JARQ 47 (1), 21 – 27 (2013) http://www.jircas.affrc.go.jp

REVIEW

Suppression of Defense Response Related to Plant Cell Wall

Tomonori SHIRAISHI*

Laboratory of Plant Pathology and Genetic Engineering, Graduate School of Environmental and Life Science, Okayama University (Okayama, Okayama 700-8530, Japan)

Abstract

In plant–parasite interactions, "effectors" are thought to play an important role in suppressing the innate immune response, but the vast majority of effector functions and host target molecules remain unclear, except for several combinations[1]. A pea pathogenic fungus, *Mycosphaerella pinodes*, secretes compounds that block defense responses of the host plants only and also induce local susceptibility ("accessibility"), even to avirulent pathogens. These compounds have been called "suppressors" or "suppressors of defense." The *M. pinodes-suppressors*, which are low-molecular weight mucin-type glycopeptides, were named supprescins and their presence markedly blocked elicior-induced resistance, such as the generation of superoxide, formation of infection-inhibitors, production of phytoalexin and so on, in host plants. For three decades from 1977, it was found that supprescins disturb the fundamental functions of the host cells, particularly apyrase and redox enzymes in the host cell wall in a species-specific manner. In this review, the role of supprescins with the plant cell wall in determining specificity was introduced.

Discipline: Plant disease/Plant protection
Additional key words: effector, elicitor, MAMPs (microbe-associated molecular pattern), plant-pathogen specificity, suppressor

Introduction

In nature, it is well known that while both plants and other organisms are resistant/immune to the vast majority of pathogens, given plant species are always prone to specific pathogens. In other words, virtually all pathogens have a very limited host range. Accordingly, in host-parasite interactions, resistance is the rule and susceptibility is the exception. This phenomenon is known as "host-parasite specificity" and elucidating this mechanism is an intriguing issue. Plants possess both static and induced resistance systems. The former includes constitutive properties such as the thickness and hardness of the cell wall, the existence of antimicrobial substances, hydrophobic surfaces and so on. Meanwhile, induced/active resistance indicates the formation of chemical and physical barriers and is considered crucial to resistance because suppression by heat shock, treatment with metabolic inhibitors or inoculation with virulent pathogens means infection may be allowed, even by avirulent pathogens on nonhost plants[22]. Conversely, even virulent pathogens find it hard to infect the host plant once active resistance has been established by inoculation with avirulent pathogens, as described in "Phytolalexin theory".

Inducers of active defenses were termed "elicitors" in 1975 by N. Keen. Meanwhile a substance causing the elicitor action to decline is designated as a "suppressor" [17, 18]. Two concepts have been used to determine specificity; 1) virulent pathogens might not produce an elicitor effective on host plants, and, 2) the virulent pathogens may produce both elicitors and suppressors. As far as we know, there is no pathogenic microorganism which does not produce elicitors (MAMPs/PAMPs) because common constituents on the surface of pathogenic microorganisms, such as chitin, β-glucan, flagella, lipopolysaccharides and so on, are recognized as alien substances by plant cells. Moreover, in the real infection court, the fungal pathogens secrete glycoprotein elicitors and/or cell wall-degrading enzymes in their spore-germination fluids or mucilage. These facts led us to believe that fungal pathogens must avoid the host resistance positively with suppressors. In this review, our 35

* Corresponding author: e-mail tomoshir@cc.okayama-u.ac.jp
Received 26 March, 2012; accepted 1 June, 2012.

years of research on the specificity mechanism will be introduced with a nonspecific glycoprotein elicitor and species-specific mucin-type suppressors found in the spore germination fluid of the causal agent of Mycosphaerella blight of pea, *Mycosphaerella pinodes*.

