

HSP70 plays an ambiguous role during viral infections in plants

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Abstract

Heat shock and almost all types of stresses associated with oxidative stress are accompanied by heat shock protein (HSP) expression. HSPs are involved in refolding denatured proteins and directing unrepairable proteins for degradation. Thus, under stress conditions, HSPs help to restore cellular balance. However, in virus-infected plants, HSP70 can have both positive and negative effects because viruses usually recruit HSP70. HSP70 can promote the replication and translation of the viral genome, the formation of viral replication complexes, and the propagation of viral particles from cell to cell and throughout the plant. *HSP* gene silencing in various virus-host plants systems and the comparison of susceptible and resistant species have shown that HSPs70 accelerate the development of infection. Conversely, during the process known as thermotherapy, the temperature increase inhibits viral replication in some host and virus systems. The success of thermotherapy depends not only on the temperature and treatment period or duration but also on the plant species and viral strain. In this review, we discuss the ambiguous role that HSPs70 play during viral infections in plants towards weighing the balance between their positive and negative functions.

Keywords: acquired tolerance, heat shock, host factor, plant viruses, thermotherapy, virus propagation.

Introduction

Heat shock proteins (HSPs) are a family of mainly stress-induced proteins whose primary function is to refold denatured proteins. HSPs are divided into six groups according to their relative molecular mass and primary structure homology (Wang *et al.* 2004, Park and Seo 2015). The genes that encode HSPs are found in different cell compartments, and HSP expression is controlled by transcriptional factors known as heat shock factors (HSFs) (Haq *et al.* 2019).

In particular, HSP70 is arguably the most conserved protein family among all the organisms, from bacteria to plants and animals. The number of members in different

HSP70 families ranges from 18 in *Arabidopsis thaliana* and 20 in *Solanum tuberosum*, through 30 in *Oryza sativa* to 61 in *Nicotiana tabacum* (Liu *et al.* 2018, Song *et al.* 2019). HSP70 are crucial for cells as constitutive and ubiquitously expressed proteins, but HSP70 expression is also induced, not only by heat shock (HS), but by almost all types of plant stresses as well (Park and Seo 2015, Usman *et al.* 2017).

In the classical model for stress activation of HSPs, the presence of stress-induced unfolded proteins in the cell causes the release of HSPs from their constitutive inhibitory association with HSF monomers, although this model could involve more pathways, especially at temperatures that do not unfold proteins. The unfolded

Received 2 October 2020, last revision 14 December 2020, accepted 7 January 2021.

Abbreviations: CP - coat protein; HS - heat shock; HSC70 - heat shock cognate 70; HSF(s) - heat shock factor(s); HSP(s) - heat shock protein(s); RdRp - viral coded RNA-dependent RNA polymerase; ROS - reactive oxygen species; UPR - unfolded protein response; VRC - viral replicase complex.

Acknowledgements: We sincerely apologize to colleagues whose publications could not be cited here owing to space constraints. The authors thank Dr. Carlos V. Melo for English proofreading this review. This work was supported by the Charles University, projects SVV260427/2020 and GA UK No. 1304119 and the Ministry of Education, Youth and Sports of CR from European Regional Development Fund-Project "Centre for Experimental Plant Biology" (grant number CZ.02.1.01/0.0/0.0/16_019/0000738).

Conflict of interest: The authors declare that they have no conflict of interest.



Fig. 1. The main events in the potyviral infection cycle (red) and during HS response (yellow). Heat shock proteins (HSP70) are formed to help plants respond to both stressors (green), but HSP70 can also be misused by viral particles to facilitate their propagation (red). The scheme represents the complexity of HSP70 involvement in the stress situation. Abbreviations: CI - cytoplasmic inclusion, CP - coat protein, HS - heat shock, HSF - heat shock factor, NIa - nuclear inclusion type a with proteolytic activity, NIB - potyviral RNA-dependent RNA polymerase, 6K2 - membrane associated protein, UPR - unfolded protein response, VPg - viral genome linked protein. The viral replication scheme was modified from [Ivanov et al. 2014](#).

protein response (UPR) is a signaling pathway activated in cells in response to stresses that impair protein stability. Changes in ambient temperatures are sensed *via* a complex network of molecular sensors located in different cell compartments: the plasma membrane sensing mechanism, which triggers a specific inward calcium flux, UPR sensors in endoplasmic reticulum and cytosol and histone decreased occupancy in the nuclei. These signals generated by different sensors are integrated by a signal transduction network that involves calcium fluxes, calmodulin, protein kinases, phosphatases, and transcriptional regulators, such as HSFs ([Mittler et al. 2012](#)).

