**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. 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 elicitor-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.

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” in 1977. 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...
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 $\beta$-D-Glc-(1,6)-$\alpha$-D-Man-(1,6)-D-Man, which is $\alpha$-glycosidically attached to serine residues in the protein moiety 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 with structures of $\alpha$-GalNAc $\alpha$-ser-ser-gly (supprescin A; Mr, 452) and $\beta$-Gal-(1,4)-$\alpha$-GalNAc $\alpha$-ser-ser-gly-asp-glu-thr (supprescin B; Mr, 959). A hypovirulent strain OMP-X76 secreted supprescins 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. 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 bruiengeri*, *Medicago sativa*, *Millettia japonica*, *Pisum sativum* and *Trifolium pratense* of 12 plant species tested in the presence of supprescins 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, supprescins, only blocked these defense responses in host plants induced by various elicitors. However, supprescins alone instead elicited defense responses in nonhost plants.

It is well known that an oxidative burst is one of...
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.\textsuperscript{3} and Halliwell\textsuperscript{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 \textit{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\textsuperscript{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 the pea and cowpea cell wall preparations with Mn\textsuperscript{2+}, \textit{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\textsuperscript{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 generat-

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

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Degree of formation of infection hyphae* M. pinodes A. alternata 15B A. alternata 15B + M. pinodes suppressors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachis hypogaea</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Glycine max</td>
<td>0-1 0 0</td>
</tr>
<tr>
<td>Lespedeza buergeri</td>
<td>2 0 2</td>
</tr>
<tr>
<td>L. bicolor</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Lotus japonicus</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Medicago satina</td>
<td>1 0 1</td>
</tr>
<tr>
<td>\textit{M. truncatula}\textsuperscript{**}</td>
<td>2-4 0 2-4</td>
</tr>
<tr>
<td>Millettia japonica</td>
<td>2 0 1</td>
</tr>
<tr>
<td>Pisum sativum</td>
<td>4 0 4</td>
</tr>
<tr>
<td>Trifolium pratense</td>
<td>1 0 1</td>
</tr>
<tr>
<td>T. repens</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Vicia faba</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Vigna sinensis</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

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, \textit{Mycosphaerella ligulicola} (Toyoda et al., 2002, unpublished).

![Fig. 2. Effects of the elicitor (□) and suppressor (●) from \textit{Mycosphaerella pinodes} on \textit{in vitro} generation of superoxide in the NaCl-solubilized fractions from pea and cowpea cell wall preparations (Kiba et al., 1997\textsuperscript{23})](image-url)
tion (Toyoda et al., unpublished data). The results suggest that the first step of O$_2$/$\text{H}_2\text{O}_2$ generation is evoked by ecto-peroxidase in the apoplast/cell wall, meaning that the subsequent O$_2$/$\text{H}_2\text{O}_2$ generation is evoked by NADPH oxidase on the plasma membrane. An amplifying system for O$_2$/$\text{H}_2\text{O}_2$ generation in cells mediated by plasma membrane NADPH oxidase has been demonstrated. Recently, Bolwell and his colleague 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. We previously demonstrated that suppresscins 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 suppresscins 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 suppresscins 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 suppresscins 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 suppresscins 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 suppresscins in a strictly species-specific manner$^{10}$. In fact, even in vitro, suppresscins 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 suppresscins 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,
INF1-elicitin, β-glucan, laminarin, lipopolysaccharide, chitin oligomer and *Escherichia coli* (JM109)-gDNA but also to MgSO$_4$, AlCl$_3$, 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. Based on blue formazan assay with nitroblue tetrazolium, inorganic phosphate induced superoxide generation (2nd phase) 6 h after treatment. 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*. 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 superoxide dismutase, diamine oxidase, peroxidase and so on. Surprisingly, it emerged that these activities were also regulated, even *in vitro*, by the elicitor and suppresscins from *M. pinodes*. Details on *PsCu/Zn-SOD1* were demonstrated previously and a study on the association between ecto-apyrase and these redox enzymes in the apoplast/cell wall is underway.

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**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\textsuperscript{86} 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 suppresscins as a means of inducing the expression of genes associated with jasmonate signaling\textsuperscript{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.

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References

23. Takahashi, H. et al. (2006) Localization and respon-


