Chapter 17
Viral, Fungal and Bacterial Disease Resistance in Transgenic Plants

Vinod Saharan, Devendra Jain, Sunil Pareek, Ajay Pal, R.V. Kumaraswamy, Sarita Kumari Jakhar, and Manvendra Singh

Abstract Continuing attention is being devoted to the development of substitute strategies in plant-disease management and reducing dependency on synthetic chemicals. Viral, fungal and bacterial diseases are unquestionably the most versatile for environmental adaption and in the destruction of plant growth. Among the strategies, resistance breeding has generated proven data and been exploited in depth. However, conventional methods alone are not sufficient to control the novel races of viral, fungal and bacterial pathogens in crops due to a scarcity in required crop variations. The current situation encourages the search for variation against biotic stress through identification of genes across species. Over the last two decades, significant efforts have been initiated in plant-disease management via genetic engineering. In addition, several molecular techniques have emerged to disentangle multifaceted plant-pathogen systems and associated disease-resistance candidate genes. Besides describing many promising candidate genes from viruses, fungi and bacteria, numerous plant disease-resistance genes have been identified and evaluated in crop improvement programs by transformation. Advancement in plant transformation techniques enables transferring useful genes for the rational creation of disease-resistant plants. Success has been achieved in transgenic crops against various diseases of important crop plants. This chapter describes genetically engineered plants and their resistant to viral, fungal and bacterial pathogens.

V. Saharan (✉) • D. Jain • R.V. Kumaraswamy • S.K. Jakhar • M. Singh
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur 313001, Rajasthan, India
e-mail: vinodsaharan@gmail.com; devroshan@gmail.com; kumarabiotech@gmail.com; saritajakhar5@gmail.com; manvendra.jhansi76@gmail.com

S. Pareek
Department of Agriculture & Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Kundli 131028, Sonepat, Haryana, India
e-mail: sunsil_ciah@yahoo.com

A. Pal
Department of Biochemistry, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar, Haryana, India
e-mail: ajaydrdo@rediffmail.com

J.M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits*, DOI 10.1007/978-3-319-22518-0_17

Keywords Antimicrobial • Disease resistance • Genetic engineering • Pathogenicity • Plantibodies • Plant pathogens • RNA interference

17.1 Introduction

Global warming, the human population explosion and shrinking arable lands are among the major issues which require a sustained solution to be able to feed the nine billion world population by 2050. Plant pathogens frequently alter their behavior to survive in changing environment (Fisher et al. 2012). Therefore, efforts made so far to control plant diseases are inadequate. Chemical pesticides in use are rapidly losing their potency against mutating plant pathogens. Furthermore, uncontrolled use of pesticides has raised serious concerns of pathogens developing resistance to them. (Bosch et al. 2014). These challenges could be efficiently met with biotechnological inventions. This review describes plant genetic engineering efforts to find novel strategies for plant disease management (Collinge et al. 2010; Saharan et al. 2008).

Advancements in transgenic technology have great potential to benefit farmers, consumers and agro-food industries worldwide. Under the specter of global warming, disease control is the primary focus in the coming years for sustained crop yield and quality under the stress of novel races of pathogenic fungi, bacteria and viruses. Since the Green Revolution, quantifiable successes have been achieved in disease resistance breeding programs by the transfer of resistant loci from wild relatives to commercial cultivars (Bruehl 1991; Gómez et al. 2009). However, pathogens continuously evolve mechanisms to overcome resistance in crop plants. The breakdown of resistance is common and an unstoppable event which induces broad epidemics in the concerned crops (Table 17.1) (Fry 1993; Talbot 2004). Pathogens have many components that promote their proliferation, establishment and the spread of disease in crops (Gururani et al. 2012; James 2003). Hence, before starting any exercise to deal with the novel pathogens, a thorough knowledge of the complexity of plant-pathogen interaction must be investigated (Gururani et al. 2012; Jackson and Taylor 1996). Development of disease-resistant crops has been stimulated by the inputs from the genomics and proteomics of plants and pathogens (Chern et al. 2001; Peremarti et al. 2010; Ramonell and Somerville 2002; Sanseverino et al. 2010). In this chapter, we critically review the basic understanding and advances in developing disease resistant transgenic crop plants.

17.2 Virus Resistant Transgenic Plants

A number of accounts of viral disease-resistant crops have been confirmed since the first report of a virus-resistant transgenic plant (Table 17.2) (Fitchen and Beachy 1993; Galvez 2012; Galvez et al. 2014; Kumar et al. 2012; Powell-Abel et al. 1986; Prins et al. 2008). The genetic engineering of virus-resistance crops has been

Table 17.1 Potential of present and future diseases of some commonly distributed crops

Disease	Distribution
Viral disease	
African cassava mosaic	Wide spread in Africa, Asia and America
Bunchy top of banana	Destructive in Asia, Australia, Africa and Pacific Islands
Bean golden mosaic	Caribbean Basin, Central America and Florida
Rice tungro disease	Severe crop losses in Southeast Asia
Fungal disease	
Downy mildew of corn and sorghum	Rapid distribution out of Southeast Asia
Late blight of potato and tomato	Emergence of new virulent races spreading worldwide
Karnal bunt of wheat	Severe crop losses in Middle East, USA and Asia
Sugarcane rust	Destructive in America and Africa
Chrysanthemum white rust	Important in Europe, Asia and USA
Citrus black spot	Severe in Central and South America
Bacterial disease	
Bacterial leaf blight of rice	Destructive in Japan, India and wide distribution
Cassava bacterial blight	Severe in Africa, America and Asia
Bacterial wilt of banana	Destructive in America and Africa

Source: www.plantwisr.org/knowledgebank/searchresult

comprehensively elevated by sequencing, isolation and cloning of a number of key genes of viruses. This along with associated advances in genetic transformation of a number of crops has opened up the possibility of an entire new approach in genetic engineering toward controlling plant-viral diseases (Young 2000).

17.2.1 Pathogen Derived Resistance

Pathogen-derived resistance (PDR) refers to the resistance obtained from a pathogenic virus. Therefore, the whole gene or a part of its sequence isolated from pathogenic virus is transferred to the susceptible plants to obtain resistance.

17.2.1.1 Viral Protein Mediated Resistance

Viral protein mediated resistance is a type of PDR exhibited when a transformed plant produces viral protein (coat protein, replicase protein and movement protein) that interferes with the life cycle of the invading virus. This type of resistance is further divided into three groups (a) coat protein mediated resistance (CPMR), (b) replicase mediated resistance (Rep MR) and (c) movement protein mediated resistance (MPMR).

Table 17.2 Virus resistance transgenic crops

Candidate PDR genes	Viral disease	Host plant	Targeted gene	References
Coat protein mediated	Ring spot virus (PRSV)	Papaya (*Carica papaya*)	PRSV-CP	Wani and Sanghera (2010)
	Zucchini yellow mosaic 2 Potyvirus	Squash (*Cucurbita maxima*)	ZYM2P- CP	Meng and Gubba (2000)
	Citrus psorosis virus (CPsV)	Citrus (*Citrus* sp.)	CPsV – CP	Zanek et al. (2008)
	Potato virus – X	Potato (*Solanum tuberosum*)	PVX- CP	Bai et al. (2009)
Replicase mediated	Rice yellow mottle virus (RYMV)	Rice (*Oryza sativa*)	RdRp	Palukaitis and Zaitlin (1997)
	Potato leaf roll virus (PLRV)	Potato	PLRV- Rp	Ehrifeild et al. (2004)
	Bean golden mosaic virus (BGMV)	Bean (*Phaseolus vulgaris*)	BGMV-Rp	Faria et al. (2006)
Movement protein mediated	Astobra, Caulimo, Nepo virus	Tobacco (*Nicotiana tabacum)*	MP	Cooper et al. (1995)
	Tobacco mosaic virus (TMV)	Tobacco	MP-P^{30}	Prins et al. (2008)
Post transcriptional gene silencing (RNAi)	Potato spindle viroid	Tomato	SiRNA of transformation vector	Schwind et al. (2009)
	African cassava mosaic virus (ACMV)	Cassava (*Manihot esculenta*)	Rep/AC-1	Vanderschuren et al. (2009)
Satellite RNA mediated	Cucumber mosaic virus (CMV)	Tomato	HV-CMV	Cillo et al. (2004)

17.2.1.2 Coat Protein Mediated Resistance

Coat protein mediated resistance (CPMR) is a type of transgenic virus resistance crop plants have developed by exploiting coat protein (CP) encoding sequences (Anna et al. 2002; Ferreira et al. 2002; Lehmann et al. 2003; Makeshkumar et al.

2002; Mundembe et al. 2009; Nomura et al. 2004). Appropriate CP sequences are isolated from the concerned virus genome with certain modifications, and transferred with regulatory sequences to target plants. Compared to control plants, resistance is observable in transgenic plants in the form of delayed appearance of symptoms as well as by reduced virus titer. Generally two mechanisms have been established for depicting CPMR (Beachy and Philos 1999). First, *recoating of invading viruses*, which describes how an expressed CP subunit recoats the invading genetic material of a virus. A recoated virus genome is incapable of exploring −ve RNA for reverse transcriptase. In the case of + ve RNA, the virus does not have access to host ribosome for viral protein synthesis. Thus invading viruses cannot multiply and therefore cannot infect the plants. The second mechanism, *blocking the receptors in transgenic cells*, could be described as various subcellular components acting as receptors or uncoating the site for invading viruses. The CP subunit expressed through a transgene, binds to receptors and prevents the association of virions with the receptor, thus making it unable to penetrate the plant. The transformed tobacco expressing TMV CP subunit not only expressed CPMR against tobacco mosaic virus, but also against the closely-related virions. This might be explained through significant homology in gene encoding CP subunits of two different viruses. Other strategies can be adapted to increase broad spectrum CPMR via multiple gene transformation for different CPs and the searching of homology sequences in gene encoding CP subunits. Specific mutation in CP coding sequence translated in transgenic cells produce defective subunits that have more inter subunit interaction and lead to aggregation of subunits with virus coded CP. Field performance of transgenic papaya and squash made CPMR a prime choice to integrate resistance in other economically-important crop plants. Tomato, cucumber, watermelon and potato are some other important crops that have been successfully transformed with CPs to achieve resistance against viral diseases (James 2014). Freedom II is another commercially-released transgenic squash which affords resistant to zucchini yellow mosaic (Meng and Gubba 2000; Gubba 2000). Similarly, in citrus introduction of a CP gene against the citrus psorosis virus (CPsV) was reported to be successful by Zanek et al. (2008). CP-transgenic papaya, namely Sun up and Rainbow, were the first such commercialized fruit trees in Hawaii (Wani and Sanghera 2010).