In response to heat stress, HSP70 is activated as shown in [Fig. 1](#). Pre-treatment of a plant with a moderately elevated temperature can enhance heat stress tolerance and accelerate recovery after stress ([Prerostova et al. 2020](#)). During this process (acquired thermotolerance), plants sense an elevation in ambient temperature and initiate signal

transduction networks, which regulate the expression of several genes, including those encoding HSPs and reactive oxygen species (ROS)-scavenging enzymes, to increase their thermotolerance. The integration of hormone and radiation signaling, circadian clock, and high ambient temperature regulates plant thermoresponsive growth ([Li et al. 2018](#)). In the absence of heat stress, some HSP family members are nevertheless constitutively expressed, and these family members are known as heat-shock cognate proteins HSC70 ([Duck and Folk 1994](#)).

HSPs70 have a highly conserved primary and 3D structure. The protein consists of two domains. The N-terminal domain binds ATP, Mg^{2+} , and K^{+} ions and has ATPase activity, whereas the β strands and loops of the C-terminal domain form the substrate-binding pocket ([Frydman 2001](#), [Hartl et al. 2011](#)). The HSP70 substrate binding and release cycle is exclusively regulated by ATP binding and hydrolysis, whereby HSP70 switches between

(ATP-bound) low- and (ADP-bound) high-affinity states. The former is the open form, which can bind to the substrate protein. Another molecular chaperone, HSP40, is also required for initial protein binding in this cycle (Mayer and Bukau 2005, Doubnerova and Ryslava 2014).

HSPs70 act as chaperones, stabilizing nascent proteins during protein folding, assembling or disassembling multi-protein complexes, assisting protein unfolding, transporting, and sorting organelle-specific and secretory proteins into subcellular compartments or the extracellular space, respectively (Doubnerova and Ryslava 2014, Asea *et al.* 2016, Anaraki *et al.* 2018). HSPs70 are also involved in preventing protein aggregation, repairing misfolded proteins or targeting irreparable proteins for degradation by the ubiquitin-proteasome system. Furthermore, HSP70 overexpression in plants positively correlates with acquired thermotolerance and results in enhanced tolerance to abiotic stress treatments (Wang *et al.* 2004, Su and Li 2008), whereas HSP70 downregulation reduces tolerance to abiotic stresses (Lee and Schoffl 1996, Su and Li 2008). Thus, the HSP70 system plays a key role in plants recovering from various biotic and abiotic stresses (Usman *et al.* 2017, Anaraki *et al.* 2018).

To respond to biotic stresses (viruses, bacteria, fungi, and nematodes), plants have evolved different strategies, signal perception, transduction, and activation of the immune system, including stress-related HSPs (Haq *et al.* 2019). Pathogen-derived homologs of HSP70 may be involved in pathogenesis (*e.g.*, facilitation of colonization) (Ghazaei 2017), but plant HSPs70 expressed in response to stress caused by pathogens with their own HSPs is mostly associated with beneficial effects. For example, HSPs play a key role in defense against virulent bacterial strains, and along with PR proteins, HSPs are more effective against fungal pathogen or work in cooperation with antioxidant-related enzymes to develop resistance against nematodes (for more examples, please refer to the review by Haq *et al.* 2019). However, the interaction between viral infection and HSP70 is highly complex and ambiguous. Unlike eukaryotes and bacteria, viruses lack HSPs and rely on host HSPs for protein folding (Bolhassani and Agi 2019). It seems that viruses do not encode a specific inducer of HSP70. The initiation of chaperons' response was associated with production of virus-specific proteins and the process could be analogous to the UPR in the endoplasmic reticulum as shown for *Turnip mosaic virus* and *Turnip crinkle virus* (Aparicio *et al.* 2005). The concentration of the insoluble viral coat protein of *Tobacco mosaic virus* was in correlation with the induction of HSP70 (Jockusch *et al.* 2001). Nascent viral protein or misfolded cellular proteins compete with HSF for HSP70 binding, which results in an increased amount of free and active HSF (Morimoto *et al.* 1994, Kim and Oglesbee 2012).

How does temperature affect the course of viral infections?