Specific production and action on the infection of the *M. pinodes*-suppressor

It is thought that the initial interaction between plants and fungal pathogens occurs at the plant surface mediated by substances in spore-containing (germination) fluids or in mucilage. *M. pinodes* secreted a nonspecific, high molecular weight glycoprotein elicitor (Mr>70 kDa) that has a partial structure of *β*-D-Glc-(1,6)-*α*-D-Man-(1,6)-D-Man, which is *o*-glycosidically attached to serine residues in the protein moiety[15] as shown in Fig. 1. Also mucin-type glycopeptide suppressors (Mr<5 kDa) are secreted in its spore suspension fluid of a virulent strain OMP-1 [17, 19, 21] with structures of *α*-GalNAc-*o*-ser-ser-gly (supprescinA; Mr, 452) and *β*-Gal-(1,4)-*α*-GalNAc-*o*-ser-ser-gly-asp-glu-thr (suppressinB; Mr, 959). A hypovirulent strain OMP-X76 secreted suppressins but the activity was lower than in the virulent case. No suppressor effective on pea plants was produced by a nonpathogen of the pea, *M. ligulicola* (the causal agent of *Chrysanthemum* ray blight) strain OML, whereas the elicitor activity produced by *M ligulicola* was almost identical to that of *M. pinodes*. Treatment of pea leaves with the *M. pinodes* suppressor allowed infection by many avirulent pea pathogens, such as *Alternaria alternata*, *M. ligulicola*, *M. melonis*, *Stemphylium sarcinaeforme* and so on[18]. Thus, the suppressor from *M. pinodes* conditioned the pea plant to be susceptible even to avirulent fungi. Meanwhile, an avirulent pathogen, *Alternaria alternata* (Japanese pear pathotype 15B) could infect *Lespedeza bruergeri*, *Medicago sativa*, *Millettia japonica*, *Pisum sativum* and *Trifolium pratense* of 12 plant species tested in the presence of suppressins and susceptible to *M. pinodes* to various extents (Table 1). Accordingly, the infection-inducing activity of the suppressors is strictly species-specific.

Effect of fungal signal molecules on superoxide generation in vitro

The *M. pinodes*-elicitor induces diverse active defenses such as phytoalexin production, superoxide generation, infection-inhibitor formation, PR-protein activation and so on, either in host or nonhost plants of *M. pinodes*. Meanwhile, the *M. pinodes*-suppressor, suppressins, only blocked these defense responses in host plants induced by various elicitors. However, suppressins alone instead elicited defense responses in nonhost plants[26].