17.2.1.3 Replicase Mediated Resistance

In replicase mediated resistance (Rep MR), viruses need a replicase enzyme to perform replication of their genetic material in the host cell. The origin of Rep MR can be explained by the fact that mutated or truncated replicase express in host plant and impede replication of virus genetic materials. Therefore, a truncated replicase encoding gene has been tried in many crops for viral resistance. Rep MR acts at two levels, one at the transcriptional level by interfering RNAs and the other at the translational level by interfering with truncated Rep protein (Lawson et al. 2001). Indeed, it is still not clear which mode of Rep MR is acceptable universally. This ambiguity is due to the contradictory reports asserting the presence of truncated rep protein or

the presence of Rep RNA species in host cytoplasm. Transgenic rice expressing the RdRp of rice yellow mottle virus (RYMV) proved to have stable resistance to RYMV strains (Palukaitis and Zaitlin 1997). C1 gene encoding Rep from tomato yellow leaf curl Sardinia virus (TYLCSV) confers resistance to viral disease in *Nicotiana benthamiana* and tomato plants (Brunetti et al. 1997). Similarly, in potato, the complete sense PLRV replicase gene provided resistance to potato leaf roll virus (PLRV) (Ehrifeild et al. 2004). Transgenic tomato carrying a truncated replication associated protein gene of tomato yellow leaf curl virus-Israel (TYLCV-Is [Mild]) conferred resistance to TYLCV-Is. *Phaseolus vulgaris* carrying the rep gene of bean golden mosaic virus (BGMV) also manifested resistance to BGMV (Faria et al. 2006). However Rep MR showed a relatively narrow spectrum of resistance, i.e. resistance manifested only for the particular virus race from which the transgene was isolated. Hence, research on Rep MR in crop plants has not been further exploited.

17.2.1.4 Movement Protein Mediated Resistance

Movement protein mediated resistance (MPMR), as the name implies, has as its proposed function to facilitate movement of nucleoprotein and/or viral particles, intercellular/intracellular, through plasmodesmata and tubules. Movement proteins (MP) and virus together make a complex of 1.5–2.0 nm diameter which can easily pass through plasmodesmata (Citovasky et al. 1992). Transgenic plants expressing MP showed delayed infection with mild symptoms of viral disease. This strategy also manifested a broad spectrum resistance as the dysfunctional MP-tobacco plants interfered with the systemic spread of distantly-related and unrelated viruses such astobra-, caulimo- and nepo-viruses (Cooper et al. 1995). It appears that two distinct plasmodesmatal transportation mechanisms are utilized. The first is involved in increasing the size exclusion limits of plasmodesmata during localized trafficking of MPs. The second involves large tubular structures composed of MPs that appear to facilitate the movement of viral particles through enlarged plasmodesmata (Jackson and Taylor 1996). Tobacco plants engineered with P^{30} MP of TMV (lacking N- terminal amino acids), showed delayed appearance of infection and symptom of disease. The expression of dysfunctional or mutated MP genes has reported the broader resistance, compared to CP/Rep mediated resistance (Prins et al. 2008).

17.2.1.5 Viral RNA Mediated Resistance

Viral RNA mediated resistance (VRMR) relates to the fact that most of the disease-causing plant viruses have a RNA genome that encodes all essential proteins viz. movement proteins, coat proteins, replicase proteins etc. Previously, it was assumed that over-expression of one or more structural or functional proteins in a normal or a dysfunctional state in transgenic plants would confer protection against the virus at protein-level interaction. Several examples have justified the above statement

(discussed in CPMR and Rep-MR) whereas in several others the above statement is not true. So transgene appears to have confirmed resistance through its mRNA rather than by its encoded proteins (Jianping et al. 2001; Jiunn et al. 2003; Khaled et al. 2002; Nomura et al. 2004). Hence, the phenomenon produced from the results of further study is known as viral RNA-mediated resistance.

Lindbo and Dougherty (1992), in experiments with the transgenic tobacco expressing CP gene, did not find a considerable concentration of CP, but reported CP transcripts in cytoplasm which provided considerable resistance against tobacco etch virus. Jiunn et al. (2003) carried out molecular analysis of nine selected transgenic lines of papaya harboring ring spot CP gene and found it to exhibit different levels of resistance. The analysis revealed that the expression level of the transgene is negatively correlated with the degree of resistance. This finding suggests that the resistance is manifested by a RNA-mediated mechanism. Baulcombe (1996) reported several VRMR characteristics which help to understand the complicated phenomenon of virus resistance. Doughorty and Parks (1995) provided considerable insight into VRMR and proposed that transgene mRNA in virus-resistant plants induce degradation of RNAs with the same or complementary sequence within cytoplasm which has arrived from infected virus. This attractive hypothesis has received much support in recent years. As a result, a well-established phenomenon of gene silencing known as post transcriptional gene silencing (details in PTGS)/co-suppression/antisense suppression/VRMR/RNA interference was given. However, instead of adapting the traditional VRMR, they found that design of an RNAi system in crop plants has more versatility against viruses (Galvez et al. 2014).

17.2.1.6 Post Transcriptional Gene Silencing/RNA Interference

Post transcriptional gene silencing and RNA interference (PTGS/RNai) is another strategy to create viral disease resistance in plants. In cross-protection, an initial viral infection generates small interfering RNAs (siRNAs) species which provide immunity to further viral attack. These siRNAs have sequence homology with infecting viral genetic material. Therefore, siRNAs commence an RNA complex pathway to viral genetic material which is a favorable substrate for endogenous RNA degrading enzymes. As a result the virus cannot proliferate in the host (Doughorty and Parks 1995; Galvez et al. 2014; Kubota et al. 2003). Despite being elicited by homologous RNA species, RNA interference is also triggered by self-complementary hairpin RNAs. This cruciform structure is a very favorable substrate for RNAi enzyme machinery. As a consequence, a large number of siRNA populations have emerged to act on complementary RNAs species in the cytoplasm.

RNAi technology has been exploited through transgenic-mediated synthesis of siRNAs (Ghildiyal and Zamore 2009; Leibman et al. 2011; Wang et al. 2010). In this strategy, key conserved sequences of the viral genome are used in designing a hairpin RNA transformation vector which has inverted repeats separated by non-coding sequences. These inverted repeats of the hairpin RNA transformation vector produce hairpin RNAs. These hairpin RNAs are further subjected to DICER and

RISC (RNA inducing gene silencing complex) enzymes for subsequent production of siRNA and further degradation of the target viral genome in the host cytoplasm. Transgenic tomato plants exhibited resistance against potato spindle viroid through siRNA using a similar transformation vector (Schwind et al. 2009). In another report, the engineered transgenic cassava plants showed resistance to African cassava mosaic virus (ACMV) by expressing dsRNAs. Transgenic cassava lines with high levels of AC1-homologous small RNAs have ACMV replication associated with protein coding sequence imparting Rep/AC1-homologous hairpin double strain immunity (Vanderschuren et al. 2009).

17.2.1.7 Satellite RNA Mediated Resistance

Certain RNA sequences packed with a viral genome cannot replicate, move and pack independently but require assistance from viral genome sequences called satellite RNA (Lin et al. 2013). A viral genome which helps satellite RNA to perform its function is known as a helper virus (HV). Some strains of CMV encapsulate the satellite RNA in addition to their own function of coding messenger RNA. CMV satellite RNA depends on its HV CMV for their essential functions. A very good example of using multiple or partial copies of CMV satellite RNA is to display reduced symptoms against CMV in tomato transgenics (Cillo et al. 2004). Little is known about the mechanism of satellite RNA mediated resistance but this has been explained by RNA gene silencing. In adopting this new concept of resistance, sufficient caution must be taken as there are chances of generating novel viral sequence *super pathogens* (Dempsey et al. 1998).

17.2.2 Non-pathogen Derived Resistance

Non-pathogen derived resistance (NPDR) refers to resistance obtained from a non-virus origin i.e. gene(s) derived either from plants or any other non-pathogenic sources.

17.2.2.1 Ribosome Inactivating Proteins (RIPs)

Ribosome inactivating proteins (RIPs) are specific N-glycosidases that eliminate a specific adenine from the sarcin/ricin loop of the 28S rRNA. These proteins are committed to arrest protein synthesis at the translocation step and are synthesized as pre-pro protein in plants and stored in cell vacuoles (Stirpe 2013). Their translation-inhibiting activity has been exploited against viral diseases. Studies have revealed that RIPs act on virus protein synthesis in the host plant cells and therefore the infected virions are not able to generate the protein for their multiplication. The pokeweed antiviral protein (PAP; RIP Type-1) coding gene expressed in transgenic tobacco shows a low level of resistance against many unrelated viruses. Besides its

resistant nature towards pathogenic viruses, it also has toxic effects on plants. However, a terminal deletion mutation in PAP has shown antiviral activity without causing toxic side effects to the host plant. Type-1 RIP from iris bulbs, called IRIP, has been transferred to tobacco. Molecular studies of the transgenic tobacco plants and characterization of purified protein have revealed that the recombinant IRIP from tobacco leaves has the same molecular structure as the native protein from iris bulbs. The tobacco transformants showed no apparent phenotypic side effects indicating that ectopically expressed IRIPs are not cytotoxic to tobacco cells. Antiviral activity and lack of cytotoxity of the expressed IRIP in transgenic tobacco renders IRIP an interesting and useful tool for the engineering of virus resistance (Baranwal et al. 2002; Desmyter et al. 2003; Wook et al. 2002).

17.2.2.2 Viral Protease Inhibitors from Plants

Viral protease inhibitors from plants, studied with respect to their viral structural and functional proteins, revealed the necessity to process their polyproteins for survival in host cells. Some groups of viruses, namely clostero-, nepo-, como- and potyviruses, require cysteine protease activity to process their nascent polyprotein for replication. The plant community expresses various protease inhibitors which impart natural resistance towards viruses. Transgenic tobacco expressing cysteine protease inhibitors from rice has been successfully tested against tobacco etch virus (TEV) (Gutierrez-Campos et al. 1999). Despite these encouraging results, this method could not be implemented where certain viruses did not require protein processing. In addition, it has been reported that cloned genes for viral protease inhibitors have deleterious effects on plant enzyme systems (Blandenvoorde et al. 2000).

