentioning that data concerning the role of AVPs produced by species of Caryophyllales as signaling plant defense against viruses are scarce. Finally, this review proposes a model for the main mode of action hypotheses of viral infection inhibitors, and highlights the importance of surveying Caryophyllales species as an excellent strategy for controlling a broad spectrum of plant viruses from different taxonomic groups.

1. Introduction

1.1. A brief history of Caryophyllales as plant virus inhibitors

Several taxonomic groups have been studied for their inhibitory action against viral infection [1]. However, Caryophyllales stand out for the promising findings obtained.

Caryophyllales Juss. ex Bercht. And J. Presl (1820), one of the largest orders in eudicots [2], with approximately 12,500 species [3], contains economically important plants, encompassing food (conventional and non-conventional) and ornamental species [4]. Based on recent molecular phylogeny studies, this order comprises 39 families and 749 genera [3,5]. Many of these plants are specially adapted to extreme habitats such as xeric conditions, high salinity, and nitrogen-poor soils, including a number of succulent, halophytic, gypsophilous and carnivorous plants [3].

In addition to being one of the major lineages of angiosperms, Caryophyllales are of great ecological and evolutionary interest because they show multiple origins of specialized morphological, anatomical, and biochemical traits. Members of this group are chemically characterized by betalains nitrogen-based pigments and, instead of anthocyanins [6].

Early studies suggesting the presence of viral infection inhibitors in extracts of vascular plants date back to the early 20th century. Allard [7] observed that, although healthy pokeweed plants (*Phytolacca decandra* L. = *P. americana* L., Phytolaccaceae) were successfully infected after artificial infection with *Phytolacca* inoculum, all attempts to transmit the virus from *Phytolacca* to tobacco plants failed. Similarly, in 1925, Doolitle and Walker [8] found that mechanical transmission of cucumber mosaic virus (CMV, genus *Cucumovirus*) present in *P. decandra* leaves to cucumber plants was also impossible. Mechanical transmission of virus from infected spinach or sugar beet (Amaranthaceae) plants to other hosts also failed [9].

In 1925, Duggar and Armstrong [10] attempted to explain transmission failures with an experiment. The authors showed that *Phytolacca* leaf extract inhibited tobacco mosaic virus (TMV, genus *Tobamovirus*).

* Corresponding author. Laboratório de Fitovirologia e Fisiopatologia, Instituto Biológico, Av. Conselheiro Rodrigues Alves, 1252, 04014-0900, São Paulo, Brazil.
E-mail address: ligia.duarte@sp.gov.br (L.M. Lembo Duarte).

https://doi.org/10.1016/j.pmpp.2020.101597
Received 13 March 2020; Received in revised form 16 June 2020; Accepted 29 December 2020
Available online 31 December 2020
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Later, Grant [11] found that TMV infectivity became null when leaf extract of *Phytolacca rigida* Small. (now *P. americana*) was added to the inoculum. Johnson [12,13] and Fulton [14] initiated chemical studies of these plant extracts, but the inhibitor compound extracted from *P. esculenta* Van Houtte was only isolated and identified in 1948 by Kassanis and Kleczkowski [15]. This compound, classified as a glycoprotein, has been shown to act on several plant viruses in addition to TMV, such as CMV, tobacco necrosis virus (TNV, family *Tombusviridae*) and tomato bushy stunt virus (TBSV, genus *Tombusvirus*).

Continuing with plant virus inhibition investigations, Weintraub and Gilpatrick [16] also failed to transmit tobacco ringspot virus (TRSV, genus *Nepovirus*) from infected *Dianthus barbatus* L. (Caryophyllaceae) to healthy tobacco plants. The inhibitory activity of leaf extracts from *Chenopodium album* L., *C. amaranticolor* (now *C. giganteum* D. Don) and *Spinacia oleracea* L. (Amaranthaceae) was also demonstrated against induced local infection of potato virus X (PVX, genus *Potexvirus*) in *Gomphrena globosa* L. [17]. The total inhibition of local infection with TMV in *Nicotiana glutinosa* L. by extracts of *Mesembryanthemum* sp. (Aizoaceae) and *Opuntia* spp. (Cactaceae) has also been reported [18]. The same effect was observed with the leaf extract of *Basella alba* L. (Basellaceae) [19]. Additionally, studies on the action of leaf extracts from *Bougainvillea spectabilis* Willd (Nyctaginaceae), *Portulaca grandiflora* Hook (Portulacaceae) and *Talinum paniculatum* (Jack.) Gaertn. (Talinaceae) on local TMV infection in *N. glutinosa* were published in the 1980s [20,21].

In addition to viral-infection inhibition in plant-pathogen systems with local responses [21–25], several other studies demonstrated that these inhibitors found in species of Caryophyllales can also inhibit systemic infection in their hosts [20,26–30], as well as mixed systemic infection induced by PVX and potato virus Y (PVY, genus *Potyvirus*) in *N. tabacum* L. [30].

In spite of all this knowledge concerning the potential viral infection inhibition of extracts from Caryophyllales species [31], of the 39 families belonging to this order, only twelve (Aizoaceae, Amaranthaceae, Basellaceae, Cactaceae, Caryophyllaceae, Didiereaceae, Nyctaginaceae, Petiveriaceae, Phytolaccaceae, Polygonaceae, Portulacaceae and Talinaceae) have been studied for their role in inhibiting viral infection. (Fig. 1).

These antiviral proteins (AVPs) present in the extracts of several Caryophyllales species are proteins or glycoproteins [15,32] with basic low-molecular weight (20–32 kDa), high isoelectric points, stable and resistant to denaturing agents and proteases [33], which act against a range of plant viruses [34]. Table 1 summarizes an exhaustive search of all Caryophyllales species with viral infection inhibiting activity, described since the early 20th century.

Several papers have been published since the discovery of virus infection inhibitors in 1918 (Fig. 2). The exponential increase after the 1960s, however, was related to the purification of PAP (Phytolacca antiviral protein), peaking in the 1970s/1980s with several studies related to AVP properties, purification and activity [22,24,27,29,50,55,57,62,105]. Despite a sharp decline in the 1990s, the discovery of AVP inhibitory activity at ribosome inactivation led to the isolation and characterization of numerous ribosome-inactivating proteins (RIPs) [34,45,89], and in the 2000s and 2010s, researchers endeavored in cloning of RIPs, as well as to understand the mode of action of AVP, focusing primarily on the *N*-glycosidase activity of RIPs [37,38,46,52,65,66,84]. Plant extracts from different species were tested in several pathosystems; however, the role of these antiviral proteins (AVPs) in the induction of resistance/protective mechanisms has yet to be elucidated [108].

2. Mode of action of plant virus infection inhibitors: main hypotheses

Three main hypotheses have been put forward to explain the mode of action of inhibitors: (**i**) direct action inactivating virus particles or forming a loose complex with the virus, (**ii**) action on the virus infection process, and (**iii**) changes in host cell metabolism altering host susceptibility [109,110].