It is well known that an oxidative burst is one of

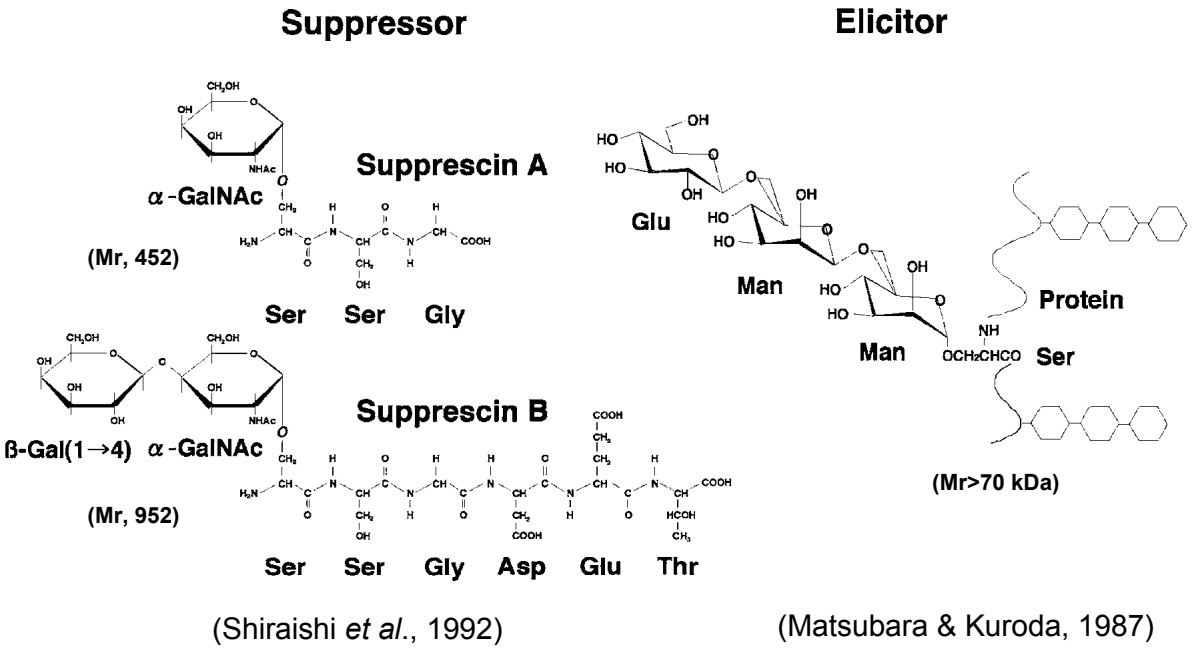

Fig. 1. Structures of the elicitor and suppressors in the spore germination fluid of a pea pathogen, *Mycosphaerella pinodes*

the rapid responses to invading avirulent pathogens and acts as one of the intracellular signal molecules. NADPH oxidase is responsible for generating a superoxide anion on a plasma membrane. Conversely, peroxidase was reported to contribute to the synthesis of O_2^-. Gross et al.[3] and Halliwell[4] pointed out the oxidation of NADH by cell wall-bound peroxidases, resulting in the generation of O_2^-/H_2O_2 through a complex pathway involving the apoplastic NADH, NAD* and NAD^+ cycle. On pea or cowpea leaves, the *M. pinodes*-elicitor induced an SOD-sensitive reduction of nitroblue tetrazolium, indicating O_2^- generation. Conversely, supprescins blocked such induction of O_2^- on pea leaves, but in contrast, evoked O_2^- generation on cowpea leaves alone [11]. Subsequently, to clarify whether the O_2^- generation was evoked in the cell wall, we analyzed the O_2^- generation in the fraction NaCl-solubilized from cell wall preparations from the pea and cowpea with Mn^{2+}, *p*-coumarate and NADH. As shown in Fig. 2, the elicitor induced O_2^- generation in pea and cowpea fractions on a dose-dependent basis. Conversely, supprescins inhibited the generation in the NaCl-solubilized fraction from the pea cell wall, but supprescins alone stimulated the generation in the cowpea fraction[12].

On plant tissues the inhibition rate of elicitor-induced O_2^- generation by diphenylene iodonium (DPI) accounted only for 30%, while a peroxidase inhibitor, salicylhydroxamic acid, perfectly inhibited the genera-

Table 1. Accessibility induction for an avirulent *Alternaria alternata*, Japanease pear pathotype 15B on several plant species by suppressors from a pea pathogen, *Mycosphaerella pinodes* (Oku et al., 1980)

Plant species	Degree of formation of infection hyphae*		
	M. pinodes	*A. alternata* 15B	*A. alternata* 15B + *M pinodes*-suppressors
Arachis hypogaea	0	0	0
Glycine max	0-1	0	0
Lespedeza buergeri	2	0	2
L. bicolor	0	0	0
Lotus japonicus	0	0	0
Medicago satina	1	0	1
*M. truncatula***	2-4	0	2-4
Millettia japonica	2	0	1
Pisum sativum	4	0	4
Trifolium pratense	1	0	1
T. repens	0	0	0
Vicia faba	0	0	0
Vigna sinensis	0	0	0

The suppressors were partially purified on TLC.
* Based on a 0~4 rating where 0=no formation and 4=abundant formation.
** The challenger was a chrysanthemum pathogen, *Mycosphaerella ligulicola* (Toyoda et al., 2002, unpublished).