17.2.2.3 Plant Antibodies

Plant antibodies (Av-plantibodies) represent an attractive approach to protect plants against pathogens and create plants that are endogenously resistant to pathogens. This can be achieved by using genetic engineering techniques such as expressing heterologous antibodies and antibody fragments for producing *designer* plants with viral resistance. These plant antibodies are known as *plantibodies*. Functional full-size antibodies and single-chain variable fragments (scFv fragments) can be targeted to different compartments of the plant cells. Cytosolic expression of specific scFv fragments can be used to protect plants from intracellular pathogens and to inhibit enzymes or hormones involved in the growth of viral pathogens. Extracellular targeting such as to the plasma membrane or retention in the endoplasmic reticulum gives high expression levels of correctly folded recombinant antibodies in plants. Targeting antiviral scFv fragments to plant cell membranes via heterologous mammalian membrane anchors has conferred resistance to tobacco mosaic virus. These surface expressions of the virus-specific scFv fragment may be a novel approach to shield the plant cell from an invading pathogen. Combining this strategy with cytosolically-expressed scFvs specific for conserved viral functional domains such

as movement proteins or replicase protein could provide an even more attractive route for generating virus-resistant plants. Recently, a cytosolical-expression system was used to achieve virus resistance based on the expression of scFvs against a conserved domain in a plant viral RNA-dependent RNA polymerase, a key enzyme in virus replication. The selected scFvs inhibits complementary RNA synthesis of different plant virus RDRP in vitro and virus replication in planta. Moreover, the scFvs are also bound to the RDRP of the distantly-related hepatitis C virus. T1 and T2 progeny of transgenic lines of *Nicotiana benthamiana* expressing different scFvs either in the cytosol or in the endoplasmic reticulum showed various degrees of resistance against four plant viruses from different genera. Virus resistance based on antibodies to RDRP adds another tool to the repertoire for combating plant viruses (Boonrod et al. 2004).

17.3 Fungal Resistant Transgenic Plants

Plant pathogenic fungi are considered the most versatile for environmental adaption and in the destruction of plant growth. Among the several approaches, genetically-engineered plants are assumed to impart resistance against fungal pathogens. Expression of antifungal compounds in transgenic plants has been a major approach to protect against fungal diseases and reduce the dependency on harmful synthetic fungicides (Wani 2010).

17.3.1 Antifungal Compounds

A wide range of antifungal compounds have been screened against fungal pathogens. Compounds which inhibit fungal growth are abundant in nature (Hegedüs and Marx 2013; Van Der Weerden et al. 2013). These antifungal compounds are natural sources of resistance in plants during various stages of development. Genes encoding such compounds for fungal-disease resistance are discussed below.

17.3.1.1 Chitinase and Glucanase

Chitinase and glucanase, the two most pivotal enzymes, have been studied in detail with respect to plant and fungal populations. Chitinase and glucanase catalyze the hydrolysis of two major structural components chitin and glucan, respectively, of the cell wall of many fungi. Chitinase genes have been identified from plants and micro-organisms and are broadly known as the PR-3 class of proteins. A number of reports of obtaining fungal-disease resistance through transformation of chitinase genes in many crops are available. The other enzyme glucanase is classified as a PR-2 class of proteins and are less studied compared to chitinase. These PR proteins

are inducible in nature and express under various conditions of pathogen attack, wounding, physico-chemical stress, etc. (Van Loon et al. 1994). Expression of chitinase and glucanase at low levels in transgenic plants has been a key issue. The low expression level of chitinase and glucanase transgenes depends on the host internal system viz. intracellular pH, cellular localization and environmental stress (Sela-Buurlage et al. 1993). Hence, isolation and selection of different chitinases and glucanases genes need to be screened to confirm their appropriate expression in a target crop. Chitinase of rice, lycopersicum, of fungal origin, has proved to be a good candidate in achieving resistance against fungal disease in certain crops (Tabei et al. 1998; Yamamoto et al. 2000). This has also proved that pyramiding of these two genes in transgenic crops promotes higher levels of resistance against fungal pathogen (Ram and Mohandas 2003; Wang et al. 2003). Studies have concluded that these enzymes hydrolyze the fungal cell walls and release oligo-N-acetyl glucosamines which function as elicitors for activation of a defense-related response in rice cells. In field trials, transgenic canola constitutively expressing a tomato endo-chitinase gene was found to exhibit increased resistance to fungal pathogens (Van Loon et al. 1994). In transgenic carrot, chitinase, β-1,3-glucanase in combination with AP24 gene gave rise to a broad spectrum fungal resistance (Stuiver et al. 2000). In general, tobacco, potato, sugar beet and rice have been transformed with chitinase gene and were found to be resistant to the fungus *Rizoctonia solani*. However, challenges still remain for those oomycetes, such as *Phytophthora* and *Pythium,* which do not contain chitin and, therefore, chitinases are ineffective (Datta et al. 2001).

17.3.1.2 Osmotin and Thaumatin-Like Proteins

Osmotin and thaumatin-like proteins (OLP and TLP) are important anti-fungal compounds. Most anti-fungal proteins found in plants share sequence homology with thaumatin, the sweet-testing proteins from the African shrub *Thaumatococcus daniellii* (Stintzi et al. 1991). These proteins have molecular masses of 22–26 kDa and are classified in the PR-5 family of pathogenesis-related proteins. These thaumatin-like proteins get induced upon microbial infection, oxidative stress, ABA, salicylic acid, methyl jasmonate, ethylene and certain wounding. Structural analysis has revealed their resistance to pH and heat denaturation by the presence of 16 cysteine residues which form 8 disulfide bonds. Broadly speaking, these PR-5 proteins induce fungal cell leakiness presumably through specific interaction with the plasma membrane which results in the formation of transmembrane pores. Transgenic potato plants expressing the tobacco osmotin (similar to thaumatin-like protein), which is basic 24 kDa pathogenesis-related protein that accumulate NaCl and regulate hormonal and environmental signals (Kononowicz et al. 1992). This showed delayed development of disease symptoms against *Phytophthora infestans* (Liu et al. 1994). Over-expression of rice TLP in rice itself, American ginseng, carrot and tobacco enhanced the resistance to various fungal diseases (Babu et al. 2003; Datta et al. 1999; Punja and Chen 2004; Velazhahan and Muthukrishnan 2004).

17.3.2 Small Cysteine Rich Proteins

Small cysteine rich proteins are usually small, cationic and amphipathic proteins having open-chain forms. The amphipathic structure with a α-helix and an anti-parallel β-sheet is highly conserved. The cationic hydrophobic residues are organized as segregate patches, resulting in a structure that is capable of forming ion channels through membrane bilayers. Furthermore, the compact and rigid structure is maintained by three or four disulfide bonds through cysteine residues. Following are two important small cysteine rich proteins which have an immense role in antifungal activities.

17.3.2.1 Defensins

Defensin, a plant antimicrobial protein, is a feasible natural candidate for fungal-disease control (Aerts et al. 2011; Carvalho and Gomes 2009; Kaur et al. 2011). Plant defensins are small cysteine-rich proteins consisting of 45–54 amino acids. They are synthesized naturally in plants, especially in seeds, and found in almost all plant organs. Although a majority of the defensins are secreted in the extracellular space, a few floral defensins are targeted to the vacuole. The best characterized defensins from radish Rs-AFP2 peptide shows enhanced resistance against the fungus *Alternaria longipipes* in transgenic tobacco. The remaining antifungal activity of the two groups, M-AMP2 and Ac-AMP2 peptides, have been proved in in vitro models only. A novel alf-AFP defensin peptide isolated from seeds of *Medicago sativa* displays robust activity against the fungal pathogen *Verticillum dahiliae* (Goa et al. 2000). The defensins peptide complex contains 4, 6 or 8 invariant cysteine residues which form intermolecular disulfide bonds. They contribute to the protection of seedlings against harmful microorganisms (analogous to the common fungicide coating of crop seeds) (Erik and Biezen 2001). However, defensins are generally not effective against bacteria (Broekaert et al. 1995).

17.3.2.2 Thionins

Thionins are small cysteine rich peptides (5 kDa) usually basic, very compact, amphipathic structures stabilized by three or four disulfide bridges and exhibit antibacterial and antifungal activities. Like defensins, the nascent protein chain of thionins is synthesized as pre-proteins and secreted into the vacuoles, intracellular spaces and cell wall. Naturally, thionins are expressed in the seeds, stems, roots of etiolated or pathogen stressed plant species. Notable results were obtained against *Fusarium oxysporium* f. sp. *matthiolae* in transgenic *Arabidopsis* expressing thionin peptide Thi2.1 (Epple et al. 1997). Accumulation of multicopy genes of the AMP group of thionins provides enhanced expression levels in transgenic crops (Isabelle et al. 2002). These small proteins are ancient systems of immune protection that

express during infection, inflammatory event and wound repair and their presence constitutes a key innate host defense against pathogens (Hancock and Diamond 2000). Thionins have a cationic charge which facilitates electrostatic attraction to negatively-charged surfaces of fungus. Their ability to assume amphipathic structures allows direct interaction with ubiquitous phosphoglycerol-lipids and their incorporation into microbial membranes. These peptides inhibit the growth of a broad range of the fungi at micro and molar levels in vitro, which is manifested by the changes in fungal morphology (i.e. reduced hyphal elongation and hyphal branching). Manipulation in attached signal peptide enables pathologists and molecular biologists to target these cysteine-rich peptides to specific sites of cells where a particular fungal attack predominates.

17.3.3 Plant Ribosome Inactivating Proteins

Plant ribosome inactivating proteins (RIPs) are RNA N-glycosidases that cleave a specific adenine residue in highly conserved sequence of 28S rRNA and inhibit the elongation factor eF-la to bind with ribosome. This irreversible modification blocks translation in ribosome assemblies. Some RIPs inactivate host-specific ribosome while others exhibit toxicity towards ribosomes from distantly-related species including animals and fungi (Stirpe et al. 1992). Based on structural diversity, plant RIPs are classified into three types (Table 17.3). As discussed in Sect. 17.2 above, these RIPs do not act on their own ribosome because they are targeted to vacuoles that sequester a certain development process. A RIP isolated from barley was shown to exhibit in vitro antifungal activity against a number of plant pathogenic fungi. Transgenic tobacco plant expressing isolated barley RIP gene under the control of inducible promoter showed increased resistance to *Rhizoctonia solani* (Logemann et al. 1992). An effective resistance was recorded in tobacco transgenics expressing

Table 17.3 Different types of ribosome inactivating proteins

RIP	Structure	Name and source
Type-1	Single chain	Pokeweed antiviral protein (PAP), pokeweed
	(N-glycosidase 29.5 kDa)	Pokeweed antiviral protein (PAPH), pokeweed hair root
	(N-glycosidase 11–30 kDa)	RIP 30, Barley
	(N-glycosidase 25 kDa)	RIP CCP 25, *Celosia cristata*
	(N-glycosidase 25 kDa)	IRIP, Iris bulbs
Type-2	Two chain	RIP, Caster
	(A chain- N-glycosidase)	
	(B chain-cell binding lectin)	
Type-3	Two dimmers of type-2	Various plants

Source: Saharan et al. 2008

a combination of RIP and chitinase (Chi-a) gene against *R. solani* (Jach et al. 1995). Rice blast caused by *Magnaporthe grisea* is one of the three major diseases that seriously affect rice production. Alpha-momorcharin (α-MC), a ribosome-inactivating protein (RIP) isolated from *Momordica charantia* seeds, has been found to exhibit in vitro antifungal activity (Qian et al. 2014). Further investigations are required into the transportation of RIP proteins and the way they bind with ribosome assembly (Stirpe 2013).

