Manipulation of Induced Resistance to Viruses

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Abstract (109 words)

Induced resistance against plant viruses has been studied for many years. However, with the exception of RNA silencing, induced resistance to viruses remains mechanistically less well understood than for other plant pathogens. In contrast, the induction processes involved in induced resistance, comprising basal resistance signaling, effector-triggered immunity, and phytohormone pathways, have been increasingly well characterized in recent years. This has allowed induced resistance to viruses to be placed in a broader conceptual framework linking it to other defense systems, which we discuss in this review. We also discuss the range of agents, including chemicals and beneficial microorganisms and application methods that can be used to induce resistance to viruses.

Highlights

- Plants possess multiple inducible defenses against viruses.
- The best understood is RNA silencing but others impact on virus replication and movement.
- It is now known that PAMP-triggered immunity inhibits infection by certain viruses.
- Various defense responses are triggered by naturally occurring plant signal molecules including the well established, such as salicylic acid, and novel signals including azelaic acid, glyceraldehyde 3-phosphate and pipecolic acid.
- A wide range of synthetic compounds and beneficial microbes that have been investigated as potential resistance inducers.

Introduction

Induced antiviral defense mechanisms attain full potency in response to microbial or chemical stimuli but are inactive or operational only at some basal level in unchallenged plants [1,2]. RNA silencing is an inducible adaptive antiviral mechanism [1,3]. However, since silencing has been recently reviewed by others [2,3], it will be discussed in detail here only where it functionally overlaps with, or reinforces, other induced resistance systems.

Induced resistance is exploitable through several approaches, including treatment with naturally occurring or synthetic chemicals [e.g. salicylic acid (SA) or BTH (benzothiadiazole/acibenzolar-S-methyl), respectively] (Figure 1), or beneficial microorganisms [2,4]. Alternatively, engineering genes encoding factors that regulate or execute induced resistance could enhance antiviral or general anti-pathogen defenses, although this will not be the focus of this article. Additional approaches may emerge from research on treatments that engender trans-generational epigenetic improvements in pathogen resistance [5,6].

Induced resistance has advantages for combating viruses because no agrochemicals analogous to fungicides or insecticides exist that can be used to prevent virus diseases under field conditions. Inducers stimulate endogenous resistance mechanisms that are less likely to harm non-target or beneficial organisms; also true for genetically-engineered plants with enhanced or faster-responding defenses. There are potential disadvantages, likely to be surmountable through additional research. For example, whilst some current inducers provide prolonged protection [7], others may engender incomplete or transient resistance (discussed in [2,4]), or decrease fitness and yield [8]. Additionally, more research is needed on the factors that control and execute anti-viral resistance, which are less well delineated than those mediating induced resistance against fungi, oomycetes and prokaryotes [1,9].

Basal resistance to viruses: a potential role for PAMP-triggered immunity

Plants are resistant to most potential cellular pathogens by detecting conserved pathogen-associated molecular patterns (PAMPs) with pattern recognition receptors (PRRs) that activate PAMP-triggered immunity (PTI) [10]. The best-studied *Arabidopsis thaliana* PRR, FLS2, perceives flagellin utilizing a partner kinase, BAK1 (SERK3), which also facilitates the activity of other PAMP and hormone-responsive PRRs including the BRI1 brassinosteroid receptor [11].

PTI manipulation may have potential for increasing crop resistance to viruses but it was once debatable whether or not it affected viruses [1]. Recently, BAK1 was found to be necessary for the limitation of the accumulation of tobamoviruses and turnip crinkle virus (TCV) [12,13]. Titer and symptoms for TCV and two tobamoviruses were enhanced in *bak1* and *bkk1* (BKK1 encodes another BRI1 partner) mutants [12,13]. Viral dsRNAs may also be able to trigger resistance via PTI, in addition to their ability to initiate antiviral silencing [14].

A geminivirus nuclear shuttle protein binds the tomato BAK1-related factor NIK1 [15,16] and the plum pox virus coat protein inhibits PTI against that virus in Arabidopsis and *Nicotiana benthamiana* [17]. These viral proteins appear to be acting as *effectors*; i.e., a pathogen-encoded counter-defense molecules [18]. In Arabidopsis, cucumber mosaic virus triggers PTI without any apparent effect on virus accumulation [19]. However, this up-regulates glucosinolate biosynthesis, which inhibits prolonged feeding by aphid vectors, and promotes their onward migration. This suggests that CMV manipulates PTI to enhance insect-mediated transmission [19].