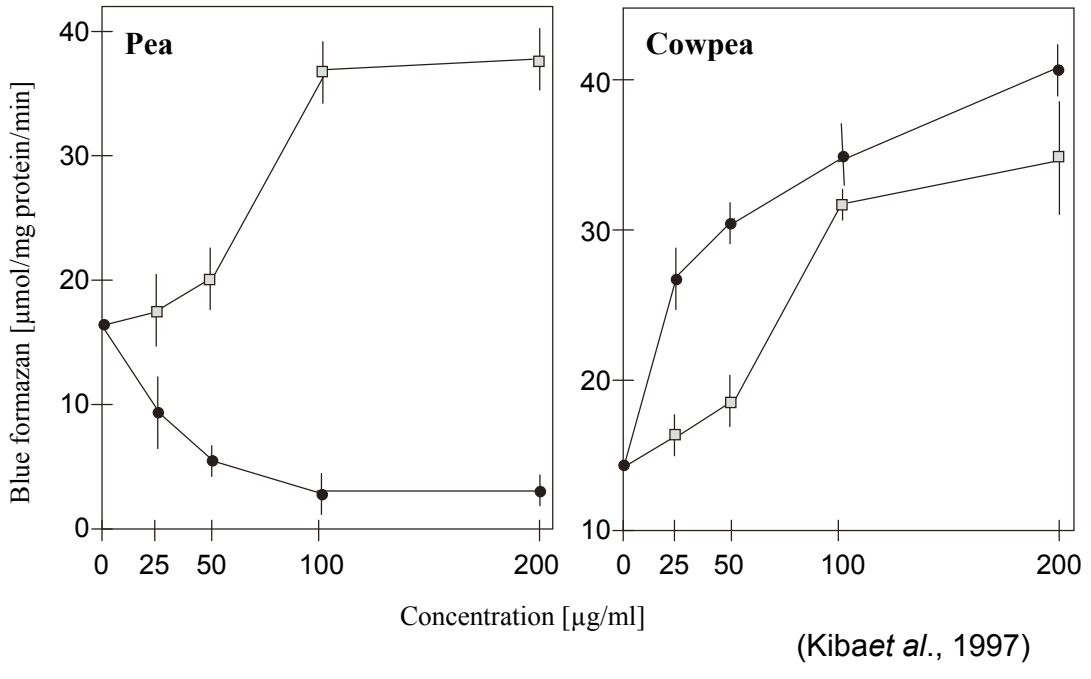

Fig. 2. Effects of the elicitor (□) and suppressor (●) from *Mycosphaerella pinodes* on *in vitro* generation of superoxide in the NaCl-solubilized fractions from pea and cowpea cell wall preparations (Kiba et al., 1997[12])

tion (Toyoda et al., unpublished data). The results suggest that the first step of O_2^-/H_2O_2 generation is evoked by ecto-peroxidase in the apoplast/cell wall, meaning that the subsequent O_2^-/H_2O_2 generation is evoked by NADPH oxidase on the plasma membrane. An amplifying system for O_2^-/H_2O_2 generation in cells mediated by plasma membrane NADPH oxidase has been demonstrated[28]. Recently, Bolwell and his colleague[2] clarified the significance of cell wall peroxidase in MAMPs-triggered immunity in *Arabidopsis thaliana*. Interestingly, ecto-apyrase-silenced *Vigna sinensis* lost the O_2^- -generating activity dependent upon peroxidase induced by several PAMPs, suggesting a tight association between ecto-apyrase, peroxidase and superoxide generation (Toyoda et al., unpublished).