17.3.4 Phytoalexins

Higher plants synthesize a wide variety of secondary metabolites. Among them, phytoalexins play an important role in plant defense systems. Phytoalexins, a term originally coined by Muller (1958), are grouped under the class of plant antibiotics. These inducible antifungal and antimicrobial compounds are produced in plants after biotic or abiotic stresses. Their frequent accumulation is correlated to hypersensitive reaction (HR) of infected cells (Fig. 17.1). Phytoalexins are produced by healthy cells adjacent to localized damaged and necrotic cells in response to materials diffusing from the damaged cells. These diffused materials are known as

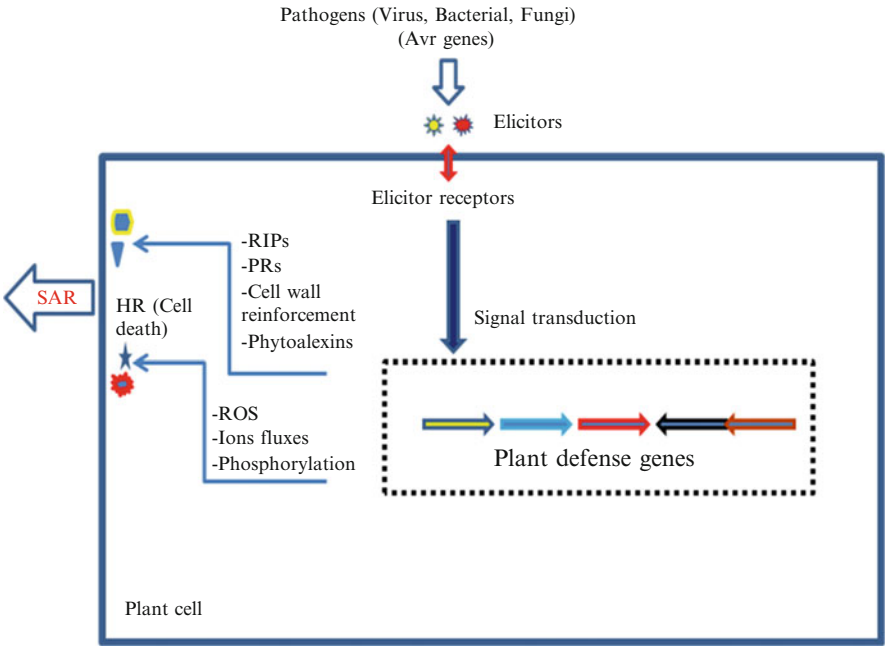

Fig. 17.1 Plant defense against pathogens

elicitors which trigger phytoalexin production. These elicitors play a key role in inducing the defense system of the plant cell. Phytoalexins accumulate around both resistant and susceptible necrotic tissues. Resistance occurs only when one or more phytoalexins along with other components reach a concentration sufficient to restrict pathogen development and therefore results in HR. The majority of biochemical and molecular evidence concerning the biosynthesis of phytoalexins has been obtained from the phenylpropanoid pathway which is also involved in lignin synthesis and to a lesser extent in terpenoid metabolism. The basic flavonoids skeleton is a derivative of two converging pathways, the acetate-mevalonate and shikimate pathways. These interconnected pathways are involved with various types of enzymes and their isomers. Leading enzymes which have a potent role are phenylalanine ammonia lyase (PAL), CoA ligase, chalcone synthase (CHS), chalcone isomerase (CHI) and stilbene synthase. PAL and CHS exist in isozymic form and are encoded by multigene families. Bean cells treated with elicitors revealed that CHI accumulates as a single polypeptide encoded by a single gene (Mehdy and Lamb 1987). The expression of grapevine stilbene synthase gene in rice plants has been shown to enhance disease resistance (Stark-Lorenzen et al. 1997). Similarly, resveratrol synthase and isoflavone methytransferase genes have been proved to enhance disease resistance in transgenic alfalfa (Hipskind and Paiva 2000). Alteration of phytoalexins through chemical engineering can be a way of stimulating more activity against fungal pathogens. Methylation of free hydroxyls has been shown to increase the antifungal activity of isoflavonoids. In addition, phytoalexins are often toxic to humans and/or animals. Consequently an inducible system may be applied for transgenic expression of phytoalexin gene(s) in plants (Großkinsky et al. 2012).

17.4 Bacterial Disease Resistant Transgenic Plants

Bacterial pathogens are responsible for numerous diseases in higher plants. Cereals, vegetables and fruits are common crops which are severely affected by bacterial diseases (Morgues et al. 1998). The development of bacterial disease resistant transgenic plants holds considerable promise to combat these pathogens.

17.4.1 Anti-microbial Protein

A large number of diverse, natural and cationic antimicrobial peptides (CAPs) have been discovered in recent years to strengthen resistance against bacterial diseases (Table 17.4). CAPs are the foremost active peptides among the antimicrobial peptides. These peptides fall in two classes: α-helical peptides, such as cecropines and maganins and β-sheet peptides, such as defensins, protegrins and lactoferrin (Huang et al. 2010). Amphipathic distribution of polar residues gives these peptides the

Table 17.4 Key achievement in transgenic production of antimicrobial proteins against bacterial disease

Transgenic protein	Crop	Resistance against	References
(Chimera protein) SP-cec B	Rice (*Oryza sativa*)	*Xanthomonas oryzae*	Sharma et al. (2000)
Msr A1 (Cecropin + chitinase chimera)	Potato (*Solanum tuberosum*)	*Erwinia carotovora*	Osusky et al. (2000)
MSI-99 (Melittin + cecropin) (synthetic protein analog)	Tomato (*Lycopersicon esculantum*)	Bacterial speck disease	Alan et al. (2004)
MB-39 (Melittin + cecropin) (synthetic protein analog)	Tomato	*Pseudomonas syringae* pv. *tabaci*	Jan et al. (2010)
Attacin E	Apple (*Malus malus*)	*E. amylovora*	Norelli et al. (1999)
Lysozyme	Potato	*E. chrysanthemi*	Rivero et al. (2012)
Lysozyme	Tobacco (*Nicotiana tabacum*)	*P. syringae* pv. *tabaci*	Nakajima et al. (1997)
Lysozyme	Potato	*E. carotovora*	During et al. (1993)
Lactoferrin (human)	Tomato	*Ralstonia solanacearum*	Lee et al. (2002)
Lactoferrin (bovine)	Pear (*Pyrus communis*)	*E. amylovora*	Malnoy et al. (2003)

ability to interact with the phospholipid membrane. This causes opening of the lipid bilayer and collapse of the trans-membrane electrochemical gradients leading to cell death (Bechinger 2004). Results have shown that these peptides are effective against plant pathogens (Alan and Earle 2002; Goyal and Mattoo 2014; Maroti et al. 2011).

17.4.1.1 Transgenic Expression of Cecropins

Cecropins are potent antimicrobial linear amphipathic peptides consisting of 31–39 amino acids residues and adapts α-helical structure on interaction with the bacterial membrane and induces pore formation. Natural cecropin and its synthetic analog (SB-37 and MB-39) gene have been introduced in tobacco plants and showed pathogen resistance (Huang et al. 1997). Norelli et al. (1999) transferred natural cecropin and its synthetic analog to enhance resistance against fire blight in Royal Gala apple. No effective resistance was observed in transgenic tobacco expressing a cecropins B gene against *Ralstonia solanaecarum* and *Pseudomonas syringae* pv. *tabaci* (the casual agent of tobacco wild fire). This was due to less expression of

transgene protein and degradation by host proteases. Therefore, to prevent cellular degradation of peptides by host peptidases, cecropins must be targeted to intercellular spaces. A transgenic rice plant carrying SP-cec B construct has been developed by fusing signal peptide (SP) of chitinase gene of rice which is known to direct the secretion of the gene product into the intercellular spaces in rice (Sharma et al. 2000). Targeted to intercellular space, cecropins sequestered from protease of the host plants provide a significant level of resistance against bacterial leaf blight in rice (Jan et al. 2010). Another cationic antimicrobial peptide called melittin, consisting of 26 amino acids, showed powerful haemolytic activity (Hancock and Diamond 2000). Osusky et al. (2000) reported broad-spectrum resistance to phytopathogens expressing an N-terminus-modified, cecropin-melittin chimera (Msr A1) in two potato cultivars. They modified the melittin peptide to reduce their toxicity towards haemolytic activity. Other small cationic peptides such as MSI-99, a synthetic analog of magainin II (MII), have been used in developing transgenic tomato plants for enhancing resistance to bacterial speck disease. Several MSI-99 expressing lines developed significantly fewer disease symptoms than controls. These results suggested that expression strategies providing continuous high expression of MSI-99 is necessary to achieve significant enhancement of plant disease resistance against bacterial speck disease (Alan et al. 2004). Co-operation between molecular modeling and engineered novel peptides provides a powerful tool to generate chimera peptides (Fox 2013).

17.4.2 Transgenic Expression of Lactoferrin Gene

Lactoferrins (~80 kDa) belong to a family of cationic iron-binding glycoprotein found in mammalian milk. A lactoferrin gene has been isolated, cloned and characterized from human and bovine sources. Its mode of action against bacteria is not only bacteriostatic but also bactericidal (Borther et al. 1989). The siderophores produced by many bacteria which are one of the virulence factors, allows bacteria to overcome the condition of iron limitations in host cells and has a protective effect against the toxicity of reactive oxygen species (Venisse et al. 2003). Thus decreasing iron availability in transgenic plants could be an attractive approach to limit bacterial survival in the host plant. Lactoferrin which has iron-chelating action could be a limiting factor of bacterial growth in transgenic host cells. Expression of the human lactoferrin gene in transgenic tobacco plants conferred increased resistance to *Ralstonia solanacearum* (Zhang et al. 1998). Similarly transgenic tomato exhibited partial resistance against bacterial wilt through the lactoferrin gene (Lee et al. 2002). Transgenic pear containing bovine lactoferrin cDNA conferred reduction in fire blight disease symptoms (Malnoy et al. 2003). Furthermore, medicinally-important ginseng and rice also produced high amounts of human lactoferrin. Besides their use for bacterial disease resistance, they are also used as food additives. Rice expressing lactoferrin may be a useful vehicle to introduce recombinant human lactoferrin to infant food (Kwon et al. 2003; Suzuki et al. 2003). Introduction of lactoferrin in transgenic cereals, fruits and vegetables

could be a new challenge to overcome bacterial diseases as well as make lactoferrin a hygienic food supplement (García-Montoya et al. 2012).