Elevated temperatures lead to HSP synthesis. On the one hand, HSP expression can help maintain homeostasis and proteostasis in the plant cell, even under stress conditions. On the other hand, it can help viral nucleic acid replication, protein synthesis and particle assembly, as well as virus spread to other cells (Nagy *et al.* 2011, Verchot 2012, Jiang *et al.* 2014, Heinlein 2015). Accordingly, HSP70 silencing or inhibition prevents efficient viral infection. This effect has been shown in plants infected with *Rice stripe virus* (Jiang *et al.* 2014), *Turnip mosaic virus* (Dufresne *et al.* 2008, Jungkuntz *et al.* 2011), *Tomato yellow leaf curl virus* (Gorovits and Czosnek 2017), *Red clover necrotic mosaic virus* (Mine *et al.* 2012), *Tomato bushy stunt tobravirus* (Wang *et al.* 2009a), *Abutilon mosaic virus* (Krenz *et al.* 2010), *Pepino mosaic virus* (Mathioudakis *et al.* 2012), and *Potato virus A, X, and Y* (Hofius *et al.* 2007, Chen *et al.* 2008, Hafren *et al.* 2010). However, elevated temperatures may aid in defensive reactions against viruses, as demonstrated in thermotherapy experiments. Thermotherapy usually involves subjecting plants to a slightly elevated temperature for a long time until HSP synthesis stops increasing and is hindered by other mechanisms. The success of thermotherapy depends not only on the temperature and treatment period or duration but also on the plant species and virus strain (Tan *et al.* 2010, Kwon *et al.* 2012, Achachi *et al.* 2014).

The balance between the success of a virus and that of a plant depends on the plant species, its health and developmental stage, on the virus species and strain, and on abiotic factors, including temperature. In this context, the role of HSP70 may vary from supportive to deleterious, depending on the nature of the infectious agent and possibly on the host itself (Asea *et al.* 2016).

When the elevated temperature helps the plant

In general, HSP70 synthesis under abiotic stress conditions is associated with positive effects on plants (Haq *et al.* 2019). Many factors affect the ability of a virus to multiply and penetrate plants and their ability to activate defense reactions and to prevent virus propagation. In addition to their antiviral innate immunity based on pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI), plants degrade viral nucleic acids *via* gene silencing. However, viral pathogens continuously develop new strategies to overcome host defenses (Gouveia *et al.* 2017, Carr *et al.* 2019, Gaffar and Koch 2019).

HSPs90 regulate stomatal development under acute heat stress in *Arabidopsis*. Acute stress suppresses stomatal development in a HSP90-dependent manner (Samakovli *et al.* 2020). Temperature strongly affects plant-virus interactions. The increase in temperature boost the defense response (RNA silencing) in *Nicotiana benthamiana* plants infected with *Tobacco ringspot virus*. In contrast, in the cold, the amount of central molecules

of RNA silencing (siRNAs) rapidly decreases, and plants become more susceptible to viruses (Szittyá *et al.* 2003).

A number of recent studies have provided evidence for connections between HSFs, HSPs, ROS, and ROS scavengers upon heat stress. In a recently proposed model, HS induced a short-term positive and a long-term negative feedback loop in the HSF signaling pathway (Driedonks *et al.* 2015). Overexpression of small HSPs in *Arabidopsis* and tobacco enhanced the activity of antioxidant enzymes (Jiang *et al.* 2009, Sun *et al.* 2012). Accordingly, HSPs may decrease the concentration of ROS by protecting the conformation of ROS scavenging proteins. This model for interactions between HSFs, HSPs, ROS, and ROS scavengers also seems applicable to stresses other than heat and may explain the phenomenon of cross-acclimation (Driedonks *et al.* 2015). Plants likely show defense signaling crosstalk and take advantage of HSP70 acquired by abiotic stress for protection against biotic stress. However, the mechanism of this process remains unknown, and the response to prolonged exposure to elevated temperatures likely differs from acute exposure to HS. Heat treatment may not eliminate viruses at all, but it reduces virus multiplication and translocation in plants, and virus elimination requires long treatment periods (Kwon *et al.* 2012).

Thermotherapy

Outbreaks of virus disease are often associated with low temperature, whereas high temperature is frequently associated with attenuated symptoms (“heat masking”) and with low virus content in virus-infected plants (Szittyá *et al.* 2003). Thermotherapy is the most common method for virus eradication because virus content can be reduced when plants are heat-treated. Thermotherapy temperature and time application depend on the virus present and plant cultivar sensitivity to heat (Aguilar-Camacho *et al.* 2016). In general, the higher the temperature and the longer the exposure are, the higher the virus eradication will be. Often, thermotherapy 35 - 42 °C for 4 - 6 weeks is applied to the target plants, albeit depending on the specific virus-plant system (Wang *et al.* 2018). Some examples of effective thermotherapy schemes for virus eradication are outlined in Table 1. Thermotherapy using alternating day/night temperatures was found to alleviate the negative effects of a high constant temperature (Lizárraga *et al.* 2017, Wang *et al.* 2018). Thermotherapy could be applied as both hot-air and hot-water (37 °C) treatments, which are suitable for example for eliminating *Potato leaf roll virus* from potato tubers (Abbas *et al.* 2016). Recently, the effect of thermotherapy has been amplified by combining cryotherapy with chemotherapy (Mathew *et al.* 2021). Salicylic acid- and H₂O₂-induced thermotolerance and their long-time effects significantly increased post-thermotherapy survival in *Potato virus X*-infected microplants (Aguilar-Camacho *et al.* 2016). Therefore, studies on oxidative stress physiology and on the fate of HSPs during thermotherapy could elucidate the balance between the positive and negative functions of HSPs in

viral propagation.

