Published in Journal of Phytopathology 153, issues 7-8, 377-383, 2005,
which should be used for any reference to this work

Review

Laboratory of Biochemistry, University of Neuchâtel, Neuchâtel, Switzerland

Abscisic Acid and Callose: Team Players in Defence Against Pathogens?

V. Flors[1], J. Ton[2], G. Jakab[3] and B. Mauch-Mani[3]

Authors' addresses: [1]Department of Experimental Sciences, Plant Physiology Section, University of Jaume, Borriol, 12071 Castellon, Spain; [2]Section of Phytopathology, Faculty of Biology, Utrecht University, 3584 CA Utrecht, The Netherlands; [3]Faculty of Science, Laboratory of Biochemistry, University of Neuchatel, 2007 Neuchatel, Switzerland (correspondence to B. Mauch-Mani. E-mail: brigitte.mauch@unine.ch)

Keywords: abscisic acid, basal resistance, callose, callose deposition, papillae, plant–pathogen interactions, resistance response, systemic acquired resistance

Abstract

Abscisic acid (ABA) plays an important role as a plant hormone and as such is involved in many different steps of plant development. It has also been shown to modulate plant responses to abiotic stress situations and in recent years, it has become evident that it is partaking in processes of plant defence against pathogens. Although ABA's role in influencing the outcome of plant-pathogen interactions is controversial, with most research pointing into the direction of increased susceptibility, recent results have shown that ABA can also be involved in rendering plants more resistant to pathogen attack. In these cases, ABA interacts with callose deposition allowing an early and efficient build up of papillae at the sites of infection. The present review tries to shed some light on a possible interplay between ABA and callose in the protection of plants against invading pathogens.

Introduction

When plants are attacked by pathogens they activate a battery of reactions including both chemical and physical defences (Agrios, 1997). Among the chemical defences the synthesis of antimicrobial compounds such as phytoalexins and defensins have been well documented to play a role in the outcome of an interaction between a host plant and a pathogen (Kuc, 1995; Penninckx et al., 1998). The pathogenesis-related (PR) proteins, comprising enzymes capable of degrading pathogen cell walls, are another example of a successful plant defence mechanism (Van Loon and Van Strien, 1999). They play an important role in basal resistance as well as in systemic acquired resistance (SAR) where they have been shown to be induced not only locally in the attacked plant parts but also systemically in distant areas of the plant (Sticher et al., 1997). This systemic induction is thought to contribute to the heightened defensive capacity of the systemic tissues in SAR. At the cellular level these defence reactions are preceded by numerous changes including the synthesis of salicylic acid, reactive oxygen species (ROS), nitric oxide (NO) and the hypersensitive reaction to name a few (Veronese et al., 2003). All these changes occur in a well-defined sequential order and are mediated by distinct signal transduction pathways picking up the signal upon recognition of the pathogen by the host and leading to the final induction of defensive measures. Classically, two different signalling pathways have been distinguished (Kunkel and Brooks, 2002). One of the pathways is based on SA-dependent signalling (Ryals et al., 1996), the other is dependent on a functional jasmonate/ethylene signalling (Thomma et al., 2001). While the former has been shown to be implicated in the defence against biotrophs such as *Hyaloperonospora parasitica* and *Erysiphe orontii* or the hemibiotroph *Pseudomonas syringae* in *Arabidopsis*, the latter one is implicated when plants are challenged by necrotrophic organisms such as *Botrytis cinerea*, *Alternaria brassicicola* or *Erwinia carotovora* (Rojo et al., 1999; Thomma et al., 2001). This clear distinction observed in *Arabidopsis*, however, might not be valid for every plant species. Achuo et al. (2004) showed that the SA pathway was effective against *Botrytis* in tomato, but not in tobacco, while the SA pathway was effective against *Oidium* in tobacco but not in tomato. Recently, increasing evidence has also been pointing to the involvement of yet another plant hormone, abscisic acid (ABA), in plant–pathogen interactions (Audenaert et al., 2002; Anderson et al., 2004; Thaler and Bostock, 2004; Thaler et al., 2004; Ton and Mauch-Mani, 2004; Ton et al., 2005).