17.4.3 Other Antimicrobial Peptides

17.4.3.1 Attacins Expression in Apple and Pear

Attacins such as cecropins are small lytic peptides which show a substantial degree of resistance against bacterial pathogens. European apple cultivars are under great threat of bacterial fire blight caused by *Erwinia amylovora*. The attacins gene has been expressed in cultivars of apple and found less susceptible to the fire blight pathogen (Norelli et al. 1999). Royal Gala apple transgenic line TG138 containing attacin E under the control of pin II promoter had only 5 % shoot length blighted (SLB) as compared with 56 % SLB in non-transgenic Royal Galas and 37 % SLB in the moderately resistant Liberty control (Norelli et al. 1999). Transgenic cultivars Royal Gala, Galaxy and M 26 rootstocks expressing attacin LP under a constitutive promoter have also shown increased fire blight resistance (Aldwinckle et al. 2003). Besides apple, European pear (*Pyrus communis*) is also affected by *E. amylovora.* Here as well the transgene attacin E has been expressed against fire blight (Reynoird et al. 1999).

17.4.3.2 Transgenic Expression of Lysozymes

Lysozymes enzymes are widely distributed in nature and can be expressed transgenically. The human, chicken and T4 bacteriophage lysozyme cleaves the α-1–4 glycosidic bond of peptidoglycan in the bacterial cell wall. The T4 bacteriophage lysozyme cannot hydrolyze chitin, so human and egg white lysozyme has been used in many studies on phytopathogen resistant transgenic plants. So far, only a few research papers have appeared on the engineering of bacterial resistance in plants. One of the earlier reports regarding the transgenic potato expressing is the T4 bacteriophage lysozyme gene. The transgenic potato secretes lysozymes into the intercellular spaces, the site of entry and spread of the bacterium *Erwinia carotovora* (Rivero et al. 2012). Although expression levels of the transgene were found to be very low, the plants appeared to be less susceptible to *E. carotovora* infection than the control plants (During et al. 1993). A human lysozyme gene was transformed into tobacco and exhibited slightly fewer symptoms against the fungus *Erysiphe cichoracearum* and the bacterium *Pseudomonas syringae* pv. *tabaci* (Nakajima et al. 1997). Transgenic potato line R93 identified as less susceptible against black leg (*Erwinia chrysanthemi*), has been transformed with a chicken lysozyme gene through *Agrobacterium*-mediated transformation. However, these less-susceptible transgenic plants showed the same phenotype as the non-transgenic cultivars (Hirai et al. 2004). Rice cultivar Taipei 309 was utilized to evaluate the expression level of the human lysozyme gene under glutelin-1 promoter in maturing rice grain. At least 12 independently-transformed lines have been found with a significant level of lysozyme. The expression level of

lysozyme reached 0.6 % of brown rice weight, or 45 % of soluble proteins. Further segregation analysis has shown Mendelian inheritance with the same level of transgene protein expression. A similar study was conducted of transgenic rice expressing the human lysozyme in the endosperm, which revealed distorted trafficking and sorting of native storage protein in the rice endosperm and affected the expression of natural storage protein (Yang et al. 2003). A significant level of resistance in cultivars for commercial purpose is still to be achieved for bacterial diseases.

17.4.4 Strategies for Bacterial Virulence Factors

Developing strategies for bacterial virulence factors involve expressions of various compounds that help pathogenic bacteria to spread infection or carry out damage to host cells, they are known as virulence factors. These include the toxins, pectin enzyme, exo-polysaccharides, hormones, etc. Any mechanism expressed by a plant to inhibit bacterial pathogenicity or virulence factors can lead to resistance or reduced susceptibility. This knowledge has not been intensely investigated to develop strategies for engineering disease resistance (Baker et al. 2010). The wildfire disease of tobacco is caused by *Pseudomonas syringae* pv. *tabaci* which produces tabotoxin, a dipeptide toxin containing an uncommon β-lactum amino acid causing the chlorotic symptoms. The tabotoxin resistance gene ttr, encoding an inactivating acetylating enzyme from the same bacterium, was expressed at high levels in transgenic tobacco and successfully enhanced resistance to this bacterium. Further evaluation in the field of up to R7 progeny has confirmed a heritable resistance (Anzai et al. 1989; Batchvarova et al. 1998).

17.5 Exploiting Natural Plant Defenses

17.5.1 Transgenic Production of Elicitors

Transgenic production of elicitors has potential in natural plant defense. A variety of substances called elicitors are released by pathogens during infection of a host plant which are recognized by the plant as signal molecules and trigger defense mechanisms. In most cases, elicitors are synthesized by pathogens themselves but in a number of instances elicitors are produced as a result of a pathogen hydrolyzing the host cell walls. Pectate lyase (PL) enzyme is a major virulence factor of bacteria; it degrades the pectin component of the cell wall into unsaturated oligogalacturonates (OG) which are known to elicit a plant-defense response. A gene coding the isoenzyme pectate lyase-3 was transferred into potato and four PL3 transgenic lines selected over a period of 4 years exhibited enhanced resistance to *Erwinia* soft rot (Wegener 2002). Therefore, production of elicitors through transgenic means could be an effective strategy to enhance disease resistance (Fig. 17.2).

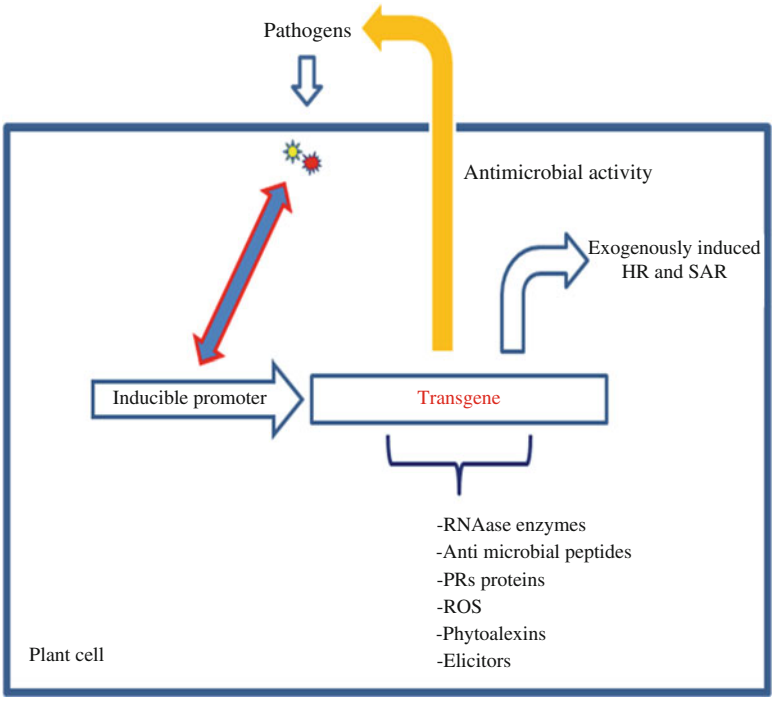

Fig. 17.2 Transgenically induced plant defense

17.5.2 Transgenic Production of Reactive Oxygen Species

Transgenic production of reactive oxygen species (ROS) also has potential in natural plant defense. The interaction of pathogen elicitors with host receptors activates a signal transduction cascade that involves other defense signals along with production of ROS. ROSs are directly related to enhancing the plant defense system, induce local hypersensitive reaction, systemic acquired response, etc. Enhancement of ROS production in plants could be an effective means to attain broad-spectrum disease resistance. The expression of the glucose oxidase (GO) gene in many plants induces hydrogen peroxide which results in an increased level of resistance to many bacterial pathogens and shows an increased level of hypersensitive response (Kachroo et al. 2003; Lee et al. 2002). High levels of GO expression in plant cells were associated with reduced growth of stem, root, less seed set and low seed germination. Hence, ROS expressing transgenes should be under precise control to protect the plant from a growth inhibitory effect from the transgene product (Fig. 17.2) (Murray et al. 1999).

17.5.3 Exogenously Induced Programed Cell Death

Exogenously induced programed cell death occurs when pathogens trigger a rapid and localized response in infected cells which kills them. This complex response is known as hypersensitive response (HR) or programed cell death (PCD). Exogenously induced programed cell death mimicking the natural HR is an alternative system to provide resistance to susceptible plants (Goyal and Mattoo 2014; Greenburg et al. 1994). However, this system may have deleterious effects on non-affected host cells. Therefore, there is need for a defined expression system which acts only on infected cells. A transgene responsible for inducing programed cell death could be attached to a pathogen inducible promoter so the transgene is expressed only in infected cells and the rest of the uninfected plant tissue not killed. This two-component system of transgenes (barnase and barstar) has successfully been expressed in transgenic potato plants against the fungus *Phytophthora infestans* (the causal agent of potato late blight). The availability of inducible promoters especially under pathogen infection is a major requirement to precisely control the transgene expression to avoid deleterious effects on healthy cells. In addition, controlled expression of transgenes saves energy which could be used in growth promotion of the host plant. Another approach which mimics the HR in plants to enhance resistance against pathogen attack is cloning of bacterio-opsin gene (bO) and proton pump. These genes are responsible for an accumulation of salicylic acid (a key chemical signal to systemic acquired resistance) and are inducers of an HR pathway. Cloning of the bO gene in tobacco increased resistance to TMV. The possibility of using a wound-inducible promoter to control the expression of bO did not develop spontaneous lesions. Nevertheless, under controlled laboratory conditions, they were found to be resistant to the pathogen. The activation of the defense mechanisms by the bO gene was not constitutive, and occurred in response to wounding or pathogen infection. Furthermore, wounding of transgenic tobacco plants resulted in the induction of systemic resistance to pathogen attack within 48 h. These findings provide a promising initial assessment for the use of wound-inducible promoters as new strategies to enhance pathogen resistance in transgenic crops by means of lesion mimic genes (Fig. 17.2) (Rizhsky and Littler 2001).