Corroborating the first hypothesis, Grasso [102] suggested that the *in vitro* antiviral activity of the inhibitor could be a consequence of ionic bonds between the virus and the inhibitor, and that this interaction could occur in the early stages of virus replication. *In vitro* studies of mixtures of TMV with antiviral proteins of *P. americana* (PAP and PAPII) suggested that the precipitation of virus particles involves a weak ionic bond [111]. Using transmission electron microscopy, Duarte et al. [88] observed an electron-dense granular mass covering the viral particles after treatment of purified PVX preparation with *Mirabilis jalapa* L. leaf extract. Awasthi et al. [112] also observed aggregation of barley stripe mosaic virus, barley yellow mosaic virus, PVX and TMV particles with the *Boerhaavia diffusa* L glycoprotein, coating virus particles. Fractured of virus particles were also observed.

Distinctly, the inhibition of tomato spotted wilt virus (TSWV, genus *Orthotospovirus*) infection in tomato only when plants were sprayed with leaf extract of *M. jalapa* before viral inoculation, reinforces the hypothesis of action at the very initial stages of viral replication [29], possibly blocking virus receptors located on the surface of the leaf [32,54,69,113]. Virus and inhibitor may compete for the receptors [69]. These putative virus receptors may have an affinity for amino groups, and the amino groups of the AVPs allow them to substitute the virus particle [64]. Furthermore, a virus charge may change on virus due to the presence of amino lysine groups in the infection-inhibiting plant extract. These amino lysine groups have been identified in *Dianthus caryophyllus* L., *Phytolacca americana*, and several Chenopodiaceae (now

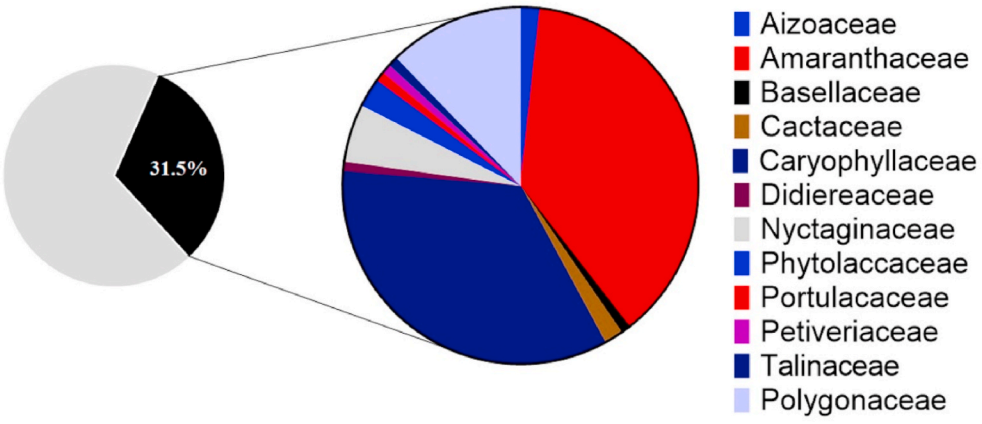

Fig. 1. Caryophyllales families with the highest number of species (31.5%) tested for their plant virus infection inhibitory activity.

Table 1
Caryophyllales species with updated name (in parentheses) and their respective plant virus infection inhibitory activity.

Families	Inhibitory species	Plant part	Viruses	References
Aizoaceae	*Mesembryanthemum* sp. (Referred to as *M. caprohetum*)	L	TMV, PVY	[18]
	Tetragonia tetragonioides (Pall.) Kuntze (Referred to as *T. expansa* Murray)	L	TMV, TRSV	[35]
Amaranthaceae	*Aerva sanguinolenta* (L.) Blume	L	TMV	[27]
	Alternanthera	L	TMV	[21]
	bettzickiana (Regel) G.	L	TSWV	[29]
	Nicholas (Referred to	L	PVX, PVY	[30]
	as *A. amoena* Back. &			
	Sloot.)			
	A. brasiliana (L.)	L	TMV	[21]
	Kuntze	L	BGMV,	[28]
			TMV	
		L	TSWV	[29]
		L	PVX, PVY	[30]
	A. ficoidea (L.) Sm.	L	TMV	[23]
		L	BGMV,	[28]
			TMV	
		L	TSWV	[29]
		L	PVX, PVY	[30]
	A. ficoidea (Referred to as *Telanthera ficoidea* (L.) Moq.)	Sh	TMV	[36]
	A. philoxeroides (Mart.) Griseb.	L	TMV	[21]
	Amaranthus albus L.	L	TNV	[22]
	A. aureus F.Dietr.	L	TNV	[22]
	A. blitum L. (Referred to as *A. lividus* L.)	L	TMV	[37]
	A. caudatus L.	L	TNV	[22]
		U	TMV	[24]
	A. deflexus L.	L	TMV	[23]
		L	PVX, PVY	[30]
	A. hybridus L.	L	TMV	[23]
	A. hypochondriacus L.	L	TNV	[22]
	A. retroflexus	L	TNV	[22]
	A. tricolor L.	L	SRV	[38]
		L	SRV	[39]
	A. tricolor L. (Referred to as *A. mangostanus* L.)	U	TMV	[24]
	Atriplex halimus L.	L	TNV	[22]
	A. portulacoides L. (Referred to as *Halimione portulacoides* (L.) Aellen)	L	TNV	[22]
	A. prostrata Boucher ex DC. (Referred to as *A. calotheca* (Rafn) Fr.)	L	TNV	[22]
	A. sagittata Borkh. (Referred to as *A. nitens* Schkuhr)	L	TNV	[22]
	Beta nana Boiss. & Heldr.	L	TNV	[22]
	B. trigyna Waldst. & Kit.	L	TNV	[22]
	B. vulgaris L.	L	TMV, CbMA	[9]
		L	SHMV	[40]
		Se	TNV	[41]
		L	TNV	[22]
		L	TNV, TRSV	[42]
		L	CMV, TNV	[43]
	Celosia argentea L. (Referred to as *C. cristata* L. and *C. plumosa* (Voss) Burv.)	U	TMV	[24]
		L	TNV	[22]
		L	PVX, SRV, TMV	[44]
		L	TMV	[45]
		L	TMV	[37]
		L	SRV	[39]
		L	TMV	[46]
	Chenopodium album L.	L	PVX	[17]
		L	SHMV	[40]
		L	TMV	[47]
		L	TMV	[48]
		L	TuMV, TMV	[49]
		L	PVX, TNV, TMV	[22]
		L	TMV	[50]
		L	SRV, TMV	[51]
		L	ULCV	[52]
	C. bonus-henricus L.	L	TNV	[22]
	C. capitatum (L.) Asch.	L	TNV	[22]
	C. ficifolium Sm.	L	TNV	[22]
	C. giganteum D.Don (Referred to as *C. amaranticolor* (H.J. Coste & A.Reyn.) H.J. Coste & A.Reyn.)	L	PVX	[17]
		L	CMV	[53]
		L	TMV	[47]
		L	TNV	[41]
		L	TNV	[22]
		L	TNV	[54]
		L	TMV	[55]
		L	TMV	[56]
		L	TMV	[57]
		L	TMV	[58]
		Se	TMV	[59]
		L	BGMV, TMV	[28]
		L	TSWV	[29]
		L	PVX, PVY	[30]
	C. glaucum L.	L	TNV	[22]
	C. hybridum L.	L	TNV	[22]
	C. murale L.	L	SRV, TMV	[60]
	C. opulifolium L.	L	TNV	[22]
	C. quinoa Willd.	L	CMV	[53]
		L, R	ACLSV	[61]
		L	TMV	[58]
		L	TSWV	[29]
		L	PVY, PVX	[30]
	C. rubrum L.	L	TNV	[22]
	C. urbicum L.	L	TNV	[22]
	C. vulvaria L.	L	TNV	[22]
	Dysphania ambrosioides (L.) Mosyakin & Clemants (Referred to as *Chenopodium ambrosioides* L.)	L	TMV	[23]
		L	SRV, TMV	[62]
		L	BGMV TMV	[28]
		L	TSWV	[29]
		L	PVX, PVY	[30]
	Dysphania botrys (L.) Mosyakin & Clemants (Referred to as *Chenopodium botrys* L.)	L	TNV	[22]
	Gomphrena globosa L.	L	PVX	[17]
		L	TMV	[24]
	Hablitzia tamnoides M. Bieb.	L	TNV	[22]
	Iresine herbstii Hook.	L	TMV	[21]
			BGMV, TMV	[28]
			TSWV	[29]
			PVX, PVY	[30]
	Patellifolia procumbens (C.Sm.) A.J.Scott, Ford-Lloyd & J.T. Williams (Referred to as *Beta patellaris* Moq.)	L	TNV	[22]
	Salsola kali L.	L	TNV	[22]
	Spinacia oleracea L.	L	CMV	[9]
		L	PRSV-l, PRSV-n,	