When the elevated temperature helps the virus

In addition to positive effects, HSP70 expression can have negative effects on virus-infected plants because viruses usually recruit HSP70. The possible involvement of HSP70 in viral infection is shown in Fig. 1. Generally, during viral infections, many viral proteins are folded by plant HSP molecular chaperones (Milani *et al.* 2016). HSP70, HSC70, the J-domain chaperones, and HSP90 were associated with some plant viruses (Table 2). As shown in Table 2, HSPs70 are an infection factor for many plant viruses.

The replication and translation of the viral genome, the formation of viral replication complexes, and the propagation of viral particles from cell to cell and throughout the plant can all be promoted by HSP70 (Mayer and Bukau 2005, Hafren *et al.* 2010, Nagy *et al.* 2011, Jiang *et al.* 2014, Lohmus *et al.* 2017, Yang *et al.* 2017). The identification of host factors recruited to the replicase complex helps to elucidate virus-cell interactions and to reveal mechanistic components required for viral nucleic acid replication (Dufresne *et al.* 2008). The viral-coded RNA-dependent RNA polymerase (RdRp) is responsible for synthesizing viral progeny in infected cells by positive strand RNA viruses (Pogany and Nagy 2015). Host HSP70 are required for RdRp within virus-induced membrane vesicles or replication complexes on intracellular membrane systems (Dufresne *et al.* 2008, Mine *et al.* 2012, Pogany and Nagy 2015, Yang *et al.* 2017). Furthermore, the RdRp of several positive-strand RNA viruses is initially inactive to avoid viral synthesis in cytosol, which would rapidly induce cellular antiviral responses. Pogany and Nagy (2015) identified co-opted cellular HSP70 and neutral phospholipids as activators of *Tomato bushy stunt tobravirus* RdRp *in vitro*. In addition, these authors found that the membrane-bound tobravirus replicase complex (VRC) consist of RdRp, the viral p33 RNA chaperone and several co-opted host proteins, one of which is HSP70. HSP70 and HSP90 were identified as host factors required for *Red clover necrotic mosaic virus* RNA replication. These chaperones promote VRC assembly by binding to p27, a virus-encoded component of the replicase complex (Mine *et al.* 2012). Furthermore, the furoviral replicase of the *Chinese wheat mosaic virus* recruits both *Triticum aestivum* L. HSP70 and *Nicotiana benthamiana* HSP70 from the cytoplasm or nucleus to the inclusion-like structures, suggesting that both HSPs70 may be recruited into VRC and promote furoviral infection (Yang *et al.* 2017). In addition, Yang *et al.* (2017) identified that the amino acid region 167-333 of the replicase is responsible for this interaction. DNA viruses such as gemmiviruses do not encode their own DNA-polymerase; instead, they utilize the host nuclear DNA replication machinery and reprogram the expression of many plant genes. To ensure a successful long-term infection, these viruses restrain their destructive effect on host cells, delaying or preventing cell death by interfering with the plant cell death pathway

Table 1. Successful thermotherapy applied to various plant viruses (* - thermotherapy was also combined with cryotherapy or chemotherapy; ** - temperature gradient with the posterior isolation and culture of apical meristems; *** - symptoms that were not mentioned in the selected reference, were derived from an online database by Brunt *et al.* 1996). Several examples, which are alphabetically ordered are shown.