17.5.4 Cloning of R Gene for Disease Resistant Transgenic Plants

The R genes, naturally present in plants, are frequently used in breeding programs to produce disease-resistant transgenic plants. These genes are dominant, monogenic and provide resistance against one or few races of pathogen species. Race-specific resistance is explained by the gene-for-gene hypothesis proposed by Flor (1971) during his historical studies on the interaction between flax *(Linum usitatissimum)* and rust fungus *Melamspora lini*. According to this hypothesis, the plant

receptor (coded by the R gene) can recognize a pathogen-derived ligand (a product of avr gene) and ultimately convey signals to other defense-related genes for battling pathogens. Many techniques used to clone R gene(s) are still being pursued along with map-based cloning and transposon tagging (Tanksfey et al. 1995). Since the isolation of the first resistant gene, Hm1, about 20 R genes have been cloned. A general feature of the products of R genes is the presence of leucine rich repeats motifs, which are believed to be involved in recognition of avr gene products. Another protein motif is the nucleotide-binding site (NBS). This is assumed to be a regulatory switch for a signal transduction cascade (Kobe and Deisenhofer 1995).

R gene mediated genetically engineered plants have several attractive features for disease control. They have the natural mode of action that is homologous to the plant defense system and the concerted response can efficiently halt the growth of the pathogen. No input is required from farmers and there are no adverse environmental effects. However, R genes often become ineffective by co-evolving pathogens. Under selective pressure, the pathogens the avr gene evolves and become virulent in nature (thus coding mutated elicitors) and as a result the concerned R gene coded receptors cannot recognize pathogen infection. However, recent advances in structure and function of R protein and elucidation of new elements involved in downstream signal pathways provide a fertile field of the future scope of recombinant novel R genes (Wally et al. 2009).

17.5.5 R Gene Pyramids

The recent concept of cloning multiple R gene pyramids might provide strategies to overcome the above mentioned deficiencies. Transgenic use of the R gene, known as Bs2, cloned from pepper, has provided longstanding resistance against bacterial spot disease caused by the bacterium *Xanthomonas campestris* in tomato expressing NB-LRR (Thilmony et al. 1995). Other R genes cloned with potential use against fungal pathogens include the barley Rpg1 gene (Whitham et al. 1996) and tomato Ve1 and Ve2 genes (Strittmatter et al. 1996). The Rpg1 gene has provided remarkably durable resistance to stem rust for decades and Ve1 and Ve2 target *Verticillum* species that cause wilt in many different crops. The Ve genes can provide resistance to different *Verticillum* species and are functional in potato when expressed as transgenes. The Rpg1 and Ve genes have novel structural features that discriminate them from earlier R genes. Novel R genes can be used as prototypes to identify additional R genes to be used in genetic engineering. The phenomenon of *non-host resistance* exists when all varieties of plant species are resistant to all strains of a particular pathogen species. For example, *Arabidopsis* and tobacco are uniformly resistant to many microbes that plague crops (e.g. *Phytophthora infestans*). Recent studies have revealed that certain signal transduction components are responsible for non-host resistance (Bent et al. 1994; Salmeron and Staskawicz 1993). A similar reason was proposed for *restricted taxonomic functionality* which restricts the function of transgenes between distantly related species (Warren et al. 1997).

17.6 Measures for GM Crops Acceptance

Measures to promote GM crops acceptance is necessary in view of the current hue and cry against them. It is imperative to use technologies which decrease the risk associated with the blending of transgenes to a different genome. Efforts seek to implement more approaches of *non-pathogen derived resistance* to avoid the contamination of unrelated genes. In this connection, the most desirable approaches are the transferring of plant origin gene to crops viz. cloning resistance R gene, antimicrobial peptides, induction of HR and subsequent systemic acquired resistance (SAR). Artificial enhancement of the HR response could be the revolutionized option of broadened resistance towards the pathogens. Introduction of certain genes associated with SAR in crops could strengthen the natural immunity to combat future disease attacks in economically-important crops (Goyal and Mattoo 2014). Anti GMO lobbies have a number of concerns about transgenic crops like ethical issues, bio safety aspects etc. Owning to the benefits of candidate genes, the major concern are the selectable marker genes which may be toxic or allergenic to human beings; antibiotic selectable markers having wide clinical and veterinary applications. The marker gene could be transferred into microorganisms in the human and animal gut, which could render the microorganism resistance towards antibiotics. In addition to this, selectable markers have no function after selection and this exerts an extra load to the plant system. Therefore it is reasonable to consider removal of these extra genetic materials from the transgenic crops. Some successful methods are under current research which has the ability to remove these marker genes through co-transformation of a marker gene and the gene-of-interest followed by segregation, Intra-genomic relocation of transgenes via transposable elements, removal of the selectable marker gene after the selection procedure via site-specific recombinases and novel zinc finger nucleases are some of the methods could be used to remove selectable marker gene from transgenic crops (Tuteja et al. 2012).

17.7 Conclusions and Prospects

A major obstacle in accelerating transgenic technology against crop diseases is the lack of defined studies of plant-pathogen interaction at the molecular level to identify the resistance product and its genetics. Furthermore, the lack of precision in cloning of resistance genes or its identification in genomic clusters of source organism adds to the problem. More inputs are needed to supplement the high throughput functional genomics to enrich large experimental data of regulatory and structural genes (Kumar and Mysore 2011). This may certainly facilitate obtaining plentiful options of resistant genes for disease management in crop plants. The current advances in crop genomics, especially functional genomics and proteomics, will no doubt boost the development of disease resistance through transgenic crops.

References

Aerts AM, Bammens L, Govaert G et al (2011) The antifungal plant defensin HsAFP1 from *Heuchera sanguinea* induces apoptosis in *Candida albicans*. Front Microbiol 2:47

Alan AR, Earle ED (2002) Sensitivity of bacterial and fungal plant engineering disease resistance in crop plants pathogens to the lytic peptides, MSI-99, magainin II, and cecropin-B. Mol Plant Microbe Interact 15:701–708

Alan AR, Blowers A, Earle ED (2004) Expression of magainin-type antimicrobial peptide gene (MSI-99) in tomato enhances resistance to bacterial speck disease. Plant Cell Rep 22(6):388–396

Aldwinckle HS, Borejsza-Wysocka EE, Malnoy M et al (2003) Development of fire blight resistant apple cultivars by genetic engineering. Acta Horticult 622:105–111

Anna G, Maria S, Hugh B et al (2002) Initial infection of roots and leaves reveals different resistance phenotypes with coat protein gene-mediated resistance to potato moptop virus. J Virol 83(5):1201–1209

Anzai H, Yoneyama K, Yamaguchi I (1989) Transgenic tobacco resistance to a bacterial disease by detoxification of a pathogenic toxin. Mol Gen Genet 219:492–494

Babu RM, Sajeena A, Seetharaman K et al (2003) Over expression of the rice thaumatin-like protein (PR-5) gene in transgenic rice plants enhancing resistance to bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. J Ecobiol 15(1):73–79

Bai Y, Guo Z, Wang X et al (2009) Generation of double-virus-resistant marker-free transgenic potato plants. Prog Nat Sci 19:543–548

Baker CM, Chitrakar R, Obulareddy N et al (2010) Molecular battles between plant and pathogenic bacteria in the phyllosphere. Braz J Med Biol Res 43:698–704

Baranwal VK, Tumer NE, Kapoor HC (2002) Depurination of ribosomal RNA and inhibition of viral RNA translation by an antiviral protein of *Celosia cristata*. India J Exp Biol 40(10):1195–1197

Batchvarova R, Nikolaeva V, Slavov S et al (1998) Transgenic tobacco cultivars resistance to *Pseudomonas syringae pv.tabaci*. Theor Appl Genet 97:986–989

Baulcombe DC (1996) Mechanism of pathogen-derived resistance to viruses in transgenic plants. Plant Cell 8:179–188

Beachy RN, Philos (1999) Coat-protein-mediated resistance to tobacco mosaic virus: discovery mechanism and exploitation. Philos Trans R Soc Lond Biol Sci 35:659–664

Bechinger B (2004) Structure and function of membrane-lytic peptides. Crit Rev Plant Sci 23(3):271–292

Bent AF, Kunkel BN, Dahlbeck D et al (1994) *RPS2* of *Arabidopsis thaliana*: a leucine rich repeat class of disease resistance genes. Sciences 265:1856–1860

Blandenvoorde MFJ, Brand HS, Henskens YMC et al (2000) Recombinant protease inhibitors in plants. In: Michaud D (ed) Protease inhibitors in health and disease control, medical and industrial aspects. Landes Bioscience, Georgetown, pp 202–213

Boonrod KJ, Galetzka D, Nagy PD et al (2004) Single chain antibodies against a plant viral RNA-dependent RNA polymerase confer virus resistance. Nat Biotechnol 22:856–862

Borther CM, Arnold RR, Miller RD (1989) Bactericidal effect of lactoferrin on *Legionella pneumophilia:* effect of the physiological state of the organism. Can J Microbiol 35:1048–1051

Bosch FV, Oliver R, Berg FV, Paveley N (2014) Governing principles can guide fungicide-resistance management tactics. Ann Rev Phytopathol 52:175–195

Broekaert WF, Terras FRG, Cammue BPA, Osborne RW (1995) Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiol 108:1353–1358

Bruehl GW (1991) Plant pathology, a changing profession in a changing world. Ann Rev Phytopathol 29:313–348

Brunetti A, Tavazza M, Noris E et al (1997) High expression of truncated viral rep protein confers resistance to tomato yellow leaf curl virus in transgenic tomato plants. Mol Plant Microbe Interact 10:571–579