(*continued on next page*)

Table 1 (*continued*)

Families	Inhibitory species	Plant part	Viruses	References
			TRSV, TMV	
		L	PVX	[17]
		L	SHMV	[40]
		L	TMV	[58]
		L	TMV	[63]
		L	TMV	[64]
Basellaceae	*Basella alba* L.	L	TMV	[19]
		L	TMV	[20]
		L	ULCV	[52]
	B. alba (Referred to as *B. rubra* L)	L	TNV	[22]
Cactaceae	*Opuntia ficus-indica* (L.) Mill.	Cla	CMV, TMV, ZYMV	[65]
		Cla	CMV	[66]
	O. robusta J.C. Wendl.	L	TMV	[18]
	Opuntia sp.	L	TMV	[58]
Caryophyllaceae	*Arenaria balearica* L.	Sh	TNV	[67]
	Agrostemma githago L.	Se	TMV	[68]
	Cerastium biebersteinii DC.	Sh	TNV	[67]
	C. tomentosum L.	Sh	TNV	[67]
	Dianthus arenarius L.	Sh	TNV	[67]
	D. barbatus L.	L	TRSV	[16]
		Se	TMV	[59]
	D. caesius Sm.	L	TRSV	[16]
	D. campestris M.Bieb.		TNV	[67]
	D. caryophyllus L.	L	TRSV	[16]
		L	TMV	[69]
		L	TMV	[70]
		L	TMV	[18]
		L	SHMV	[40]
		Se	TMV	[19]
		Se	TMV	[58]
		L	TMV	[71]
		L	BYMV	[72]
	D. chinensis L. (Referred to as *C. sinensis* Link)	L	TRSV	[16]
		Se	TMV	[58]
		L	TMV	[37]
	D. gratianopolitanus Vill.	Sh	TNV	[67]
	D. henteri Heuff. ex Griseb. & Schenk	L	TRSV	[16]
	D. hyssopifolius L. (Referred to as *D. monspessulanus* L.)	Sh	TNV	[67]
	D. knappii (Pant.) Asch. & Kanitz ex Borbás	Sh	TNV	[67]
	D. petraeus Waldst. & Kit.	Sh	TNV	[67]
	D. plumarius L.	Sh	TNV	[67]
	D. superbus L.	L	TMV	[37]
	Gypsophila elegans M. Bieb.	Sh	TNV	[67]
	G. paniculata L.	Sh	TNV	[67]
		L, St	TMV, TNV	[67]
	Herniaria glabra L.	Sh	TNV	[67]
	Minuartia capillacea (All.) Graebn.	L, St	TMV, TNV	[67]
	Petrorhagia saxifraga (L.) Link	Sh	TNV	[67]
	Saponaria ocymoides L.	Sh	TNV	[67]
	S. officinalis L.	Se	TMV	[68]
	Silene alpestris Jacq.	Sh	TNV	[67]
	S. bacifera (L.) Roth (Referred to as *Cucubalus baccifer* L.)	Sh	TNV	[67]
	S. chalcedonica (L.) E. H.L.Krause (Referred to as *Lychnis chalcedonica* L.)	Sh	TNV	[67]
	S. coeli-rosa (L.) Godr.	Sh	TNV	[67]
	S. coronaria (Desr.) Clairv. ex Rchb. (Referred to as *Lychnis coronaria* Desr.)	Sh	TNV	[67]
	S. dioica (L.) Clairv.	Sh	TNV	[67]
	S. flos-jovis (L.) Greuter & Burdet (Referred to as *Lychnis flos-jovis* (L.) Desr.)	Sh	TNV	[67]
	S. saxifraga L.	Sh	TNV	[67]
	S. schafta J.G.Gmel. ex Hohen.	Sh	TNV	[67]
//		L, St, R, Se	EMV, PVX, TMV, ToMV	[73]
	S. spathulata Schur ex Nyman	L	BGMV, TMV	[28]
	S. uniflora Roth (Referred to as *S. maritima* With.)	Sh	TNV	[67]
	S. viridiflora L.	L	BGMV, TMV	[28]
	S. viscaria (L.) Jess. (Referred to as *Lychnis viscaria* L.)	Sh	TNV	[67]
	S. vulgaris	L	BGMV, TMV	[28]
	Telephium imperati L.	Sh	TNV	[67]
	Vaccaria hispanica (Mill.) Rauschert (Referred to as *V. pyramidata* Medik.)	Sh	TNV	[67]
Didiereaceae	*Portulacaria afra* Jacq.	L	TMV	[18]
Nyctaginaceae	*Boerhavia diffusa* L.	St, L	TMV	[36]
		R	TMV, SRV, GMV	[74]
		R	TMV, TRSV	[75]
		L	PVX	[26]
		R	CMV, CGMMV, SRV, GMV, TMV	[76]
		R	PRSV, MBCMV	[77]
			BCMV	[78]
		R	CMV	[79]
		R	CGMMV	[80]
		L	BCMV	[81]
		L	ULCV	[52]
		R	PRSV	[82]
		R	TMV	[83]
	Bougainvillea x buttiana Holttum & Standl.	L	TMV, SRV	[84,85]
		L	GBNV, MYMV, TMV	[86]
	B. glabra Choisy	L	SRV, TMV	[85]
	B. peruviana Bonpl.	L	SRV, TMV	[85]
	B. spectabilis Willd.	L	TMV	[19]
		L	TMV	[23]
		L	TMV	[20]
		L	PhySMV, SRV, TMV, TmYMV	[87]
		L	BGMV, TMV	[28]
		L	TSWV	[29]
		L	PVX, PVY	[30,88]
		R	TSWV	[34]
		L	CMV	[89]
		L	SRV, TMV	[85]
		L	ULCV	[52]
		L	SFNV	[90]
		L	LMV	[91]
		L	ZYMV	[92]
	Mirabilis jalapa L.	L	TMV,	[23]