Ecto-apyrase, a target molecule of the *M. pinodes*-suppressor

It was long believed that fungal signal molecules are recognized initially by receptors or binding proteins on plasma membrane. At present several reports indicate that the receptors or target proteins for fungal signal molecules (MAMPs/PAMPs or effectors) exist in the plasma membrane or intracellular organelles. For example, a high affinity binding protein for chitin oligosaccharide elicitor (chitin elicitor-binding protein; CEBiP) was detected in the plasma membrane preparation from rice cells[5]. We previously demonstrated that suppressins inhibited the ATPase activity in isolated pea plasma membrane and pea cells as did orthovanadate[7,20,25]. Orthovanadate blocked the defense responses of all plant species tested as well as the activity of p-type ATPase[25,26,27]. These results suggest that the inhibition of the p-type ATPase is closely correlated with suppression of plant immune responses. However, unexpectedly, the action of suppressins was nonspecific on the plasma membrane ATPase of the host and nonhosts of *M. pinodes*, while *in situ* cytochemical observation with TEM and EDX showed that suppressins only inhibited ATPase activity in pea cells but not those of 4 nonhost plants such as cowpea, kidney beans, soybean and barley[20]. In other words, the action of suppressins on isolated plasma membranes is nonspecific but species-specific on living cells. This fact led us to the hypothesis that upstream of the plasma membrane, the outermost organelle, the plant cell wall, contains a molecule, which recognizes and responds to suppressins on a species-specific basis. In conclusion, an apyrase (NTP/NDPase) bound to cell wall preparations could respond to the *M. pinodes*-elicitor nonspecifically and to suppressins in a strictly species-specific manner[10]. In fact, even *in vitro*, suppressins decreased the ATP-hydrolyzing activity of pea cell wall-bound apyrase, but, conversely activated those of nonhost plants of *M. pinodes* (Fig. 3). A recombinant pea ecto-apyrase, PsAPY1 and a recombinant cowpea ecto-apyrase VsNTPase1, could also respond to suppressins and the elicitor of *M. pinodes* like the defense responses *in vivo*[8,14,23]. Furthermore, the activity of the recombinant VsNTPase1 could respond not only to microorganisms' elicitors (MAMPs) such as harpin,

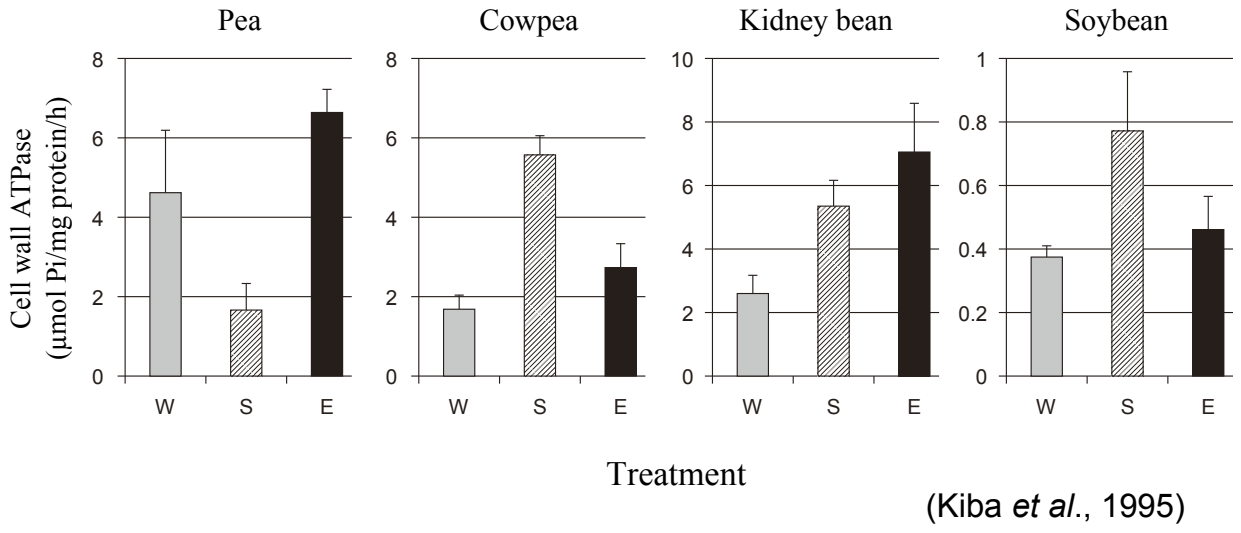

Fig. 3. Effects of the elicitor and suppressor from *Mycosphaerella pinodes* on *in vitro* ATPase activity in the NaCl-solubilized fractions from pea and cowpea cell wall preparations (Kiba et al., 1995[10])

W, water control; S, 100 μg/ml suppressor; E, 100 μg/ml elicitor.