Effector-triggered immunity and systemic acquired resistance

Pathogens that overcome PTI exert powerful selection pressures on plants, driving evolution of dominant resistance (*R*) genes [18]. Most *R* genes encode leucine-rich-nucleotide-binding domain (NB-LRR) proteins enabling direct or indirect detection of effectors (effector-triggered immunity, ETI) [18]. ETI induces a strong local hypersensitive response (HR) that restricts pathogens to inoculation sites, which may trigger plant-wide resistance enhancement (systemic acquired resistance, SAR). Several of the best-characterized *R* genes provide virus resistance, although antiviral NB-LRRs are not different in overall structure to those that condition resistance to other pathogens [1,18]. However, it is notable that viruses (in contrast to cellular pathogens) are less able to evolve to generate viable mutants able to overcome genetic resistance [20].

ETI and SAR depend upon local and systemic signaling. SA and jasmonic acid (JA) are the best-studied defensive signal molecules [1], although recent findings have pointed to important roles in SAR induction for azelaic acid, glycerol-3-phosphate, and pipecolic acid as local and systemic defense signals [2,21] (Figure 1). SA plays important roles in virus resistance; however, the JA signaling network can influence susceptibility to infection. Transgenic tomato plants with increased systemin levels were less susceptible to CMV infection and necrosis induction by a satellite RNA [22]. Systemin is a peptide hormone that is part of the JA-mediated signaling network in tomato, associated most notably with wound-induced resistance to chewing insects [23], which makes its effect on CMV surprising.

Beneficial bacteria can stimulate or prime increased resistance to pathogens, as seen with increased resistance of Arabidopsis to CMV engendered by strains of *Serratia marcescens* and *Bacillus pumilus* [24,25]. The identities of microbial signals responsible for resistance stimulation remain unclear but the bacteria have been shown to trigger induced systemic resistance (ISR) [24]. ISR is a resistance phenomenon induced by non-pathogenic microbes that is dependent predominantly on JA- and ethylene-mediated signaling, which often takes the form of priming of defense-related gene expression, rather than immediate transcriptional activation [1] (Figure 2).

Salicylic acid-induced resistance to viruses

SA was first associated with plant defense through its effects on virus infection over 30 years ago, but SA-induced virus resistance in plants remains imperfectly understood [1,9,26,27]. SA is well known to have protective effects in plants and animals. Recent work by Klessig and colleagues suggest that organisms of both Kingdoms share some of the same target molecules for SA (reviewed in [26,27]), suggesting that this simple molecule has a long evolutionary history in regulation of stress and defense responses in diverse living organisms.

Depending upon the virus-plant combination, SA can inhibit replication, intercellular trafficking or systemic movement [1,2,21,28]. For at least one virus, SA has direct antiviral effects, rather than acting as a resistance-inducing signal molecule. Via an interaction with the host enzyme glycerol 3-phosphate dehydrogenase, SA inhibits positive-strand viral RNA synthesis by the replicase protein of tomato bushy stunt virus [29], but it is not known how common such direct antiviral effects of SA on replication are for other viruses. Plastids and mitochondria facilitate SA-induced resistance through signal transduction leading to altered nuclear gene expression, or through indirect effects on plasmodesmal function [1,30,31]. SA-induced virus resistance is not dependent on factors vital for resistance to cellular pathogens, such as pathogenesis-related proteins or NPR1 ('non-expressor of PR1') [1]. Although RNA silencing may contribute to SA-induced virus resistance, experiments with silencing mutants showed it to be dispensable [32]. SA stimulates gene expression and enzyme activity of the phytohormone-inducible silencing factor RNA-dependent RNA polymerase 1 (RDR1) [28]. RDR1 does not contribute to SA-induced resistance in inoculated tissues and appears to work in co-ordination with other, unknown, SA-induced mechanisms to inhibit viral invasion of developing tissues to ameliorate symptoms [28]. A recent study by Alazem and colleagues [33] indicates that another phytohormone, abscisic acid, has wider ranging effects than SA on silencing components.