Virus	Plant	Symptoms	Reference
* <i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Malus domestica</i> (4 different cultivars)	apple mosaic disease: bright yellow patterning on leaves	(Hu <i>et al.</i> 2019)
* <i>Apple necrosis mosaic virus</i> (ApNMV)			
* <i>Apple stem pitting virus</i> (ASPV)			
* <i>Apple stem grooving virus</i> (ASGV)			
<i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Pyrus pyrifolia</i> cv. Fengshui	ACLSV: plum pseudo pox ASGV: tatter leaf symptoms in citrus	(Tan <i>et al.</i> 2010)
<i>Apple stem grooving virus</i> (ASGV)		ASPV: pear vein yellowing, leaf necrotic spot and fruit stone pitting	
<i>Apple stem pitting virus</i> (ASPV)			
** <i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Malus domestica</i> (6 cultivars)	growth retardation and loss of productivity	(Lizárraga <i>et al.</i> 2017)
** <i>Apple mosaic virus</i> (ApMV)	<i>Pyrus communis</i> (2 cultivars)		
<i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Pyrus pyrifolia</i>	Generally asymptomatic in most cultivars. ACLSV can cause leaf malformation and chlorotic rings or line patterns in some pear cultivars, ASGV: pear black necrotic leaf spot	(Hu <i>et al.</i> 2012)
<i>Apple stem grooving virus</i> (ASGV)			
* <i>Apple stem grooving virus</i> (ASGV)	<i>In vitro</i> -cultured apple shoots <i>Malus domestica</i>	latent virus causing graft incompatibility, reduced growth vigor and decreased fruit yield	(Zhao <i>et al.</i> 2018)
<i>Grapevine leafroll-associated virus Pr</i> (GLRaV-Pr)	<i>Vitis vinifera</i>	GRSPaV-rugose wood syndrome of grapevine	(Maliogka <i>et al.</i> 2009)
<i>Grapevine rupestris stem pitting associated virus</i> (GRSPaV)			
* <i>Grapevine leafroll-associated virus 3</i> (GLRaV-3)	<i>Vitis champini</i>	grapevine leafroll disease: apparent downward curling and rolling of leaves. Virus can minimize shoot vigor and fruit yield and quality	(Hu <i>et al.</i> 2020)
<i>Lily symptomless virus</i> (LSV)	<i>Lilium × elegans</i>	LSV decreases plant vigor and production despite an apparent symptomless behavior	(Nesi <i>et al.</i> 2009)
<i>Onion yellow dwarf virus</i> (OYDV)	<i>Allium sativum</i>	leaf striping and curling, stunting***	(Robert <i>et al.</i> 1998)
<i>Plum pox virus</i> (PPV)	<i>Prunus persica</i> var. <i>nectarina</i>	poor fruit quality, reduction of productivity and shortening of tree life	(Manganaris <i>et al.</i> 2003)
<i>Prunus necrotic ringspot virus</i> (PNRSV)			
<i>Potato leaf roll virus</i> (PLRV)	<i>Solanum tuberosum</i>	upward curling of leaves and vein swelling on young plants***	(Abbas <i>et al.</i> 2016)
<i>Potato virus Y</i> (PVY)	<i>Solanum tuberosum</i>	mild to severe leaf mottling or streak or ‘leaf-drop streak’ with vein necrosis***	(AlMaarri <i>et al.</i> 2012)
* <i>Raspberry bushy dwarf virus</i> (RBDV)	<i>Rubus idaeus</i>	lower fruit mass and drupelet number per fruit, crumbly fruit, leaf yellowing and reduced cane growth	(Mathew <i>et al.</i> 2021)
<i>Raspberry bushy dwarf virus</i> (RBDV)	<i>Rubus idaeus</i>	small or crumbly berries, yellow ring spots on leaves that soon disappear***	(Wang <i>et al.</i> 2008)
<i>Tomato spotted wilt tospovirus</i> (TSWV)	<i>Nicotiana benthamiana</i>	firstly, chlorotic spots on apical leaves, rapidly turning to bronze and dark-brown (necrosis) in tomato and pepper (Vaira <i>et al.</i> 1995)	(Roggero and Pennazio 1997)

Table 2. Function of HSP70 as host factors during viral infections in plants. Studies targeting both upregulation (overexpression) or down regulation (loss of function mutants or silencing) of a protein have been used to confirm the function of HSP70 as a host factor. If HSP mutant plants have not been studied, N.A. (not applied) is indicated. Symptoms were derived from an online database by [Brunt et al. 1996](#), Chl - chloroplastic, Mit - mitochondrial.