INF1-elicitin, β-glucan, laminarin, lipopolysaccharide, chitin oligomer and *Escherichia coli* (JM109)-gDNA but also to $MgSO_4$, $AlCl_2$, $FeSO_4$, jasmonic acid and salicylic acid (Takahashi et al., 2006, unpublished results). These findings suggest that plant ecto-apyrases play an important role in sensing environmental organisms and/or substances.

Induction of defense responses by an apyrase product

So what happened when ecto-apyrases were activated? We studied the effect of apyrase products such as ADP, AMP and inorganic phosphate on the defense response. Pretreatment of pea tissues with inorganic phosphate for 6-12 h prior to inoculation was capable of inducing resistance to *M. pinodes* on pea tissues[9]. Based on blue formazan assay with nitroblue tetrazolium, inorganic phosphate induced superoxide generation (2nd phase) 6 h after treatment[9]. Inorganic phosphate also induced transcriptional activation of *PsPOX11*, *POX14* and *POX21* but not *POX13* and *POX29*. However, ATP, ADP and AMP showed little effect on the O_2^- generation and induction of the rejection reaction to *M. pinodes*. In other words, a product of apyrase, inorganic phosphate, seems to be one of the 2nd messengers for defense signaling, suggesting the significance of activated ecto-apyrase in induced resistance.

A transformed *Nicotiana tabacum* SR1 with pea ecto-apyrase gene, *PsAPY1*, showed resistance to virulent *Alternaria* sp. and *Pseudomonas syringae* pv. *tabaci* as shown in Fig. 4 (Kiba et al., unpublished data). Conversely, apyrase-silenced *Nicotiana benthamiana* by VIGS decreased the resistance to *Ps. syringae* pv. *tabaci*[24]. These facts suggest that the ecto-apyrases play a crucial role in determining resistance/susceptibility by sensing pathogenic microorganisms.

Concluding remarks

In the NaCl-solubilized fraction from cell wall pea and cowpea preparations, we found the activities of several redox enzymes such as ascorbate oxidase, Cu/Zn speroxide dismutase, diamine oxidase, peroxidase and so on. Surprisingly, it emerged that these activities were also regulated, even *in vitro*, by the elicitor and supprescins from *M. pinodes*. Details on PsCu/Zn-SOD1 were demonstrated previously[6] and a study on the association between ecto-apyrase and these redox enzymes in the apoplast/cell wall is underway.