(*continued on next page*)

Table 1 (*continued*)

Families	Inhibitory species	Plant part	Viruses	References
		L	BGMV TMV	[28]
		L	TSWV	[29]
		L	PVX PVY	[30]
		L	PVX	[88]
		L, R, Se	CGMMV, CMV, TMV, TuMV	[93]
		R	PVX, PVY, PLRV, PSTVd	[94]
		L	CMV	[89]
		L, R, Se	TSWV	[95]
		L	CMV	[96]
		Sh, R	BYMV	[72]
		L	AltMV	[97]
		L, R	ULCV	[52]
		L	PVY	[98]
		R	BCMV	[99]
Petiveriaceae	*Petiveria alliacea* L.	L	TMV	[21]
Phytolaccaceae	*Phytolacca acinosa* Roxb.	L	PVY	[98]
	P. americana L.	L	SBMV	[100]
		L	CMV	[101]
		L	SBMV, CPMV	[102]
		L	TMV	[63, 103–105]
		L	TSWV	[95]
		L, R	BYMV	[72]
		L	PVY	[98]
		L, St	PVY	[106]
	P. americana (Referred to as *P. decandra* L.)	L	TMV	[10]
		L	TAMV, TBSV	[43]
	P. americana (Referred to as *P. rigida* Small	L	TMV	[11]
		L	TMV	[13]
		L	BeanMV, CMV, PRSV, TMV, TRV	[14]
	P. esculenta Van Houtte	L	TMV	[15]
	P. tyrsiflora Fenzl ex J. A.Schmidt	L	BGMV TMV	[28]
		L	TSWV	[29]
		L	PVX, PVY	[30]
		L	PVX	[88]
Polygonaceae	*Coccoloba peltata* Schott	L	TNV	[107]
	C. uvifera (L.) L.	L	TNV	[107]
	Muehlenbeckia varians Meisn.	L	TNV	[107]
	Oxyria sinensis Hemsl.	L	TNV	[107]
	Persicaria amphibia (L.) Delarbre (Referred to as *Polygonum amphibium* L.)	L	TNV	[107]
	P. amplexicaulis (D. Don) Ronse Decr. (Referred to as *Polygonum amplexicaule* D.Don)	L	TNV	[107]
	P. bistorta (L.) Samp. (Referred to as *Polygonum bistorta* L.)	L	TNV	[107]
	P. wallichii Greuter & Burdet (Referred to as *Polygonum polystachyum* Wall. ex Meisn.)	L	TNV	[107]
	Polygonum affine D. Don	L	TNV	[107]
	Reynoutria japonica Houtt.	L	TNV	[107]
	R. sachalinensis (F. Schmidt) Nakai (Referred to as *Polygonum sachalinense* F.Schmidt)	L	TNV	[107]
	Rheum palmatum L.	L	TNV	[107]
	R. pichonii Pierre ex F. B.Forbes & Hemsl.	L	TNV	[107]
	Rumex lanceolatus Thunb.	L	TNV	[107]
Portulaccaceae	*Portulaca grandiflora* Hook.			[10]
Talinaceae	*Talinum paniculatum* (Jacq.) Gaertn.	L	TMV	[21]

ACLSV = apple chlorotic leaf spot virus, AltMV = Alternanthera mosaic virus, BCMV = bean common mosaic virus, BeanMV = bean mosaic virus, BGMV = bean golden mosaic virus, BYMV = bean yellow mosaic virus, CbMA = cabbage mosaic virus, CGMMV = cucumber green mottle mosaic virus, Cla = cladode, CMV = cucumber mosaic virus, CPMV = cowpea mosaic virus, EMV = eggplant mosaic virus, GBNV = groundnut bud necrosis virus, GMV = Gomphrena mosaic virus, L = leaf, LMV = lettuce mosaic virus, MGMV = Mungbean yellow mosaic virus, PhySMV = Physalis shoestring mosaic virus, PLRV= Potato leafroll virus, PRSV-l = potato ringspot virus (latent strain), PRSV-n = potato ringspot virus (necrotic strain), PVX = potato virus X, PVY = potato virus Y, PSTVd = potato spindle tuber viroid, R = root, SBMV = southern bean mosaic virus, Se = seed, SFNV = sunflower necrosis virus, Sh = shoot, SHMV = sunn-hemp mosaic virus, SRV = sunn-hemp rosette virus, St = stem, TAMV = tomato aucuba mosaic virus (TMV strain), TBSV = tomato bushy stunt virus, TMV = tobacco mosaic virus, TmYMV = tomato yellow mottle mosaic virus, TNV = tobacco necrosis virus, ToMV = tomato mosaic virus, TRSV = tobacco ringspot virus, TSWV = tomato spotted wilt virus, TuMV = turnip mosaic vius, U = uninformed, ULCV = urd-bean leaf crinkle virus, ZYMV = zucchini yellow mosaic virus.

Amaranthaceae) species [32,100,101]. The inhibition of virus-receptor complex formation by *Chenopodium* sap was also reported by Yoshi and Sako [49]. Interaction between the inhibitor compounds and viruses may also hinder viral movement from cell to cell, preventing it from spreading through the plant [88].