Virus	Symptoms	Factor	Up- (↑) or down- (↓) regulation	Nucleic acid	Plant	Function
<i>Red clover necrotic mosaic virus</i> (Mine et al. 2012)	necrotic local lesions, mosaics, leaf deformation	HSP70 HSP90	↓	(+) ssRNA	<i>Nicotiana benthamiana</i>	HSP70 and HSP90 promote the assembly of the VRC through interactions with viral p27
<i>Tomato bushy stunt tobusvirus</i> (Pogany and Nagy 2015)	stunting, mottling, deformed fruit,	HSP70	N.A.	(+) ssRNA	recombinant <i>RdRp</i>	HSP70 is host factor required for the activation on N-terminal truncated recombinant TBSV RdRp. interaction with viral p33 in VRC
<i>Chinese wheatchlorotic mosaic virus</i> (Yang et al. 2017)	chlorotic and necrotic spots or netting	NbHSP70 TaHSP70	↑	(+) ssRNA	<i>Nicotiana benthamina</i> (silenced HSP70) <i>Triticum aestivum</i> (transiently overexpressed HSP70)	interaction with the region aa 167-333 of replicase. Both HSP70 are very probably recruited into VRC to promote furoviral replication.
<i>Rice stripe virus</i> (Jiang et al. 2014)	systemic necrotic or chlorotic spots and streaking	HSP70	↓	(-) ssRNA	<i>Oryza sativa</i> <i>Nicotiana benthamiana</i> (silenced HSP70)	HSP70 plays a role in viral replication and probably in interaction with RdRp
<i>Turnip mosaic virus</i> (Dufresne et al. 2008)	chlorotic local lesions, mosaic, mottling, puckering or rugosity	HSC70-3	N.A.	(+) ssRNA	<i>Arabidopsis thaliana</i>	HSC70-3 is potentially integral compartment of the replicase complex and could has important roles to play in the regulation of RdRp functions.
<i>Turnip mosaic virus</i> (Jungkunz et al. 2011)		HSP70	↓	(+) ssRNA	<i>Arabidopsis thaliana</i> (<i>AtHSP70-15</i> deficient)	key factor in proper folding of cytosolic proteins
<i>Beet yellows virus</i> (Peremyslov et al. 1999 , Alzhanova et al. 2001 , Prokhnevsky et al. 2002)	vein clearing, then leaves become thick, brittle, yellow with necrotic spots	HSP70h (viral homolog)	N.A.	(+) ssRNA	the mutant and wild type full length cDNA viral clones transcribed into tobacco protoplasts	HSP70 functions in intercellular translocation, viral tail (special device to cell-to cell movement) assembly and transport and severs as molecular link between local and systemic spread of a plant virus by docking a long-distance transport protein (viral p20) to virions.
<i>Tomato bushy stunt virus</i> (Wang et al. 2009a)	stunting, mottling, deformed fruit	HSP70	↓	(+) ssRNA	<i>Nicotiana benthamiana</i>	part of virus replicase complex. HSP70 affects the subcellular localization and membrane insertion of the viral replication proteins as well as assembly of the viral replicase
<i>Potato virus A, X, and Y</i> (Hafren et al. 2010)	PVA: mild mosaic, leaf surface rough with wavy margins, or symptomless PVX: symptoms vary, some strains symptomless, others induce necrotic streaks PVY: mild to severe leaf mottling or streak or 'leaf-drop streak' with vein necrosis	HSP70	↓	(+) ssRNA	<i>Nicotiana benthamiana</i> (downregulated HSP70)	HSP70 together with its cochaperone CPIP regulates the function of a potyviral CP during replication-associated translation. HSP70 as a crucial component of a distinct translational activity that is associated with potyvirus replication

Virus	Symptoms	Factor	Up- (↑) or down- (↓) regulation	Nucleic acid	Plant	Function
<i>Tomato yellow leaf curl virus</i> (Gorovits and Czosnek 2017)	plants stunted with small chlorotic puckered leaves; fruit yield much decreased	HSP70, not HSP90	↓	DNA	<i>Solanum lycopersicum</i>	HSP70 participates in the translocation of CP from the cytoplasm into the nucleus
<i>Tomato yellow leaf curl virus</i> (Moshe <i>et al.</i> 2016)	plants stunted with small chlorotic puckered leaves; fruit yield much decreased	HSP90	↓	DNA	<i>Solanum lycopersicum</i>	the suppression effect on cell death is induced by the inhibition effect of HSP90 and its co-chaperone SGT1.
<i>Tomato yellow leaf curl virus</i> (Anfoka <i>et al.</i> 2016)	plants stunted with small chlorotic puckered leaves; fruit yield much decreased	HSP90, HSP17	↓	DNA	<i>Solanum lycopersicum</i> (with downregulated HSF and thus HSP90)	combined heat and viral stresses induce the aggregation of HSP90 and HSP70. The maintenance of cellular chaperones in the aggregated state prevents the circulation of free soluble forms causing an additional decrease in stress response efficiency.
<i>Abutilon mosaic virus</i> (Krenz <i>et al.</i> 2010)	Yellow mosaic	cpHSC70-1	↓	ssDNA	<i>Nicotiana benthamiana</i> silenced cpHSC70	functional relevance of viral movement protein-chaperone interaction for viral transport and symptom induction
<i>Pepino mosaic virus</i> (Mathioudakis <i>et al.</i> 2012)	mosaic, dark green enations on the abaxial surfaces	HSC70	N.A.	(+) ssRNA	<i>Solanum lycopersicum</i>	interaction with PepMV CP during infection
<i>Potato virus Y</i> (Hofius <i>et al.</i> 2007)	mild to severe leaf mottling or streak or 'leaf-drop streak' with vein necrosis	Dna-J like proteins, designated Nt CIPs	↑	(+) ssRNA	<i>Nicotiana tabacum</i>	<i>in vivo</i> confirmation for the essential role of plant chaperones in virus movement.
<i>Tobacco mosaic virus</i> (Chen <i>et al.</i> 2008)	leaf mosaic, severe crop losses	HSP70	↓,↑	(+) ssRNA	<i>Nicotiana benthamiana</i>	enhancement of <i>N. benthamiana</i> viral infection by diverse viruses
<i>Potato virus X</i> (Chen <i>et al.</i> 2008)	symptoms vary, some strains symptomless, others induce necrotic streaks	HSP70	↓,↑	(+) ssRNA	<i>Nicotiana benthamiana</i>	enhancement of <i>N. benthamiana</i> viral infection by diverse viruses
<i>Watermelon mosaic virus</i> (Chen <i>et al.</i> 2008)	mosaic, mottling, leaves and fruit malformed	HSP70	↓,↑	(+) ssRNA	<i>Nicotiana benthamiana</i>	enhancement of <i>N. benthamiana</i> viral infection by diverse viruses
<i>Cucumber mosaic virus</i> (Chen <i>et al.</i> 2008)	mosaics and stunting, reduced fruit yield	HSP70	↓,↑	(+) ssRNA	<i>Nicotiana benthamiana</i>	enhancement of <i>N. benthamiana</i> viral infection by diverse viruses
<i>Tobacco mosaic virus</i> (Jockusch <i>et al.</i> 2001)	leaf mosaic, severe crop losses	HSP18, HSP70	N.A.	(+) ssRNA	<i>Nicotiana tabacum</i> temperature sensitive CP mutants	the induction of HSPs paralleled the amount of insoluble CP in leaf extracts.
<i>Potato virus Y</i> (Makarova <i>et al.</i> 2018)	mild to severe leaf mottling or streak or 'leaf-drop streak' with vein necrosis	HSP70 HSP90	N.A.	(+) ssRNA	<i>Solanum tuberosum</i> thermosensitive and thermoresistant cultivar	response to heat stress and PVY in cultivar-specific manner.