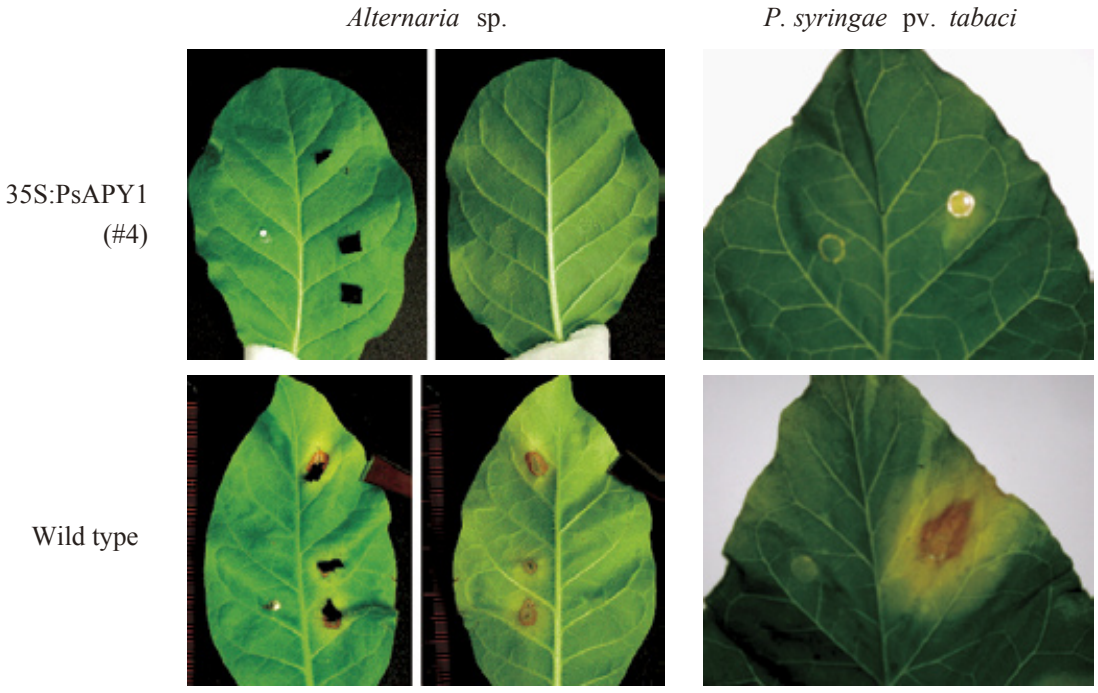

Fig. 4. Resistance to *Alternaria* sp. and *Pseudomonas syringae* pv. *tabaci* on a tobacco (SR1) transformed with 35S promoter and the pea ecto-apyrase gene (*PsAPY1*)
Lesion development was observed 5 dpi.

In this review, the significance of the combination of the plant cell wall and a fungal effector was introduced in determining host-parasite specificity. However, as an excellent work demonstrates how a host-specific toxin, ACR, targets the mitochondrial membrane in rough lemon cells[16], we know that host-parasite specificity is also determined inside cells in the other combinations. Here, an analog phytopathologist emphasizes that ultimately, the key question is whether the effector(s) exists in the real infection court and guarantees the pathogen's infection/proliferation. Recently, we found a new function of suppprescins as a means of inducing the expression of genes associated with jasmonate signaling[24]. Moreover, we also found that plant cell walls participate in ion-effluxes and the production of infection-inhibitors when treated with elicitors. Details will be presented elsewhere due to lack of space.

Acknowledgements

I would like to express sincere thanks to colleagues in our laboratory for their kind comments and technical support. Financial support from the Ministry of Education, Science, Sport and Culture, Japan is also deeply acknowledged.