The virus-SA relationship is an ambiguous one. SA is needed for a successful HR and either SA pre-treatment or induction of endogenous SA biosynthesis renders plants less susceptible to viruses. However, some viruses (potyviruses and CMV, for example) trigger SA accumulation (discussed in [9]) and the CMV 2b protein possesses domains facilitating this [34]. It is unknown why these viruses increase levels of this potent defense signal.

Engineering and application of induced resistance

In addition to genetic methods (see [35-37] of this issue), three general approaches have been used to inhibit virus accumulation and virus disease (usually, but not always linked), involving ectopic application to plants: (i) various metabolites from plants; (ii) synthetic chemicals, including phytohormone derivatives; and (iii) plant growth-promoting rhizobacteria (PGPR) plus bacterial-encoded proteins. The first of these approaches has been explored over many years with mixed and largely limited success, but the search continues. In addition, many of these plant substances have been shown to be activators of defense responses (reviewed in [2,4]). In fact, all three approaches seem to lead to one or more defense pathways mediated by the phytohormones: SA, JA, ethylene, abscisic acid and brassinosteroids (BR) [38] (Figure 1). Direct application of phytohormones can provide resistance; e.g., BR [39,40]. While SA or JA can also be used, the direct application of SA and JA to plants was superior, with JA followed by SA (1-3 days later) giving the best response [41,42]. Table 1 lists many of the various inducers or primers of defense that have been used and Figure 1 shows several resistance inducing chemicals and endogenous signals. In other cases, short peptides have been used, which are described in [43].

Better living through chemistry

A number of approaches have been made to put into practice the knowledge gained from molecular analysis of the pathways involved in SAR and ISR (see Table 1). These include direct application of SA analogs such as BTH; chemicals that induce SAR, such as BABA, chitosans, dufulin, eudesmanolides, eugenol, laminarins, lentinan, *p*-aminobenzoic acid, probenazole, strobilurin and tiadinil; chemicals that induce SAR and ISR, such as ningnanmycin; chemicals that induce an ABA-mediated response, such as chitosans; proteins inducing SAR, such as harpin, lactoferin and PeaT1; and rhizobacteria that induce either ISR or a mixture of ISR and SAR. Many of these substances have only been tested for SAR responses against TMV in tobacco plants containing the *N* gene for resistance to TMV. Since the treated leaves were inoculated with TMV, this test is actually for local acquired resistance rather than SAR. In most cases, the ability of the compound to either induce SAR or affect the systemic spread of a virus in the absence of an HR has not been evaluated.

The modes of action of many of these substances have been studied and generally affect one or both of the two major resistance pathways: SAR and ISR (Table 1). However, BR can activate a brassinosteroid disease resistance pathway independent of SAR [39,40], chitosans can induce SA and/or ABA mediated responses (reviewed in [44] and [38]), and laminarin, an algal β-1-3-glucan polymer induces an ethylene-mediated response, whereas sulfated laminarin induces an SA-mediated response [45]. While dufulin binds to harpin-binding protein 1 and activates SAR [46], dufulin or a derivative also interacts directly with viral capsid proteins of TMV [47] and cucumber mosaic virus [48], as well as a non-structural protein of southern rice black streaked dwarf virus [49]. Ningnanmycin also binds to the capsid proteins of TMV and inhibits particle formation [50]. The mechanism of action of soluble orthosilicic acid against viruses is not known, but for fungi, it appears that resistance involves pathways activated by SA, JA and ethylene [38].

What does not kill you makes you stronger

Although researchers from numerous countries have contributed significantly to this field, a large effort has been made in China over recent years to discover new antivirals that can be used in agriculture. These include the use of esterified milk whey (lactoferin), neutral polysaccharides from shiitake mushrooms (*Lentinus edodes*) (lentinan and sulfonated lentinan), oil of cloves (eugenol), sesquiterpine lactones (eudesmanolides) from *Wedelia trilobata*, degraded triterpene lactones (quassinoids) from *Brucea javanica,* a phytohormone (epibrassinolide), a cytidine-derivative antibiotic (ningnanmycin) from the bacteria *Streptomyces noursei var xichangensis*, a protein (PeaT1) from the fungus *Alternaria tenuissima*, a harpin protein (PopW) from the bacteria *Ralstonia solanacearum*, and synthetic compounds such as bis-pyrazoles, cyano-acrylates and α-amino phosphonate (dufulin). These induce varying ranges of resistance, from 25% to nearly 100%, yet the search goes on. Factors such as the cost of the agent, its required frequency of application, and to the extent to which it has a broad spectrum of activity and affects the plant all come into play as to its success in the field.