Carvalho A, Gomes VM (2009) Plant defensins – prospects for the biological functions and biotechnological properties. Peptides 30:1007–1020
Chern MS, Fitzgerald HA, Yadav RC et al (2001) Evidence for a resistance signaling pathway in rice similar to the *NPR1*-mediated signaling pathway in *Arabidopsis*. Plant J 27:101–113
Cillo F, Sialer F, Papanice MA, Gallitelli D (2004) Analysis of mechanism involved in the cucumber mosaic virus satellite RNA-mediated transgenic resistance in tomato plants. Mol Plant Microbe Interact 17(1):98–108
Citovasky V, Wong ML, Sha AL et al (1992) Visualization and characterization of tobacco mosaic virus movement protein binding to single stranded nucleic acid. Plant Cell 4:397–411
Collinge DB, Jørgensen HJL, Lund OS, Lyngkjær MF (2010) Engineering pathogen resistance in crop plants: current trends and future prospects. Ann Rev Phytopathol 48:269–291
Cooper B, Lapidot M, Heick JA et al (1995) A defective movement protein of TMV in transgenic plants confers resistance to multiple viruses whereas the functional analog increases susceptibility. Virology 206:307–313
Datta K, Velazhahan R, Oliva N et al (1999) Over expression of cloned rice thaumatin-like protein (PR-5) gene in transgenic rice plants enhances environmental friendly resistance to *Rhizoctonia solani* causing sheath blight diseases. Theor Appl Genet 98:1138–1145
Datta K, Tu J, Oliva N et al (2001) Enhanced resistance to sheath blight by constitutive expression of infection-related rice chitinase in transgenic elite indica rice cultivars. Plant Sci 160:405–414
Dempsey DA, Silva H, Klessig DF (1998) Engineering disease and pest resistance in plants. Trends Microbiol 54(2):54–61
Desmyter S, Vandenbussche F, Qiang H et al (2003) Type-1 ribosome-inactivating protein from iris bulbs: a useful agronomic tool to engineer virus resistance. Plant Mol Biol 51(4):567–576
Doughorty WG, Parks TD (1995) Transgenes and gene suppression telling us something new? Curr Opin Cell Biol 7(3):399–405
During K, Porsch P, Fladung M, Lorz H (1993) Transgenic potato plants resistant to the phytopathogenic bacterium *Erwinia carotovora*. Plant J 3:587–598
Ehrifeild N, Romano E, Serrano C, Arce-Johnson P (2004) Replicase mediated resistance against potato leaf roll virus disease plants. Biol Res 37(1):71–82
Epple P, Apel K, Bohmann H (1997) Over expression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum*. Plant Cell 9:509–520
Erik A, Biezen VD (2001) Quest for antimicrobial genes to engineer disease-resistant crops. Trends Plant Sci 6(3):89–91
Faria JC, Albino MMC, Dias BBA et al (2006) Partial resistance to bean golden mosaic virus in atransgenic common bean (*Phaseolus vulgaris* L.) line expressing a mutated rep gene. Plant Sci 171:565–571
Ferreira SA, Pitz KY, Manshard R et al (2002) Virus coat protein transgenic papaya provides practical control of papaya ringspot virus in Hawaii. Plant Dis 86(2):101–105
Fisher MC, Henk DA, Briggs CJ et al (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194
Fitchen JH, Beachy RN (1993) Genetically engineered protection against viruses in transgenic plants. Ann Rev Microbiol 47:739–763
Flor HH (1971) The current status of the gene-for-gene concept. Ann Rev Phyto Pathol 9:275–296
Fox JL (2013) Antimicrobial peptides stage a comeback. Nat Biotechnol 31:379–382
Fry WE (1993) Historical and recent migrations of *Phytopthora infestans*: chronology, pathways and implications. Plant Dis 79:113–121
Galvez LC (2012) Broad-spectrum virus resistance in transgenic plants. Dissertation, University of Nebraska
Galvez LC, Banerjee J, Pinar H, Mitra A (2014) Engineered plant virus resistance. Plant Sci. doi:10.1016/j.plantsci.2014.07.006
García-Montoya IA, Cendón TS, Arévalo-Gallegos S, Rascón-Cruz Q (2012) Lactoferrin a multiple bioactive protein: an overview. Biochim Biophys Acta 1820:226–236

Ghildiyal M, Zamore PD (2009) Small silencing RNAs: an expanding universe. Nat Rev Genet 10:94–108
Goa AG, Salim MH, Cindy AM et al (2000) Fungal pathogen protection in potato by expression of a plant defensin peptide. Nat Biotechnol 18:1307–1310
Gómez P, Rodríguez-Hernández AM, Moury B, Aranda MA (2009) Genetic resistance for the sustainable control of plant virus diseases: breeding, mechanisms and durability. Eur J Plant Pathol 125:1–22
Goyal RK, Mattoo AK (2014) Multitasking antimicrobial peptides in plant development and host defense against biotic/abiotic stress. Plant Sci. doi:10.1016/j.plantsci.2014.05.012
Greenburg JT, Guo A, Klessing DF, Ausulbel FM (1994) Programmed cell death in plants: a pathogen-triggered response activated coordinately with multiple defense function. Cell 77:551–563
Großkinsky DK, van der Graaff E, Roitsch T (2012) Phytoalexin transgenics in crop protection-fairy tale with a happy end? Plant Sci 195:54–70
Gubba A (2000) Transgenic and natural resistance: towards development of tomato and pepper plants with broad resistance to virus infection. PhD dissertation, Cornell University
Gururani MA, Venkatesh J, Upadhyaya CP et al (2012) Plant disease resistance genes: current status and future directions. Physiol Mol Plant Pathol 78:51–65
Gutierrez-Campos R, Torres-Acosta JA, Saucedo-Arias AJ, Gomez-Lim M (1999) The use of cysteine proteinase inhibitors to engineer resistance against poty viruses in transgenic tobacco plants. Nat Biotechnol 17(12):1223–1226
Hancock REW, Diamond G (2000) The role of cationic antimicrobial peptides in innate host defenses. Trends Microbiol 8(9):402–410
Hegedüs N, Marx F (2013) Antifungal proteins: more than antimicrobials? Fungal Biol Rev 26:132–145
Hipskind JD, Paiva NL (2000) Constitutive accumulation of a resveratrol glucoside in transgenic alfalfa increases resistance to *Phoma medicaginis*. Mol Plant Microbe Interact 13:551–562
Hirai D, Suzuki T, Yanagida D et al (2004) An evaluation of disease resistance of *Agrobacterium-mediated* transgenic potato (*Solanum tuberosum* L.) containing the chicken lysozyme gene or the wild spinach chitinase gene. Bull Hokkaido Prefect Agric Exp Stat 86:19–26
Huang Y, Nordeen RO, Di M et al (1997) Expression of an engineered cecropin gene cassette in transgenic tobacco plants confers disease resistance to *Pseudomonas syringae* pv *tabaci*. Phytopathology 87:494–499
Huang Y, Huang J, Chen Y (2010) Alpha-helical cationic antimicrobial peptides: relationships of structure and function. Prot Cell 1:143–152
Isabelle EJA, Miguel FCD, Geoff D et al (2002) Transgenic expression in Arabidopsis of a polyprotein construct leading to production of two different antimicrobial proteins. Plant Physiol 128:1346–1358
Jach G, Gornhardt B, Mundy J et al (1995) Enhanced quantitative resistance against fungal disease by combinational expression of different barley antifungal proteins in transgenic tobacco. Plant J 8:97–109
Jackson AO, Taylor CB (1996) Plant-microbe interactions: life and death at the interface. Plant Cell 8:1651–1668
James C (2003) Global review of commercialized transgenic crops. Curr Sci 84(3):303–325
James C (2014) GM Events with viral disease resistance. http://www.isaaa.org
Jan PS, Huang HY, Chen HM (2010) Expression of a synthesized gene encoding cationic peptide cecropin B in transgenic tomato plants protects against bacterial diseases. Appl Environ Microbiol 76:769–775
Jianping X, Joerg S, Fredy A (2001) Dissection of RNA-mediated ryegrass mosaic virus resistance in fertile transgenic perennial ryegrass (*Lolium perenne* L.). Plant J 26(3):265–274
Jiunn BH, Huey Y, Ann YT et al (2003) Broad spectrum resistance to different geographic strains of papaya ringspot virus coat protein gene transgenic papaya. Phytopathology 93(1):112–120
Kachroo A, He J, Patkar R et al (2003) Induction of H_2O_2 in transgenic rice leads to cell death and enhanced resistance to both bacterial and fungal pathogens. Transgenic Res 12(5):577–586

Kaur J, Sagaram US, Shah D (2011) Can plant defensins be used to engineer durable commercially useful fungal resistance in crop plants. Fungal Biol Rev 25:128–135
Khaled M, Ines Y, Afif H et al (2002) Tobacco plants transformed with an untranslatable from of the coat protein gene of the potato virus Y are resistance to viral infection. Eur J Plant Pathol 108(4):285–292
Kobe B, Deisenhofer J (1995) A structural basis of the interactions between leucine-rich repeats and protein ligands. Nature 374:183–186
Kononowicz AK, Nelson DE, Sing NK et al (1992) Regulation of the osmotin gene promoter. Plant Cell 4:513–524
Kubota K, Tsuda S, Tamai A, Meshi T (2003) Tomato mosaic virus replication protein suppression virus-targeted posttranscriptional gene silencing. J Virol 77(20):11016–11026
Kumar MS, Mysore KS (2011) New dimensions for VIGS in plant functional genomics. Trends Plant Sci 16(12):656–665
Kumar S, Raj SK, Sharma AK, Varma HN (2012) Genetic transformation and development of cucumber mosaic virus resistant transgenic plants of *Chrysanthemum morifolium* cv. Kundan. Sci Hortic 134:40–45
Kwon SY, Jo SH, Lee OS et al (2003) Transgenic ginseng cell lines that produce high levels of a human lactoferrin. Planta Med 69(11):1005–1008
Lawson EC, Weiss JD, Thomas PE, Kaniewski WK (2001) Replicase mediated resistance to potato leaf roll virus. Mol Breed 7(1):1–12
Lee YH, Yoon IS, Suh SC, Kim HI (2002) Enhanced disease resistance in transgenic cabbage and tobacco expressing a glucose oxidase gene from *Aspergillus niger*. Plant Cell Rep 20(9):857–863
Lehmann P, Jenner CE, Kozubek E et al (2003) Coat protein-mediated resistance to turnip mosaic virus in oilseed rape (*Brassica napus)*. Mol Breed 11:83–94
Leibman D, Wolf D, Saharan V et al (2011) A high level of transgenic viral small RNA Is associated with broad potyvirus resistance in cucurbits. Mol Plant Microbe Interact 24(10):1220–1238
Lin KY, Hsu YH, Chen HC, Lin NS (2013) Transgenic resistance to bamboo mosaic virus by expression of interfering satellite RNA. Mol Plant Pathol 14:693–707
Lindbo JA, Dougherty WG (1992) Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. Virology 189:725–733
Liu D, Raghothama KG, Hasegawa PM, Bressan RA (1994) Osmotin overexpression in potato delays development of disease symptoms. Proc Natl Acad Sci U S A 91:1888–1882
Logemann J, Jach G, Tommerup H et al (1992) Expression of a barley ribosome-inactivating protein leads to increased fungal protection in transgenic tobacco plants. Biotechnology 10:305–308
Makeshkumar T, Varme A, Singh KK et al (2002) Coat protein gene mediated resistance to potato virus Y in transgenic tobacco. Indian J Phytopathol 55(2):187–194
Malnoy M, Venisse JS, Brisset MN, Chevreau E (2003) Expression of bovine lactoferrin cDNA confers resistance to *Erwinia amylovora* in transgenic pear. Mol Breed 12(3):231–244
Maroti G, Kereszt A, Kondorosi E, Mergaert P (2011) Natural roles of antimicrobial peptides in microbes, plants and animals. Res Microbiol 162:363–374
Mehdy MC, Lamb CJ (1987) Chalcone isomerase cDNA cloning and mRNA induction by fungal elicitor, wounding and infection. EMBO J 6:1527–1533
Meng B, Gubba A (2000) Genetic engineering a novel and powerful tool to control plant virus. APSnet Features. doi:10.1094/APSnetFeature-2000-0500B
Morgues F, Brisset M, Chevreau E (1998) Strategies to improve plant resistance to bacterial diseases through genetic engineering. Trends Biochem Technol 16:203–209
Muller KO (1958) Studies on phytoalexin: The formation and the immunological significance of phytoalexin produced by *Phaseolus vulgaris* in response to infections with *Sclerotinia fructicola* and *Phytophthora infestans*. Aust J Biol Sci 11:275–300