References

1. Alfano, J. R. (2009) Roadmap for future research on plant-pathogen effectors. *Mol. Plant Pathol*., **10**, 805-813.
2. Daudi, A. et al. (2012) The apoplastic oxidative burst peroxidase in *Arabidopsis* is a major component of pattern-triggered immunity. *Plant Cell*, **24**, 275-287.
3. Gross, G. G. et al. (1977) Involvement of malate, monophenols and the superoxide radical in H2O2 formation by isolated cell wall from horseradish. *Planta*, **136**, 271-276.
4. Halliwell, B. (1978) Lignin synthesis: The generation of hydrogen peroxide and superoxide by horseradish peroxidase and its stimulation by manganese (II) and phenols. *Planta*, **140**, 81-88.
5. Kaku, H. et al. (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proc. Natl. Acad. Sci. USA*, **103**, 11086-11091.
6. Kasai, T. et al. (2006) Pea extracellular Cu/Zn-superoxide dimutase responsive to signal molecules from a fungal pathogen. *J. Gen. Plant Pathol*., **72**, 265-272.
7. Kato, T. et al. (1993) Inhibition of ATPase activity in pea plasma membranes by fungal suppressors from *Mycosphaerella pinodes* and their peptide moieties. *Plant Cell Physiol*., **34**, 439-445.
8. Kawahara, T. et al. (2003) Cloning and characterization of pea apyrases: involvement of PsAPY1 in response to signal molecules from the pea pathogen, *Mycosphaerella pinodes. J. Gen. Plant Pathol*., **69**, 33-38.
9. Kawahara, T. et al. (2006) Induction of defense responses in pea tissues by inorganic phosphate. *J. Gen. Plant Pathol*., **72**, 129-136.
10. Kiba, A. et al. (1995) Specific inhibition of cell wall-bound ATPase by fungal suppressor from *Mycosphaerella pinodes. Plant Cell Physiol*., **36**, 809-817.
11. Kiba, A. et al. (1996) Species-specific suppression of superoxide anion generation on surface of pea leaves by the suppressor from *Mycosphaerella pinodes. Ann. Phytopathol Soc. Jpn*., **62**, 508-512.
12. Kiba, A. et al. (1997) Superoxide generation in extracts from isolated plant cell walls is regulated by fungal signal molecules. *Phytopathology*, **87**, 846-852.
13. Kiba, A. et al. (2006) A binding protein for fungal signal molecules in the cell wall of *Pisum sativum. J. Gen. Plant Pathol*., **72**, 228-237.
14. Kiba, A. et al. (2006) A pea apyrase, PsAPY1, recognizes signal molecules from microorganisms. *J. Gen. Plant Pathol*., **72**, 238-246.
15. Matsubara, M. & Kuroda, H. (1987) The structure and physiological activity of glycoprotein secreted from conidia of *Mycosphaerella pinodes II. Chem. Pharm. Bull*., **35**, 249-255.
16. Ohtani, K. et al. (2002) Sensitivity *to Alternaria alternata* toxin in citrus because of altered mitochondrial RNA processing. *Proc. Nat. Acad. Sci. USA*, **99**, 2439-2444.
17. Oku, H. et al. (1977) Suppression of induction of phytoalexin, pisatin. *Naturwissenschaften*, **64**, 643.
18. Oku, H. et al. (1980) A new determinant of pathogenicity in plant disease. *Naturwissenschaften*, **67**, 310.
19. Shiraishi, T. et al. (1978) Elicitor and suppressor of pisatin induction in spore germination fluid of pea pathogen, *Mycosphaerella pinodes. Ann. Phytopathol. Soc. Jpn*., **44**, 659-665.
20. Shiraishi, T. et al. (1991) Inhibition of ATPase activity in plasma membranes *in situ* by a suppressor from a pea pathogen, *Mycosphaerella pinodes. Plant Cell Physiol*., **32**, 1067-1075.
21. Shiraishi, T. et al. (1992) Two suppressors, suppprescins A and B, secreted by a pea pathogen, *Mycosphaerella pinodes. Plant Cell Physiol*., **33**, 663-667.
22. Shiraishi, T. et al. (1997) The role of suppressors in determining host-parasite specificities in plant cells. *Int. Rev. Cytol*., **172**, 55-93.
23. Takahashi, H. et al. (2006) Localization and respon-

siveness of a cowpea apyrase VsNTPase1 to pathogenic microorganisms. *J. Gen. Plant Pathol*., **72**, 143-151.

24. Toyoda, K. et al. (2011) Suppression of defense - The role of fungal suppressors in conditioning plant susceptibility. *In* Genome–enabled analysis of plant-pathogen interactions. eds. Wolpert, T. et al., APS Press, St. Paul, MN, USA, 139-147.
25. Yoshioka, H. et al. (1990) Suppression of pisatin production and ATPase activity in pea plasma membranes by orthovanadate, verapamil and a suppressor from *Mycosphaerella pinodes*. *Plant Cell Physiol*., **31**, 1139-1146.
26. Yoshioka, H. et al. (1992) Suppression of the activation of chitinase and β-1, 3-glucanase in pea epicotyls by orthovanadate and suppressor from *Mycosphaerella pinodes*. *Ann. Phytopathol. Soc. Jpn*., **58**, 405-410.
27. Yoshioka, H. et al. (1992) Orthovanadate suppresses accumulation of phenylalanine ammonia-lyase mRNA and chalcone synthase mRNA in pea epicotyls induced by elicitor from *Mycosphaerella pinodes*. *Plant Cell Physiol*., **33**, 201-204.
28. Yoshioka, H. et al. (2003) *Nicotiana benthamiana* gp-91^{phox} homologs *NbrbohA* and *NbrbohB* participate in H_2O_2 accumulation and resistance to *Phytophthora infestans*. *Plant Cell*, **15**, 706-716.