When a pathogen tries to invade plant tissues, the first barrier it encounters is the plant cell wall. If ingress can be stopped at this stage, these results in a

large reduction of cellular damage and can also make further defensive actions as described above obsolete. A typical reaction of an attacked plant is the build up of papillae (Zeyen et al., 2002). Such papillae are apposed on the inner side of epidermal cell walls in the apoplasm directly below the attempted entry point of a pathogen. A major constituent of such papillae is callose, a β-1,3-glucan, but other substances such as polysaccharides, phenolic compounds, reactive oxygen intermediates and proteins have also been found (Smart et al., 1986; Bolwell, 1993; Bestwick et al., 1997; Thordal-Christensen et al., 1997; Heath, 2002).

In the present review we will try to shed some light on the role of callose and ABA in plant resistance against pathogens and discuss what connections might exist between these two compounds.

Where is Callose Found and Where Does it Come From?

Callose is an amorphous β-1,3-D-glucan found in numerous locations in higher plants. It is easily visualized through its UV light-induced fluorescence with the aniline blue fluorochrome (Stone et al., 1985). Callose has been observed on sieve plates in dormant phloem and in abscission zones, it appears transitorily during cell plate formation, is a major component of pollen and pollen tube cell walls, and is also found in plasmodesmata (Stone and Clarke, 1992). Callose deposition can be induced by biotic and abiotic stresses and the polymer is then deposited between the plasma membrane and the cell wall (Stone and Clarke, 1992). Upon pathogen attack a rapid deposition of callose at the point of attempted penetration by the pathogen has been observed (Zimmerli et al., 2000; Donofrio and Delaney, 2001; Roetschi et al., 2001; Zeyen et al., 2002; Ton and Mauch-Mani, 2004).

Callose is supposed to be generated by callose synthase (CalS) complexes encoded by glucan synthase-like genes. There are 12 CalS isozymes in *Arabidopsis*, and each may be tissue-specific and/or regulated under different physiological conditions responding to biotic and abiotic stresses (Verma and Hong, 2001).

The Role of Callose in Plant Defense

A role for callose as constituent of papillae in plant defence has been accepted for a long time (Aist, 1976). One of the best-investigated systems in this field is the interaction between barley and the powdery mildew fungus *Blumeria graminis* f. sp. *hordei*. *Blumeria* attacks cereal epidermal cells and tries to grow a haustorium inside of them. To prevent penetration, these cells respond by local reinforcement of the cell wall beneath the site of the penetration attempt by forming a papilla. This process involves deposition of the callose matrix together with the accumulation of components such as H_2O_2, phenolics and various proteins and glycoproteins with hydrolytic and antifungal properties (reviewed in Zeyen et al., 2002). In barley the *Mlo* gene regulates cell wall repair processes, based on intracellular papilla formation at the site of injury. Mutations in this gene generally lead to excessive papilla growth causing a very high level of resistance to powdery mildew (although it made the plants more susceptible to pathogens such as *Magnaporthe* and *Bipolaris*). In *mlo*-mutants the absence of the regulatory function of the *Mlo* gene although often leads to pleiotropic effects (Wolter et al., 1993). Another example illustrating the importance callose deposits can have for the outcome of a host–pathogen interaction has been shown for the interaction between lettuce and *Plasmopara lactucae-radicis*. Resistance of lettuce to this oomycete is based on callose deposits around the haustoria. Treatment of a genetically resistant lettuce cultivar with 2-deoxy-D-glucose (DDG), an inhibitor of callose synthesis, resulted in susceptibility (Stanghellini et al., 1993). DDG also allowed highlighting the role of callose in the interaction between *Arabidopsis* and the two necrotrophs *A. brassisicola* and *Plectospherella cucumerina*, respectively. Here too, resistance was tightly correlated to the presence of callose-containing papillae (Ton and Mauch-Mani, 2004). In *Arabidopsis*, induction of resistance against *H. parasitica* with the non-protein amino acid β-aminobutyric acid (BABA) functions via deposition of callose at the point of attempted penetration of the oomycete (Zimmerli et al., 2000). In a collection of *Arabidopsis* mutants with increased resistance towards the powdery mildew pathogen *E. cichoracearum*, one mutant, *pmr4*, has been isolated that shows loss of callose deposition following wounding or pathogen attack. Interestingly, in this case the absence of callose deposition, due to a mutation in a CalS gene, lead not as expected to a higher susceptibility but to resistance (Nishimura et al., 2003). By crossing this mutant with mutants in the SA pathway the authors were able to show that a block in the SA pathway was sufficient to restore the susceptibility of the plants pointing to a negative regulation of the SA pathway by callose or CalS (Nishimura et al., 2003).