Evidence of changes in host plants has already been addressed. As early as 1954, Bawden [109] reported that most, if not all, inhibitors act on the host plant rather than directly on the virus particles. Since then, researchers have hypothesized that the ability to inhibit infection could be correlated with the ability to interfere with host-plant metabolism [17,18,35,114]. Introducing foreign compounds into a host plant can cause greater physiological disturbances in the metabolism Gendron & Kassanis [43]. Several studies have shown a stronger preventive effect of AVPs against infection in heterologous than autologous plants [22,31, 43,106,115]. These findings support the idea that AVPs act more preferentially on host plants than on viruses [17,18,35,43,45,114]. Additionally, the persistence of plant and/or animal virus infectivity despite their previous mixing with plant extract containing AVPs has been demonstrated [88,101]. Ragetli [113] found that TMV infectivity was completely restored after separation of the inhibitor-virus mixture, suggesting that the inhibitor compound does not act directly on the viral particle. Gupta and Naqvi [116] also reported that leaf and stem extracts of *Chenopodium amaranticolor* (now *C. giganteum*) inhibited the infection process, altering the susceptibility of the host plants rather than directly affecting the virus particles. Similar results were obtained by Duarte et al. [88], who suggested that inhibitors from *B. spectabilis*, *M. jalapa* and *Phytolacca thyrsiflora* Fenzl ex J. A. Schmidt acted on plant host susceptibility to PVX.

Leaf extract of Caryophyllales species can also alter the cellular

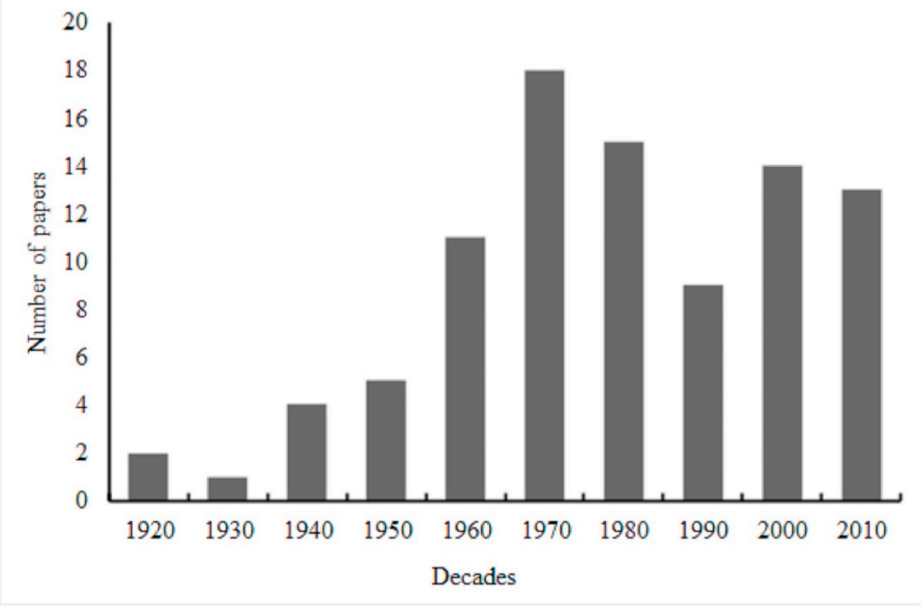

Fig. 2. Number of papers concerning Caryophyllales inhibitors of viral infection by decade.

metabolism of the host, leading to the formation of virus-interfering compounds (Virus Inhibitory Agent - VIA) [87,112,114,117,118] and precluding the need for direct contact between the inhibitor and virus [51,62]. In other words, some AVPs may not act directly to prevent infection, rather these glycoproteins would induce an antiviral state in the plants, perhaps acting as a signal activating the defense mechanism in susceptible hosts, leading to *de novo* synthesis of other proteins [80]. In addition to the direct changes in host metabolism due to inhibitory plant compounds, VIA production by proteins related to the induction of systemic resistence has already been reported [87,112,114,117]. However, this antiviral state is short and remains only for brief periods after treatment [87,112]. The VIA induced by *B. diffusa* root extract, for example, was detected only within 2 h–48 h after treatment [112,117]. The stimulus provided by bio molecules such as *B. difusa*-glycoprotein triggers signaling events that affect the entire plant, increasing the steady–state levels of defence gene transcripts throughout the plant [119]. Some studies have shown that the inhibitor compounds of *Spinacea oleracea* L., *Celosia cristata* (now *C. argentea* L.) and *Bougainvillea* × *buttiana* Holttum & Standl act when applied immediately after inoculation, and that the inhibitory effect decreases gradually over time [44,63,85]. On the other hand, extracts of *B. spectabilis*, *M. jalapa* and *P. thyrsiflora* did not inhibit TSWV infection in tomato plants when applied after inoculation [29]. Upon incubation with the VIA, the *in vitro* and *in vivo* infectivity of the virus declined. Sharma & Awasthi [120] denominated these VIA-inducing proteins systemic resistance inducing proteins (SRIPs).

Owens et al. [121] suggested that the inhibitor compound of species of *Phytolacca* could act *in vivo* blocking of the messenger function of potentially infectious viral RNA. Several studies have shown that, in addition to *N*-glycosidase activity, namely the ability to remove one or more adenine residues from ribosomal RNA precluding protein synthesis, some AVPs also exhibit antiviral activity [122–127]. These ribosome-inactivating proteins (RIPs) are widely distributed in vascular plants and have been associated with defense, protecting the plant from predator or pathogen attack [123,124,128–131].

3. Ribosome-inactivating proteins (RIPs) from caryophyllales

RIPs are classified into three categories according to their physical properties, the number of polypeptide chains, and posttranscriptional modifications: **(i)** Type I, the most widely distributed type of RIPs, are single-chained proteins with a molecular mass of approximately 30 kDa, exhibiting *N*-glycosidase activity, and involved in defense against diseases caused by viruses and perhaps microorganisms; **(ii)** Type II contains two distinct subunits: a catalytic subunit (A chain), functionally identical to those of type I, and a lectin subunit linked to a sugar-binding B chain. These are defense proteins that directly target plant-eating organisms; **(iii)** Type III contains an *N*-terminal domain associated with the A domain of RIPs fused to an unknown functional *C*-terminal domain. This type is less frequent and has been identified in barley and maize [122,125,126,130,132].

In the 1970s, an AVP able of inhibiting protein synthesis, changing interactions in the EF 1 and EF 2 elongation factors of eukaryotic ribosomes [133,134] was already known. In 1975, Irvin [134] purified and partially characterized the antiviral protein (AVP) from *P. americana*, suggesting a polypeptide with approximately 27 KDa. However, the denomination of RIP was introduced years later [135], after their damaging effect on ribosomes was discovered [123]. From these findings, several RIPs have been isolated and identified due to the antiviral action of some plant extracts [136] and, since then, is one of the most widely studied defensive properties [122–125,137].

RIPs and related proteins have been reported for at least 24 families, encompassing about 125 plant species [131]. The presence of type I RIP has been described for at least 20 families, six of which are Caryophyllales. To date, all known Caryophyllales RIPs are type I [130,131]. It is worth important to underscore that after the discovery of the effect of the antiviral proteins of *P. americana* on ribosomes, the hypothesis that protein synthesis inhibition was the mechanism by which RIPs exerted their antiviral activity was proposed [106,134].