PGPR and other saprophytes have been used successfully to induce systemic resistance in several crops, resulting in loss of symptoms and reductions in viral titers. In most cases, the effects are through ISR, although *Trichoderma* species can also induce SAR (Table 1). In addition, bacterial proteins such as harpins [51] and PeaT1 [52] induce resistance to virus infection through SAR/ISR or just SAR, respectively. Both have seen application to the field, with PeaT1 being used in 4 million ha in China during the first two years of production [53], although the nature of the crops and breadth of virus resistance have not been reported. Therefore, there are currently a number of promising and apparently successful inducers available to engender virus resistance. And yet, the search goes on.

Conclusions and Future Potential

Although we have gained an improved understanding of how induced antiviral resistance mechanisms work, our knowledge is incomplete and perhaps we know more about factors (such as RNA silencing) that do not execute resistance, than those that do. The problem remains that induced resistance using activators – chemicals or resistance inducing microbes – does not necessarily provide virus resistance that is complete or prolonged [4]. However, the field has been far from stagnant. There have been some surprises in recent work such as the similarities between some induced resistance mechanisms shared between plant and animals [26,27] and PTI has been shown to affect viruses [12-14,17], which may provide new targets for novel resistance inducing chemicals. Additionally, recent progress in formulation of resistance-inducing compounds for improved treatment of plant tissues has allowed the delivery of molecules to induce RNA silencing of viruses [54] without the need for plant transformation or expression from engineered viral vectors (Figure 2). This offers the potential of improving induced resistance through direct effects on viruses by RNA silencing, in combination with other resistance inducers or through using the system to inhibit expression of negative regulators of resistance.

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Conflicts of interest

All authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Author declaration

All authors reviewed the final draft. The corresponding author had final responsibility for the decision to submit the manuscript for publication.

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The authors show that the non-protein amino acid BABA, long used as an ‘artificial’ resistance inducer, occurs *in planta* and may be an endogenous resistance priming agent.

Table 1. Agents used to trigger or prime induced resistance in plants.

Inducer/Primer	**Virus**[a]	**Host plant**	**Infection**	**Response**	**Reference**
β-aminobutyric acid (BABA)	TMV	Tobacco	HR[b,c]	SAR	[55]
Benzothiadiazole (BTH)	CCYV CMV CMV TMV TSWV	Melon Tomato Cantaloupe Tobacco Tobacco	Systemic Systemic Systemic Systemic Systemic	SAR SAR SAR SAR SAR	[56] [57] [58] [59] [60]
Bis-pyrazoles	TMV	Tobacco	HR	ND	[61]
Brassinosteroids	CMV TMV	Zucchini Tobacco	Systemic HR	BDR BDR	[40] [39]
Chitosans	TBSV, TNV 7 viruses AMV, PSV 6 viruses 4 viruses	Bean Bean Pea Solanaceae Quinoa	HR HR/Systemic Systemic HR/Systemic HR	ABA/SAR ND ND ND ND	[38] [44] [44] [44] [44]
Cyano-acrylates	TMV	Tobacco	HR	ND	[62]
Dufulin	SRBSDV TMV	Rice Tobacco	Systemic HR	SAR SAR	[63] [47]
Eudesmanolides	TMV	Tobacco	Systemic	SAR	[64]
Eugenol	TYLCV	Tomato	Systemic	SAR	[65]
Harpin (PopW)	TMV	Tobacco	HR	SAR, ISR	[51]
Lactoferrin	TMV TYLCV	Tobacco Tomato	HR Systemic	SAR ND	[66] [67]
Laminarin (sulfated)	TMV	Tobacco	HR	SAR/ET	[45]
Lentinan	TMV	Tobacco	HR	SAR?	[68]
Ningnanmycin	TMV	Tobacco	Systemic	SAR, ISR	[69]
p-aminobenzoic acid (PABA)	CMV	Capsicum	Systemic	SAR	[70]
PeaT1	TMV	Tobacco	HR	SAR	[52]