ABA Interactions with other Hormones and Pathways involved in Resistance

The sesquiterpenoid plant hormone ABA participates in the control of numerous essential physiological processes, such as seed development and germination, but also in plant responses to different stresses (Zeevaart and Creelman, 1988; Koornneef and Karssen, 1994; Rock and Quatrano, 1995; Leung and Giraudat, 1998). The level of ABA increases in plants during seed development and under many environmental stresses, particularly drought and salinity. An increasing body of information also points to an involvement of ABA in the plants' responses to pathogen attack (Audenaert et al., 2002; Anderson et al., 2004; Ton and Mauch-Mani, 2004; Ton et al., 2005). As other plant hormones are also known to play a role in signal transduction during pathogenesis (Ryals et al., 1996; Thomma et al., 2001), it is of crucial importance to understand the interplay between the different hormones and pathways to obtain a complete picture of the events taken place during infection.

In recent years, the availability of microarray data on expression profiling showing the influence of ABA and other hormone treatments on a very large number of genes has shown that there is a considerable amount of overlap and cross talk between the different pathways (Bray, 2002; Hoth et al., 2002).

Role of ABA in Susceptibility or Resistance to Pathogens

The implication of ABA in abiotic stress interactions have been widely studied (Zhu, 2002), but much less reports exist about the influence of this hormone in biotic interactions. In recent years there has been increasing interest in determining the function of ABA in plant–pathogen interactions, although its exact role in susceptibility or resistance of plants against different pathogens remains unclear.

In the late-1980s Cahill and Ward (1989) studied the regulatory capacity of ABA in soya bean–*Phytopthora sojae* interactions. This and posterior publications showed that ABA decreased during incompatible interactions with non-pathogenic strains of *P. sojae* as in interactions where metalaxyl-treated plants displayed resistance towards pathogenic races of *Phytophthora*. No changes in ABA concentrations were observed in compatible interactions (Cahill et al., 1993). From these studies, one might have deduced that ABA decreases when plants recognize the pathogen. However, in studies with rust fungi, ABA decreased in beans infected with either *Uromyces appendicularis* (pathogenic) or *U. vignae* (non-pathogenic) ABA decreases in both cases. Therefore, this decrease seems a non-specific result of early events prior to cell penetration and is not determinant for successful fungal invasion (Ryerson et al., 1993).

ABA can regulate phenylalanine ammonia lyase (PAL) at the transcriptional level in the soya bean–*P. sojae* interaction. This leads to an increase in susceptibility and a change to a compatibility when ABA-treated soya bean plants are inoculated with non-pathogenic races of *P. sojae* (McDonald and Cahill, 1999). Application of norfluorazon, which leads to an inhibition of ABA biosynthesis, to soybeans also leads to a shift towards incompatibility when such treated plants are infected with virulent races of *Phytophthora*. Norflurazon causes a systemic closure of stomata and an increase of PAL activity, typical reactions observed during incompatible interactions (McDonald and Cahill, 1999).

Because many pathogens use stomata to penetrate the leaves it seems obvious that ABA might play an important role by closing the stomata upon pathogen attack. Recently, Desikan and co-workers (Desikan et al., 2002; Neill et al., 2002) showed that NO is a signalling molecule necessary for ABA-induced stomatal closure in *A. thaliana*. Other reports point to NO as an interesting ROS molecule that plays a regulatory role in signaling during pathogen-induced oxidative burst (Zeier et al., 2004). It is tempting to speculate that NO is the possible link between ABA and resistance to pathogens, although other possible ABA responses such as ABA-mediated callose accumulation are also likely to play an important role in pathogenesis (Ton and Mauch-Mani, 2004). For this reason, recent work by Prats et al. (2005) seems especially interesting. They showed that in barley NO generation was one of the earliest responses of epidermal cells against *B. graminis* attack and this may play an important role both in the initiation and the development of effective papillae.

A second alternative pathway, independent of ROS species, is that ABA induces the closure of stomata and activates phospholipases and generation of inositol trisphosphate (IP_3) which stimulates an increase of cytoplasmatic Ca^{2+} that induces later events in stomatal closure (Buchanan et al., 2000). The *Arabidopsis* mutant *ibs2* with a mutation in a polyphosphoinositide phosphatase and mutant *ibs3*, mutated in a zeaxanthin epoxydase both show impaired priming following BABA treatment against *H. parasitica* (Ton et al., 2005). Additionally, these mutants are unable to accumulate callose after BABA treatment and oomycete infection, showing that ABA and callose deposition can be tightly related in the regulation of resistance against *H. parasitica*. There is also evidence that fungal elicitors can activate a shared branch with ABA in the stress signal transduction pathway in guard cells that activate plasma membrane Ca^{2+}-channels and support a requirement for extracellular Ca^{2+} to start the stomatal closure process (Klüsener et al., 2002).