The first type I RIP (pokeweed antiviral protein - PAP) was obtained from American pokeweed (*P. americana*) [138]. A review by Zhu et al. [126] strengthens the role of RIPs as antiviral agents, and reinforces the importance of Caryophyllales as a prominent source of these compounds. Of the 12 RIPs reported by the authors, eight have been obtained from species of Caryophyllales, as follows: PAP and PAP I (*P. americana*), new single-chain RIPs (*Basella rubra*), CCP 25 (*Celosia cristata*), 27-kDa RIP (AAP-27 from *Amaranthus tricolor*), RIP from *Bougainvillea* × *buttiana*, ME1 (*Mirabilis expansa*), BDP-30 (*Boerhavia difusa*). All these RIPs have been tested against TMV, artichoke mottled crinkle virus (AMCV, genera *Tobamovirus*), BMV, pokeweed mosaic virus (PMV, genera *Potyvirus*), sunn-hemp rosette virus (unclassified virus) and zucchini yellow mosaic virus (ZYMV, genera *Potyvirus*).

The common pokeweed plant (*P. americana*) produces several isoforms of PAP [139], which might optimize the plant's response to several types of pathogens [127]. Another interesting point is the presence of more than one RIP in the same plant, depending on seasonality and/or plant part. Pokeweed forms PAP, PAPII, and PAP-S can appear in spring leaves, summer leaves and seeds, respectively [140]. In addition, cluster analysis of the most differentially expressed genes revealed that some PAP isoforms shared expression patterns with genes involved in terpenoid biosynthesis, JA-mediated signaling, and amino acid and carbohydrate metabolism [141].

One PAP mechanism of action is related to translation inhibition, possibly by the protein binding to the cap structure and depurinating the mRNA [38,137]. According to the authors, PAP degrades capped luciferase transcripts and behaves like an RNase at high concentrations. Studies have shown that PAP binds to the 5′-cap of mRNA and depurinates portions of gene transcripts adjacent to the cap, inhibiting the *in vitro* translation of several viruses, without depurinating the host ribosomes [139,142,143]. The activity of two RIPs, in addition to PAP, against capped and uncapped viral RNAs has been reported. PAP, *M. expansa* RIP (ME1), and *Saponaria officinalis* RIP (saporin) depurinated capped TMV and brome mosaic virus (BMV, genus *Bromovirus*) RNAs, but did not depurinate uncapped luciferase RNA, indicating that in addition to PAP, other type I RIPs can distinguish between capped and uncapped RNAs [143]. In addition, PAP (located in the apoplast) may be part of a general suicide strategy, making wounded tissue inefficient for viruses or parasitic fungi to establish an infection. PAP can trigger such events not only by ribosome inactivation, but also by deadenylation of DNA or RNA, including capped and uncapped mRNAs or poly (ADP-ribose) [127,143], by the induction of caspase pathways by other means or even by the generation of reactive oxygen species, as reported for BE27 (*Beta vulgaris* RIP) [144]. These events could lead to cell death through a complex combination of necrosis, apoptosis, necroptosis and even autophagy [127].

PAP can also directly depurinate viral RNA and inhibit virus replication [145]. Two distinct steps in the BMV reproductive cycle were impeded by PAP treatment, RNA replication and subgenomic RNA transcription. These findings not only extend the known antiviral activities of PAP, but also provide two additional viral targets for inhibiting viral infections [146]. In addition, PAP protected plants from infections caused by CMV, alfalfa mosaic virus (AMV, genus *Alfamovirus*), PVX and PVY, African cassava mosaic virus (ACMV, genus *Begomovirus*) and cauliflower mosaic virus (CaMV, genus *Caulimovirus*) [147,148]. Later, Chen et al. [149] suggested that almost all plant and animal viruses could be inhibited by PAP.

Besides all activities described for PAP, another example of RIP from Caryophyllales was isolated from *Bougainvillea* × *buttiana* has been described as non-phytotoxic, resistance inducing, and, when purified, exhibited RNase activity against TMV and SRV, causing complete degradation of viral RNAs in a concentration-dependent manner [85,110]. Thus, the antiviral properties of RIPs seem to be quite complex, and the mechanism of viral inhibition may vary among different viruses and RIPs [129].

4. Caryophyllales antiviral proteins as resistance inducers

Antiviral proteins are also resistance inducers in plants, with both local and systemic responses [75,87,112,114,119]. The role of RIPs as plant resistance inducers has also been demonstrated. PAP sprayed on squash plants before inoculation could enable the plant to avoid ZYMV infection. PAP also increases plant systemic resistance to TMV infection in *Nicotiana benthamiana* Domin [132,150]. qRT-PCR analysis showed that TMV accumulation levels were significantly lower in the systemic leaves of PAP-treated *N. benthamiana* plants when compared with the levels observed in their PBS-treated counterparts [150].

Plants exhibit constitutive and inducible mechanisms of resistence to pathogen invasion, such as morphological barriers, secondary metabolites and antimicrobial proteins. However, upon contact with pathogens, the elicitors produced and released induce new defenses, including cell wall reinforcement, phytoalexin production, and the synthesis of defense-related proteins (DRPs). Most of these DRPs correspond to pathogenesis-related proteins (PRs) or to the products of so-called SAR genes, which were identified several years ago as being associated with plant resistance reactions to various pathogens [151].

These DRPs have been classified into 17 families of PRs, and most are induced by signaling compounds such as salicylic acid (SA), jasmonic acid (JA) or ethylene (ET), as well as wound responses that activate protein production, which also accumulate during infections, and display antimicrobial activities *in vitro* through different enzymatic activities [151–154]. As expected, *PR* genes are upregulated by different types of pathogens, including viruses, and by the addition of chemical compounds that mimic the effect of pathogen infection or cause similar stress conditions [155]. In addition, these *PR* genes are also induced by signaling compounds such SA, JA or ET, as well as wound responses that activate protein production [144,151,153–155]. Thus, it is expected that upon infection by pathogens, plants often exhibit increased production of reactive oxygen species (ROS), SA, JA, ET, and nitric oxide (NO). These molecules can serve as secondary signals to activate plant defense. In addition, they are well-known inducers of *PR* gene expression [156,157]. However, it is also important to highlight that *PR* gene activation does not always coincide with enhanced SA levels [158].

Would these antiviral proteins, including RIPs from Caryophyllales, be able to induce PR and ROS accumulation and, consequently, increase virus resistance in plants? It is important to underscore that data concerning the role of Caryophyllales AVPs as signaling plant defense against viruses remain scarce. Tobacco plants treated with *Basella rubra* (now *B. alba,* Basellaceae) extract showed significantly decreased leaf levels of O_2- production, malondialdehyde (MDA) content and plasmalemma permeability, in addition to increased superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity in the first 6 days after treatment [159]. On the other hand, tobbaco leaves treated with AVPs of *Bougainvillea* × *buttiana* only showed increased CAT activity, while SOD and POD activity decreased. However, in TMV + AVP treated leaves, the activities of all three enzymes were found to be midway between those obtained with AVP or TMV treatments [110]. The authors suggest that bougainvillea AVPs could maintain the host's antioxidant status, suppressing the viral disease, probably partly due to their own ROS scavenging action or their effect on antioxidative enzymes.