3-pentanol	CMV	Capsicum	Systemic	SAR, ISR	[71]
Probenazole (& saccharin)	TMV	Tobacco	HR	SAR	[72]
Quassinoids	PepMoV TMV	Capsicum Tobacco	Systemic Systemic	ND ND	[73] [74]
Silicon (orthosilicic acid)	TMV,TRSV	Tobacco	Systemic	ND	[75]
Spermine (polyamines)	CMV	Arabidopsis	Systemic	ND	[76]
Strobilurin (fungicide)	TMV	Tobacco	HR	SAR	[77]
Tiadinil	TMV	Tobacco	HR	SAR	[78]
PGPR: *Bacillus amyloliquefaciens*	BBWV,CMV, PepMoV	Capsicum	Systemic	ISR	[79]
PGPR: *Bacillus pumilus*	CMV	Arabidopsis	Systemic	ISR	[24]
PGPR: *Pseudomonas fluorescens*	CMV	Tobacco	Systemic	ISR?	[80]
PGPR: *Serattia marcescens*	CMV	Arabidopsis	Systemic	ISR	[24]
Penicillium simplicissimum	CMV	Tobacco	Systemic	ISR	[81]
Trichoderma harzianum	CMV	Tomato	Systemic	SAR/ISR	[82]

[a] Viruses: alfalfa mosaic virus (AMV), broad bean wilt virus (BBMV), cucumber chlorotic yellows virus (CCYV), cucumber mosaic virus (CMV), peanut stunt virus (PSV), pepper mottle virus (PepMoV), southern rice black-streaked dwarf virus (SRBSDV), tobacco mosaic virus (TMV), tobacco necrosis virus (TNV), tobacco ringspot virus (TRSV), tomato bushy stunt virus (TBSV), tomato spotted wilt virus (TSWV), and tomato yellow leaf curl virus (TYLCV).

[b] Other abbreviations: ABA = abscisic acid-mediated resistance; BDR = brassinosteroid-mediated disease resistance; ET = ethylene-mediated resistance; HR = hypersensitive response; ISR = induced systemic resistance; ND = not determined; SAR = systemic acquired resistance.

[c] HR indicates that there was an enhancement of HR/ETI-type resistance, which strictly is local acquired resistance.

FIGURE LEGENDS

Figure 1. Chemical resistance inducers and defense signals. (a) A selection of chemicals used as plant treatments for studies of induced resistance. Acetylsalicylic acid (Aspirin) (**I**) and 2,6-dichloroisonicotinic acid (**II**) were used in earlier studies of SAR induction [83,84]. Other inducers shown are benzothiadiazole (BTH) (**III**), probenazole (**IV**), *p*-aminobenzoic acid (**V**), and the non-protein amino acid β-aminobutyric acid (BABA) (**VI**). The inducers I-IV are synthetic chemicals. BABA (**VI**) was recently found to occur in plant tissue and to accumulate in response to pathogen attack [85]. **(b)** A selection of plant defensive signal molecules mentioned in this article: salicylic acid (**VII**), ethylene (**VIII**), azelaic acid (**IX**), jasmonic acid (**X**), brassinolide, a brassinosteroid (**XI**), abscisic acid (**XII**), pipecolic acid (**XIII**), and glycerol-3-phosphate (**XIV**). A number of the chemicals in (b), in particular VII, have also been used experimentally as exogenous inducers of resistance.

Figure 2. Overview of approaches used to induce resistance by treatment of plants with exogenous agents. A simplified representation of exogenous agents used to elicit induced systemic resistance, systemic acquired resistance (including enhancement of the hypersensitive response/effector-triggered immunity) and RNA silencing. Some of the endogenous signals involved in induction of these resistance mechanisms are indicated (also refer to main text, Table 1, and Figure 1). Abbreviations: abscisic acid (ABA); azelaic acid (Aza); glycerol 3-phosphate (G3P); jasmonic acid (JA), pipecolic acid (PA) and salicylic acid (SA).