In other systems, in which the SA signalling pathway and PAL activity are essential for pathogen resistance, ABA induces susceptibility (Audenaert et al., 2002). Exogenous applications of ABA can inhibit PAL activity and transcription in response to the oomycete *P. megasperma* (Ward et al., 1989). The tomato mutant *sitiens*, with reduced ABA levels, shows an enhanced resistance against *B. cinerea*. However, *Nah*G transgenic tomato plants are more susceptible to *Botrytis*. This shows that SA is important for resistance against *B. cinerea* in tomato and therefore repression of the SA pathway by ABA induces a higher susceptibility to the fungus. The antagonistic effects of SA and ABA have been shown in several studies. Overexpression of the activated disease resistance 1 (ADR1) encoding a coiled-coil nucleotide-binding site leucine-rich repeats protein confers significant drought tolerance in *Arabidopsis*. This phenotype required SA and functional enhanced disease susceptibility (EDS1) and ABI1 proteins indicating interactions of the SA and ABA signalling (Chini et al., 2004). Expression of the salt-induced protein (SALT) in rice was induced by fungal elicitor, JA, ABA, but strongly inhibited by SA indicating an antagonistic effect of these hormones (Kim et al., 2004). Suppression of a stress-responsive MAPK gene from rice, OsMAPK5 that is inducible by ABA as well as various abiotic and biotic stresses, resulted in the constitutive expression of SA-regulated PR genes and enhanced resistance against pathogens (Xiong and Yang, 2003). These plants, however,

showed reduced drought, salt and cold tolerance demonstrating that a MAPK cascade is involved in the antagonistic regulation of the SA and ABA responses in plants.

In *A. thaliana*, exogenous applications of ABA also induce susceptibility to *P. syringae* but do not affect the resistance against *H. parasitica*. In contrast, an ABA-deficient mutant, *aba1-1*, displays reduced susceptibility to virulent isolates of *H. parasitica* while *abi1-1* (ABA-insensitive) resistance does not change in respect to the wild type (Mohr and Cahill, 2003). These experiments show that a high endogenous concentration of ABA at the moment of pathogen infection can contribute to develop susceptibility.

All these observations suggest that ABA can contribute to the susceptibility of a plant to those pathogens for which the SA signalling pathway is essential to stop the disease, mainly biotrophic pathogens and *Pseudonomas syringae* (Thomma et al., 1998). On the contrary, ABA can induce resistance and mimic the BABA-induced resistance (IR) to necrotrophic pathogens against which callose accumulation and the JA/ET signalling are implicated (Thomma et al., 1998; Ton and Mauch-Mani, 2004). This is also supported by the fact that ABA can repress the SA signalling pathway, which is known to negatively regulate the JA/ET pathway as well as callose deposition (Jacobs et al., 2003; Nishimura et al., 2003). Pretreatments of tobacco with ABA result in an increased resistance against tobacco mosaic virus (TMV) (Fraser, 1982). Deposition of callose is also an effective barrier against viral agents and there is a negative correlation between the β-1,3-glucanases (known as callose-degrading enzymes) content in tobacco mutants and their resistance to TMV (Beffa et al., 1996). The influence of ABA on viral infections is probably due to the fact that it can down-regulate the β-1,3-glucanase mRNA accumulation and subsequently its activity (Rezzonico et al., 1998), therefore, the inhibition of callose-degrading enzymes could be another way to increase ABA-mediated callose deposition and ABA-induced resistance in response to pathogens. A TMV-induced cell wall protein, CaTin2, containing a repeated helix-turn-helix motif and involved in the virus-resistance reaction of pepper, is also induced by ABA (Shin et al., 2003).

Recently, a new leucine-rich repeat gene named *CALRR1* has been found in pepper and was proposed to act as a receptor of pathogenic signals as it is highly stimulated by *Xanthomonas campestris* and *P. capsici* (Jung et al., 2004). Surprisingly, this putative receptor is also induced by ABA, suggesting that this hormone is probably involved in the signal transduction pathway for induction of *CALRR1*.