Mundembe R, Matibiri A, Sithole-Niang I (2009) Transgenic plants expressing the coat protein gene of cowpea aphid-borne mosaic potyvirus predominantly convey the delayed symptom development phenotype. Afr J Biotechnol 8:2682–2690

Murray F, Llewellyn D, McFadden H et al (1999) Expression of the *Talaromyces flavus* glucose oxidase gene in cotton and tobacco reduces fungal infection, but is also phytotoxic. Mol Breed 5(3):219–232

Nakajima H, Muranaka T, Ishige F et al (1997) Fungal and bacterial resistance in transgenic plants expressing human lysozyme. Plant Cell Rep 16:674–679

Nomura K, Ohshima K, Anai T et al (2004) RNA silencing of the introduced coat protein gene of turnip mosaic virus confers broad spectrum resistance in transgenic *Arabidopsis*. Phytopathology 94(7):732–736

Norelli JL, Mills JAZ, Momol MT, Aldwinckle H (1999) Effect of cecropin-like transgenes on fire blight resistance of apple. Acta Horticult 489:273–278

Osusky M, Zhou G, Osuska L et al (2000) Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance to phytopathogens. Nat Biotechnol 18:1162–1166

Palukaitis P, Zaitlin M (1997) Replicase-mediated resistance to plant virus disease. Adv Virus Res 48:349–377

Peremarti A, Twyman RM, Gómez-Galera S et al (2010) Promoter diversity in multigene transformation. Plant Mol Biol 73:363–378

Powell-Abel P, Nelson RS, De B et al (1986) Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 236:738–743

Prins M, Laimer M, Noris E et al (2008) Strategies for antiviral resistance in transgenic plants. Mol Plant Pathol 9(1):73–83

Punja ZK, Chen WP (2004) Transgenic carrots expressing enhanced tolerance to herbicide and fungal pathogen infection. Acta Horticult 637:295–302

Qian Q, Huang L, Yi R et al (2014) Enhanced resistance to blast fungus in rice (*Oryza sativa* L.) by expressing the ribosome-inactivating protein alpha-momorcharin. Plant Sci 217:1–7

Ram MSN, Mohandas S (2003) Transformation of African violet (*Saintpaulia ionantha)* with glucanase-chitinase genes using *Agrobacterium tumefacien*. Acta Horticult 624:471–478

Ramonell KM, Somerville S (2002) The genomics parade of defense responses: to infinity and beyond. Curr Opin Plant Biol 5:291–294

Reynoird JP, Mourgues F, Norelli J et al (1999) First evidence for improved resistance to fire blight in transgenic pear expressing the attacin E gene from *Hyalophora cecropia*. Plant Sci 149(1):23–31

Rivero M, Funrman N, Mencacci N et al (2012) Stacking of antimicrobial in potato transgenic plants conferring resistance to bacterial and fungal pathogen. J Biotechnol 157(2):334–343

Rizhsky L, Littler R (2001) Inducible expression of bacterio-opsin in transgenic tobacco and tomato plants. Plant Mol Biol 46(3):313–323

Saharan V, Yadav RC, Mishra R, Yadav NR (2008) Engineering disease resistance in crop plants. In: Rao GP, Yipeng Z, Radchuk V, Bhatnagar SK (eds) Advances in plant biotechnology. Studium Press, Houston, pp 525–559

Salmeron JM, Staskawicz BJ (1993) Molecular characterization and *hrp* dependence of the avirulence gene, *avrPto,* from *Pseudomonas syringae* pv. tomato. Mol Gen Genet 239:6–16

Sanseverino W, Roma G, De Simone M et al (2010) PRGdb: a bioinformatics platform for plant resistance gene analysis. Nucleic Acids Res 38:814–821

Schwind N, Zwiebel M, Itaya A et al (2009) RNAi-mediated resistance to potato spindle tuber viroid in transgenic tomato expressing a viroid hairpin RNA construct. Mol Plant Pathol 10(4):459–469

Sela-Buurlage MB, Ponstein AS, Bres-Vloemans SA et al (1993) Only specific tobacco (*Nicotiana tabacum*) chitinases and 1,3- â glucanases exhibit antifungal activity. Plant Physiol 101:857–863

Sharma A, Sharma R, Imamura M et al (2000) Transgenic expression of cecropin B, an antibacterial peptide from *Bombyx mori,* confers enhanced resistance to bacterial leaf blight in rice. FEBS Lett 484:7–11

Stark-Lorenzen P, Nelke B, Hanbler G et al (1997) Transfer of a grapevine stilbene synthase gene to rice (*Oryza sativa* L.). Plant Cell Rep 16:668–673
Stintzi A, Heitz T, Kauffmann S et al (1991) Identification of basic pathogenesis-related, thaumatin like protein of virus-infected tobacco as osmotin. Physiol Mol Plant Pathol 38:137–146
Stirpe F (2013) Ribosome-inactivating proteins: from toxins to useful proteins. Toxicon 67:12–16
Stirpe F, Barbieri L, Battelli MG et al (1992) Ribosome inactivating proteins from plants: present status and future prospects. Biotechnology 10:405–412
Strittmatter G, Gheysen G, Hahn K et al (1996) Infections with various types of organisms stimulate transcription from a short promoter fragment of the potato gst1 gene. Mol Plant Microbe Interact 9:68–73
Stuiver MH, Custers JHH, Sela-Buurlage MB et al (2000) Antifungal proteins, DNA coding therefore and host incorporating same. Australian Patent No: Au 718274
Suzuki YA, Kelleher SL, Yalda D et al (2003) Expression, characterization and biologic activity of recombinant human lactoferrin in rice. J Pediatr Gastroenterol Nutr 36(2):190–199
Tabei Y, Kitade S, Nishizawa Y et al (1998) Transgenic cucumber plants harboring a rice chitinase gene exhibit enhanced resistance to gray mold (*Botrytis cinerea)*. Plant Cell Rep 17:159–164
Talbot NJ (2004) Plant pathogen interactions. Blackwell Publishing, Oxford
Tanksfey SD, Ganal MW, Martin GB (1995) Chromosomal landing: a paradigm for map-based cloning in plants with large genomes. Trends Genet 11:63–68
Thilmony RL, Chen Z, Bressan RA, Martin GB (1995) Expression of the tomato *Pto* gene in tobacco enhances resistance to *Pseudomonas syringae* pv *tabaci* expressing *avrPto*. Plant Cell 7:1529–1536
Tuteja N, Verma S, Sahoo RK et al (2012) Recent advances in development of marker-free transgenic plants: regulation and biosafety concern. J Biosci 37:167–197
Van Der Weerden NL, Bleackley MR, Anderson MA (2013) Properties and mechanisms of action of naturally occurring antifungal peptides. Cell Mol Life Sci 70:3545–3570
Van Loon LC, Pierpoint WS, Boller T, Conejero V (1994) Recommendations for naming plant pathogenesis-related proteins. Plant Mol Biol 12:245–264
Vanderschuren H, Alder A, Gruissem W, Zhang P (2009) Dose-dependent RNAi-mediated geminivirus resistance in the tropical root crop cassava. Plant Mol Biol 70(3):265–272
Velazhahan R, Muthukrishnan S (2004) Transgenic tobacco plants constitutively overexpressing a rice thaumatin-like protein (PR-5) show enhanced resistance to *Aiternaria alternata*. Biol Plant 47(3):347–354
Venisse JS, Barn MA, Paulin JP, Brisset MN (2003) Involvement of three pathogenicity factors of *Erwinia amylovoraI* in the oxidative stress associated with compatible interaction in pear. FEBS Lett 537:198–202
Wally O, Jayaraj J, Punja ZK (2009) Broad-spectrum disease resistance to necrotrophic and biotrophic pathogens in transgenic carrots (*Daucus carota* L.) expressing an *Arabidopsis* NPR1 gene. Planta 231:131–141
Wang YX, Kausch AP, Chandlee JM et al (2003) Co-transfer and expression of chitinase, glucanase, and bar genes in creeping bentgrass for conferring fungal disease resistance. Plant Sci 165(3):497–506
Wang XB, Wu Q, Ito T et al (2010) RNAi-mediated viral immunity requires amplification of virus-derived siR-NAs in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 107:484–489
Wani SH (2010) Inducing fungus-resistance into plants through biotechnology. Not Sci Biol 2(2):14–21
Wani SH, Sanghera GS (2010) Genetic engineering for viral disease management in plants. Not Sci Biol 2(1):20–28
Warren EJ, Waterhouse PM, Upadhyaya MN (1997) Molecular biology of rice. In: Shimamoto K (ed) Genetic engineering of virus resistance. Springer, Tokyo, pp 257–281
Wegener CB (2002) Induction of defense responses against *Erwinia* soft rot by an endogenous pectate lyase in potatoes. Physiol Mol Plant Pathol 60(2):91–100

Whitham S, McCormick S, Baker B (1996) The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. Proc Natl Acad Sci U S A 93:8776–8781

Wook PS, Lawrence CB, Linden JC, Vivanco JM (2002) Isolation and characterization of a novel ribosome-inactivating protein from root cultures of pokeweed and its mechanism of secretion from roots. Plant Physiol 130(1):164–178

Yamamoto T, Iketani H, Leki H et al (2000) Transgenic grapevine plants expressing a rice chitinase with enhance resistance to fungal pathogens. Plant Cell Rep 19:639–646

Yang DC, Guo FL, Liu B et al (2003) Expression and localization of human lysozyme in the endosperm of transgenic rice. Planta 216(4):597–603

Young ND (2000) The genetic architecture of resistance. Curr Opin Plant Biol 3:285–290

Zanek MC, Reyes CA, Cervera M et al (2008) Genetic transformation of sweet orange with the coat protein gene of citrus psorosis virus and evaluation of resistance against the virus. Plant Cell Rep 27:57–66

Zhang Z, Coyne DP, Vivade AK, Mitra A (1998) Expression of lactoferrin cDNA confers resistance to *Ralstonia solanacearum* in transgenic tobacco plants. Phytopathology 88:732–734