Sunflower plants treated with *B. spectabilis* extract exhibited induction of PR proteins and oxidative enzymes β-1,3 glucanase, POD, polyphenoloxidase (PPO), and phenylalanine ammonia lyase (PAL) [90]. Similarly, the pre-application of MAP (antiviral protein of *M. jalapa*) induced phenol, PO, PPO and PAL activity, leading to suppression of TSWV-induced local and systemic lesions [95].

Gholizadeh et al. [108] also reported that multifunctional CPCs (RIP from *Celosia argentea*) inhibited viral infection, possibly through their antioxidant processes, preventing ROS induction/accumulation and/or ribosome inactivation. The same author described the RIP from *Celosia plumosa* (now *C. argentea*) as an active antioxidant protein, an important protective tool against viral infection and the subsequent oxidative damage to the plant system [46].

Zhu et al. [150] suggested that PAP may enhance a plant's systemic resistance against virus infection by regulating ROS levels. The results showed that antioxidant enzymes were activated by PAP treatment, enabling plants to activate defenses and acquire resistance. By contrast, Faoro et al. [160] observed a slight oxidative burst in leaves treated with D2 (*Phytolacca dioica* antiviral protein), indicating that the defense responses were activated. However, only when D2 and viruses were present in the same cell did cell death occur, accelerating the hypersensitive response, thereby limiting the spread of the virus. Nevertheless, the authors suggested that systemic acquired resistance (SAR) was probably not induced, since the small number of dead cells would not be sufficient to achieve a significant level of signals to induce resistance. The authors

suggested that D2 cannot translocate into the phloem, nor induce signals to activate systemic acquired resistance (SAR). Thus, it seems that D2 antiviral activity is the result of the combined effect of its deadenylation properties on cell and viral nucleic acid and the activation of the plant's own defense response.

RIPs can also act indirectly in plant defense signaling, inducing resistance or even tolerance to certain viruses [47,83,150]. These RIPs may act indirectly by activating the plant's defense system, resulting in systemic resistance irrespective of SA or PR accumulation [38]. Multi-functional in nature, these proteins may be inhibiting viral infection through their antioxidative and/or ribosome-inactivation processes [108]. As such, the proposed model for the role of RIPs in the defense against pathogens and insects indicates that discovering additional crosstalk mechanisms between RIPs and phytohormones or ROS against pathogen and insect infections will be a significant issue in the study of biotic stress [126].

5. Caryophyllales AVPs and agriculture

As shown in Table 1, more than 100 species of Caryophyllales can potentially control phytoviruses, with natural compounds (proteins or glycoproteins) able to stimulate resistance in economically important host crops against a broad spectrum of viruses. Moreover, plant extracts are natural, safe, effective, ecofriendly and durable in managing plant viruses [99]. Thus, the ecofriendly management of viral diseases in different crops could also be achieved [161].

In the early 1950s, due to several diseases caused by viruses and their difficult control, researchers began exploring diffrent alternatives. Bawden [109] suggested spraying tobacco and tomato seedlings with glycoprotein extract of *Phytolacca* sp before transplanting in order to reduce the spread of the tobacco mosaic virus; workers immersed their hands in solutions of the extract in an attempt at reducing the spread during crop operations; through frequent spraying of tomato crops under glass, inhibitors might also result in profitable return by reducing the incidence of mosaic virus, which now commonly infect the entire crop.

B. diffusa root extracts has shown to be promising in field studies. Twice-monthly spraying of this extract was effective in preventing natural infection by several viruses in *Solanum tuberosum* L. cutures. In addition, growth and tuber productions were enhanced in treated plants [26]. Increased nodulation, plant growth, fruiting and grain yield, in addition to an 80–90% reduction in yellow mosaic disease related to mung bean yellow mosaic (MYMV) in mungbean and urdbean, were detected after field treatment with *B. diffusa* aqueous root extract [112,161]. Weekly spraying of cucurbitaceous crops prevented infection, multiplication and spread of CMV, bottle gourd mosaic virus, cucumber green mottle mosaic virus and pumpkin mosaic virus. Treating tobacco plant explants with root systemic resistance-inducing protein from *B. difusa* made plants less susceptible to TMV infection [162]. Awasthi & Verma [163] published examples of prevention and control of viral diseases by *B. diffusa* antiviral agent in field crops.

Treating bean seeds with *M. jalapa* root extract before field planting increased BCMV inhibition in plants inoculated 8 days after seeding. Treating bean plants with *M. jalapa* extract inhibits BCMV infection by 81 and 74% in greenhouse and field plants, respectively [99]. The induction of systemic resistance by aqueous extracts of *P. americana* leaves and roots, *D. caryophyllus* leaves, as well as *M. jalapa* roots and young shoots to prevent natural virus infection in bean plants under field conditions was evaluated. Three sprays were performed (15, 30 and 45 days after planting) and all extracts induced high inhibition percentages, compared to control plants [72].

Brazilian field experiments confirmed the economic feasibility of applying Caryophyllales extract in virus disease control. Treating lettuce plants with leaf extracts from *B. spectabilis* and *M. jalapa* increased net profit by more than 25% [91]. Zuchini plants treated with leaf extracts of *B. spectabilis* and *M. jalapa* since cotyledon emergence, sprayed at 72 h intervals until flowering, achieved significantly higher fruit yield than that of control plants, even after ZYMV infection [unpublished data].

In addition to traditional agricultural methods, transgenic plants have been used as an important strategy to achieve more resistant and productive crops. Research on transgenic plants expressing RIP genes, especially those from *Phytolacca*, has been conducted since the 1990s. Transgenic tobacco and potato plants expressing *PAP* or a variant (*PAP-V*) were shown to be resistant to a broad spectrum of plant viruses [140,164]. The expression of *PIP* gene from *P. insularis* also conferred resistance on transgenic potato plants against a broad spectrum of plant viruses, infecting through mechanical and aphid transmission. Mature PIP contains amino acid residues (IQMVSEAARFKYI), which is a putative active site involved n depurination of ribosomal RNA by RIPs [165]. In addition, recombinant PIP (rPIP) inhibited *in vitro* protein synthesis in rabbit reticulocyte lysate through *N*-glycosidase activity, suggesting that the *PIP* gene encodes a functional RIP [165,166]. The *PIP2* gene encodes a biologically active protein with ribosome-inactivating and antiviral activities [166]. Transgenic potato plants expressing the CaMV35S-*PIP* gene also showed no symptoms of infection by PVX, PVY or potato leafroll virus (PLRV, genus *Polerovirus*), having the same shape and characteristics as uninfected healthy plants, despite exhibiting slightly delayed growth when compared with nontransgenic plants, which showed necrosis throughout the veins [165].