New evidence points to a complex interplay between ABA and JA-ethylene signalling pathways concerning the regulation of plant defense gene expression and disease resistance. Anderson et al. (2004) showed that exogenous application of ABA led to a suppression of both basal and JA-ethylene-activated transcription of defence genes. Low ABA levels in contrast were accompanied by an up-regulation of transcription from JA-ethylene responsive defence genes. Thus, antagonistic interactions between the ABA and the JA-ethylene signalling pathways modulate defence responsive gene expression in response to biotic stress.

Other possible mechanisms for ABA resistance or susceptibility still remain unknown, but ABA is definitively involved in the regulation and signalling of plant–pathogen interactions and the classical view of ABA as an abiotic stress signalling hormone should be reconsidered.

ABA: a Regulator of Pathogen-induced Callose Deposition

It is clear that both ABA and callose influence the outcome of many plant–pathogen interactions. Recently, evidence has emerged that ABA can play a regulatory role in the intensity and speed by which callose is deposited. First indications for this relationship came from experiments in our laboratory about the mode of action behind BABA-IR against necrotrophic fungi. Two *Arabidopsis* mutants impaired in ABA signalling, as well as the callose-deficient mutant *pmr4-1* (Nishimura et al., 2003), failed to express BABA-IR against the necrotrophic fungus *P. cucumerina*, demonstrating that BABA-IR against this pathogen requires both intact ABA signalling and callose synthesis. Monitoring the extent of callose accumulation upon infection by *P. cucmerina* revealed that the BABA-induced augmentation of callose was absent in ABA-insensitive *abi4-1* plants, providing a causal link between ABA signalling and callose deposition. This relationship was further confirmed by the finding that exogenous application of ABA mimicked the effect of BABA on both callose deposition and resistance against *P. cucumerina* and *A. brassicicola* (Ton and Mauch-Mani, 2004; Fig. 1). Hence, BABA-IR in *Arabidopsis* against necrotrophic pathogens is based on primed accumulation of callose, which is regulated by a novel ABA-dependent defence pathway. More evidence for the relationship between ABA signalling and callose came from the characterization of *Arabidopsis* mutants that are impaired in BABA-induced sterility (Ton et al., 2005). One of these BABA response mutants, *ibs3*, was found to be affected in the regulation of the zeaxanthin epoxidase gene *ABA1*, which mediates the biosynthesis of ABA. Further characterization of this mutant revealed that it expressed reduced levels of BABA-IR against *H. parasitica* that correlated with reduced levels of callose deposition, indicating a causal relationship between ABA signalling and callose deposition. Together, both studies provide clear evidence for a regulatory role of ABA in the BABA-induced augmentation of pathogen-induced callose deposition. However, it is important to keep in mind that ABA does not directly regulate callose deposition, but rather modulates the speed and intensity (priming) of its deposition. Mutants in the ABA pathway such as *ibs3* are not impaired in basal callose deposition upon

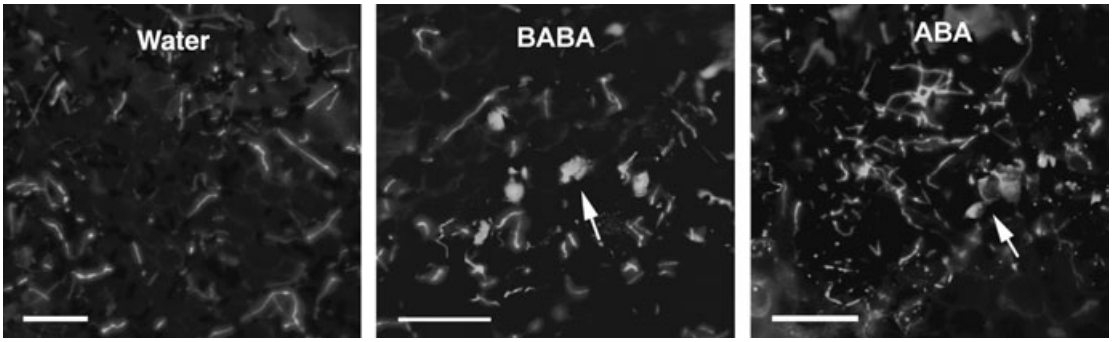

Fig. 1 Potentiation of callose deposition by β-aminobutyric acid (BABA) and abscisic acid (ABA) in *Arabidopsis* upon infection by *Alternaria brassicicola*. About 5-week-old *Arabidopsis pad3-1* plants (phytoalexin-deficient mutant) were soil-drenched with water, 150 μM BABA, or 80 μM ABA at 2 days prior to challenge inoculation with *A. brassicicola*. Leaves were collected at 2 days after inoculation, stained with calcofluor/aniline blue, and analysed by epifluorescence microscopy (UV). Germ tubes fluoresce blue and callose yellow. Arrows indicate callose depositions (bar = 50 μm)

pathogen infection, but they are clearly impaired in the (BABA-induced) priming for callose (Ton et al., 2005).