Virus	Symptoms	Factor	Up- (↑) or down- (↓) regulation	Nucleic acid	Plant	Function
<i>Potato virus Y</i> , strain <i>NTN</i> , (Hyskova <i>et al.</i> 2021)	necrotic middle veins and chlorotic, crinkled leaves with mosaic	cytosolic HSP70, Chl. HSP70, Mit. HSP70, HSP90-1	N.A.	(+) ssRNA	<i>Nicotiana tabacum</i>	the order of HS application and inoculation affect the virus propagation. In the beginning HSPs help the virus, but at a later stage specific isoforms could be also part of the plant's defense.

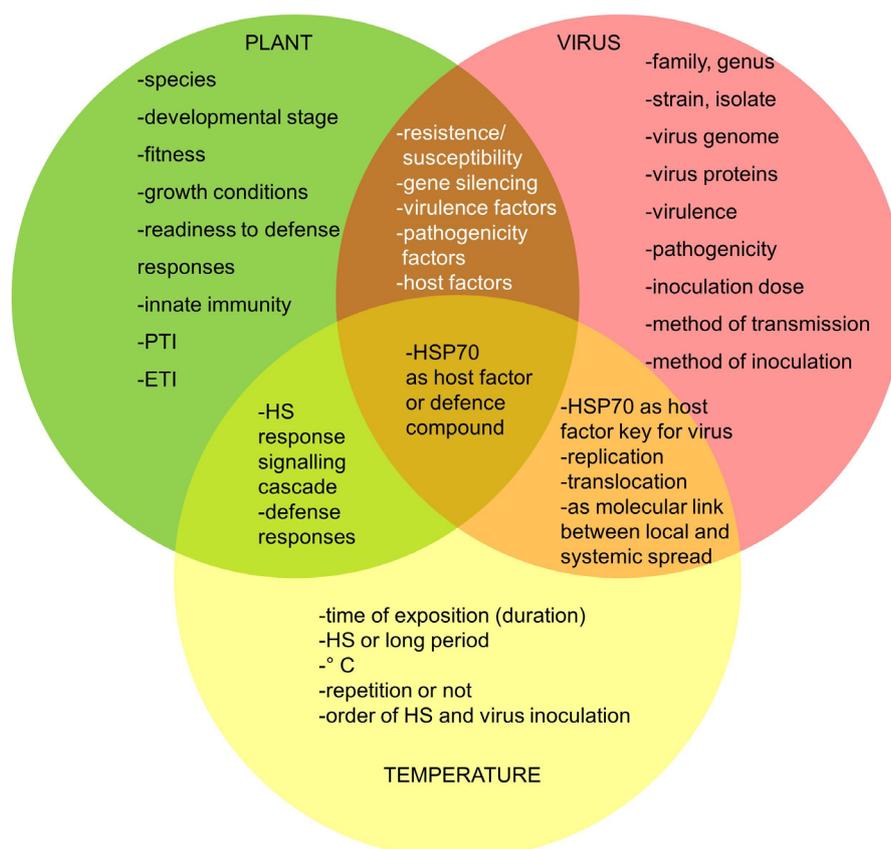


Fig. 2. Factors and conditions that determine whether HSP70 will act as host factor accelerating virus replication and spread or as a defense compound.