The *PAP* gene exhibits different functional domains. One of these has been shown to be toxic to host plants, leading to a change in their growth performance [167,168], but without interfering in antiviral activity. Transgenic cucumber plants expressing toxic-free PAP genes (*PacPAP*) displayed no change in their growth, but maintained their antiviral properties [168]. As such, non-toxic PAP mutants from *P. americana* and *P. acinosa* Roxb. were isolated and characterized [167,168]. Transgenic cucumber plants with toxic-free PAP genes (*PacPAP*) displayed no observable change in their growth and maintained their antiviral properties [168].

Thus, despite the increase in research focused on RIPs as broad-spectrum antiviral resistance inducers in plants during the 1990–2000s, their use in daily agricultural practices remains a challenge due to the potent cytoplasmic toxicity to the plant itself and the animals consuming it [148].

6. Conclusions

Caryophyllales is a special group of plants with broad potential for discovering new species with antiviral activity. Although this taxon contains the highest number of species with antiviral potential in Angiosperms, only 110 species from comprised in 30% of the families belonging to this order have been studied for their inhibitory potential against viral infection. Antiviral proteins (AVPs) have attracted considerable attention, especially those with ribosome inhibitor action (RIPs), such as PAP (*Phytolacca americana* antiviral protein). These proteins, already tested against human and plant viruses, have been described as one of the best strategies for controlling a broad spectrum of plant viruses from different taxonomic groups. Exhibiting multiple biological activities, the AVP mechanism of action has been associated with enzyme, antioxidant, plant defense system signaling and SAR functions, depending on the plant/host system. However, despite all recent findings, the exact mode of action has yet to be clarified. AVPs and/or RIPs might induce the synthesis of some new virus-interfering compounds or enhance the production of their constitutive counterparts, thereby altering host plant susceptibility and favoring plant resistance against a wide variety of pathogens [38,144,152] as in the proposed model (Fig. 3). There is no indication, however, that all the plants display the same type of inhibitor or that the antiviral mechanism is the same in all cases [112]. It is also important to underscore that more than virus-inhibitor infection compound may be present in the same extract, as reported for *Chenopodium album* sap [47]. However, despite so many doubts about the mode of action, Caryophyllales AVPs

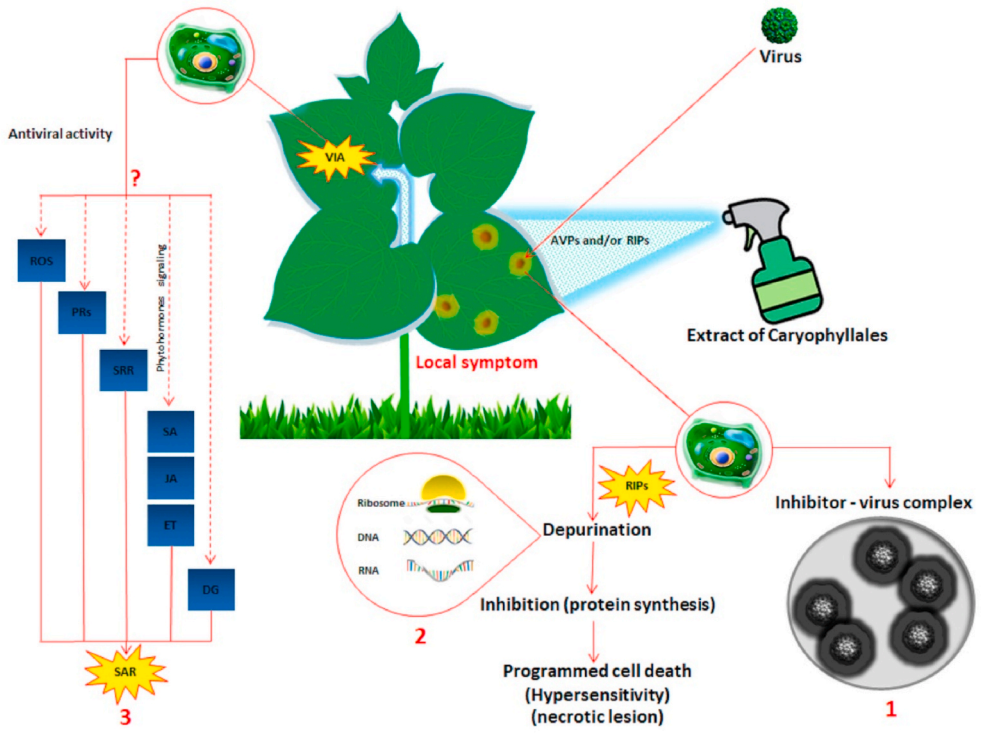

Fig. 3. Schematic representation indicating the main hypotheses of Caryophyllales antiviral protein (AVP) activity. **1.** Direct action inactivating virus particles or forming a loose complex with the virus. An electron-dense granular mass or aggregation of virus particles is also observed coating the viral particles after mixing Caryophylalles extract with the virus. Viruses and inhibitors can compete for receptors located on the leaf surface. The virus-inhibitor complex can prevent cell-to-cell movement, preventing infection and the virus from spreading throughout the plant. **2.** Action on the virus infection process: Ribosome inhibitory proteins (**RIPs**) block the messenger function of potentially infectious viral RNA through the *N*-glycosodase activity by removing one or more adenine residues of ribosomal RNA, preventing protein synthesis. In addition, RIPs can act directly on the virus particles or viral nucleic acids (RNA or DNA) through their polynucleotide: adenosine glycosidase activity. They can enter the cytosol of infected cells by a vector or mechanical injury and destroy protein synthesis machinery, preventing the virus from replicating and infecting neighboring cells. ('local suicide'). **3.** Changes in host cell metabolism altering host susceptibility: Caryophyllales extract can also alter the cellular metabolism of the host, leading to the formation of virus-interfering compounds (Virus Inhibitory Agent – **VIA**), inducing an antiviral state in the plants, and possibly acting as a signal activating the defense mechanism in susceptible hosts. RIPs can increase the systemic resistance of plants against viral infection by regulating the level of reactive oxygen species (**ROS**). They can induce a systemic resistance response (**SRR**) by activating the plant's defense system, with or without accumulation of pathogenesis-related proteins (**PRs**), whether or not they activate defense signaling phytohormones salicylic acid (**SA**), jasmonic acid (**JA**), ethylene (**ET**), and the defense genes (**DG**).

are important candidates to minimize the problem of world hunger by increasing the production of high quality food in a sustenable way, without increasing synthetic pesticides.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This study was supported by FAPESP (Research Support Foundation of São Paulo State, Brazil) (proc. 2016/25708-4).

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