(Anfoka *et al.* 2016, Moshe *et al.* 2016). *Tomato yellow leaf curl viruses* suppress cell death induced by the inhibition of HSP90 (Moshe *et al.* 2016).

Viral coat proteins (CP) act as multifunctional proteins involved in almost every stage of the viral infection cycle. Their versatility is achieved *via* specific CP interactions with viral and host components in the infected cell (Ivanov and Makinen 2012). Hafren *et al.* (2010) found that the function of a potyviral CP (which can interfere with viral gene expression) is regulated by HSP70 together with its co-chaperone capsid protein interacting protein (CPIP). Other studies also suggest that HSP70 affects viral CP local movement from cell to cell or long-distance transport through the vascular system (Table 2). Some viruses (Closteroviruses) even form viral HSP70 homologs, which enable their translocation (Karasev 2000, Alzhanova

et al. 2001). Thus, Closteroviruses, for example, need a specialized movement device with HSP70h most likely because they are exceptionally long (Alzhanova *et al.* 2001). Similarly, a subsequent study highlighted a new function of the HSP70 viral homolog as a molecular link between the local and systemic spread of *Beet yellows virus* by docking the long-distance transport factor p20 to virions (Prokhnovsky *et al.* 2002).

Cytoplasmic HSP70 enhances *Nicotiana benthamiana* infection with *Tobacco mosaic virus*, *Potato virus X*, and *Watermelon mosaic virus* (Chen *et al.* 2008). Heat stress inhibits the tobacco and potato hypersensitive response to *Tobacco mosaic virus* and *Potato virus X* (Kiralý *et al.* 2008, Wang *et al.* 2009b). For example, in plants, HSP70 silencing by RNAi technology or chemical inhibition of HSP70 expression impairs a number of positive sense,

single-stranded RNA viruses (Wang 2015). Mutant HSP70-deficient plants exhibit reduced virus content and delayed infection onset (Hafren *et al.* 2010, Jiang *et al.* 2014, Gorovits and Czosnek 2017, Huang *et al.* 2017). Furthermore, HSP-deficient *Arabidopsis* plants are more tolerant to *Turnip mosaic virus* (Jungkunz *et al.* 2011), and HSP70 overexpression has been correlated with the accumulation of furoviral genomic RNAs (Yang *et al.* 2017). Interestingly, the potato cultivar resistant to *Potato virus Y* is also thermotolerant, with HSP70 increasing only at elevated temperature and not due to viral infection. Conversely, the *Potato virus Y* and thermosensitive potato cultivar also shows increased HSP70 synthesis in response to viral infection (Makarova *et al.* 2018).

The order of HS application and inoculation affects the virus propagation. HSP70 cytosolic, chloroplastic, and mitochondrial isoforms and stress-induced Hsp90-1 were significantly increased due to HS immediately after inoculation and then declined. On the other hand, cytosolic and mitochondrial Hsp70 with lower molecular mass were enhanced simultaneously with viral propagation and increase of salicylic acid content.

Hatch-Slack cycle enzymes that serve as markers of *Potato virus Y* infection do not respond to HS, so the activity of phosphoenolpyruvate carboxylase, NADP-malic enzyme, and pyruvate, phosphate dikinase corresponds to infection, regardless of previous HS treatment (Hýsková *et al.* unpublished results).

During human diseases as well, several HSPs70 are associated with viral particles, e.g., *rabies virus*, *hepatitis C virus*, *adenovirus*, *the human immunodeficiency virus type 1*, and *Zika virus*. In such infections, HSPs70 usually act as an infection factor promoting viral transcriptional activity and spread (Milani *et al.* 2016, Bolhassani and Agi 2019). HSP70 even serves as a biomarker and drugs targeting HSP70 (HSP70 inhibitors) are attractive candidates for therapeutics targeting various viruses, such as the *Zika virus*, which is intensively studied in connection with HSP70 inhibitors (Taguwa *et al.* 2019).

Conclusions

Many factors, conditions, and circumstances determine whether elevated temperature or HS inducing HSP70 promote or inhibit viral replication and propagation in plants (Fig. 2). In addition to predetermined factors (Fig. 2), such as plant species, innate immunity, or virus pathogenicity, the combination in time and space of plant predispositions, HSP70 content and viral infection affect the resulting function of HSP70 in the plant during a viral infection. Crosslinking individual signaling pathways (UPR, HSF, ROS, ROS scavengers and hypersensitive response, gene silencing) temporally and spatially determines the role of HSP70 in a virus-infected plant. Furthermore, the process known as acquired thermo-tolerance can interfere with plant defense responses.

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