Are SNARE-proteins Molecular Mediators of ABA-dependent Callose Deposition?

The molecular mechanisms behind the modulation of callose by ABA remain to be elucidated. However, studies on cell plate formation provide useful indications about possible mediators of this response. During the onset of cell division, Golgi-derived vesicles travel along microtubules and accumulate in the plane of division. Among many other plasma membrane-associated proteins, these vesicles contain CalS proteins that deposit callose onto the cell plate (Verma, 2001). The fusion of these vesicles to the plasma membrane is mediated by proteins known as soluble *N*-ethyl-maleimide-sensitive fusion protein attachment protein receptors (SNAREs; Pratelli et al., 2004). Interestingly, various SNAREs have been implicated in ABA-dependent responses to osmotic stress. For instance, Leyman et al. (1999) isolated the syntaxin-like protein NtSyp121 (NtSyr1) in a screen for an ABA receptor. The expression of this SNARE protein was strongly induced by ABA, and overexpression of a truncated (dominant-negative) form of this protein resulted in obstruction of ABA-dependent modulation of K^+ and Cl^- channels in guard cells. Furthermore, the *Arabidopsis* mutant *osm1*, carrying a mutation in the SNARE encoding gene *AtSyp61*, is hypersensitive to salt stress and affected in stomatal movement (Zhu et al., 2001). Hence, different plant SNAREs seem to mediate ABA-dependent responses to osmotic stress. Recently, SNAREs have also been linked to disease resistance at the plant cell wall. Genetic screens for fungal pathogen resistance in *Arabidopsis* and barley identified two SNARE encoding genes, *PEN1* and *ROR2* (Collins et al., 2003). Mutations in these genes cause partial loss of cell wall resistance resulting in enhanced cell penetration by non-host pathogens. It is thought that both *PEN1* and *ROR2* mediate transport of vesicles that deliver toxic compounds and CalS proteins to the sites of fungal penetration. Strikingly, both SNARE genes are highly homologous to the tobacco *NtSyp121*, which had previously been characterized as an ABA-inducible SNARE gene (Leyman et al., 1999; Collins et al., 2003). It seems tempting, therefore, to speculate that ABA controls callose deposition by transcriptional regulation of specific SNAREs that direct vesicle-mediated transport of CalS proteins to the sites of pathogen attack.

Conclusions

There is a growing body of evidence pointing to an active and important role of ABA signalling in the plants' defence against pathogens. ABA can interact positively or negatively with other signal transduction pathways implicated in defence and as our own studies have shown, ABA seems also to play a role in callose deposition during the resistance response of plants to pathogens. The influence of this hormone on the outcome of plant–pathogen interactions definitively deserves our full attention.

Acknowledgements

Authors thank Felix Mauch for critical reading of the manuscript. Financial support to the authors for research described in this review was obtained from the Swiss National Foundation (Grant 3100AO-105884/1), the National Center of Competence on Plant Survival in Natural and Agricultural Ecosystems (B.M.M.), the Netherlands Organization for Scientific Research (Grant 863.04.019 to J.T.) and a grant from the Agéncia Valenciana de Ciència i Tecnologia (Generalitat Valenciana) to V.F.

References

Achuo EA, Audenaert K, Meziane H, Höfte M. (2004) The salicylic acid dependent pathway is effective against different pathogens in tomato and tobacco. *Plant Pathol* **53**:65–72.

Agrios GN. *Plant Pathology*. CA, USA, Academic Press, 1997.

Aist JR. (1976) Papillae and related wound plugs of plant cells. *Annu Rev Phytopathol* **14**:145–163.

Anderson PJ, Badruzsaufari E, Schenk PM et al. (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. *Plant Cell* **16**:3460–3479.

Audenaert K, De meyer GB, Höfte MM. (2002) Abscisic acid determines basal susceptibility of tomato to *B. cinerea* and suppresses

salicylic acid dependent signaling mechanisms. *Plant Physiol* **128**:491–501.

Beffa RS, Hofer R-M, Thomas M, Meins F Jr. (1996) Decreased susceptibility to virus disease of β-1,3-glucanase-deficient plants generated by antisense transformation. *Plant Cell* **8**:1001–1011.

Bestwick CS, Brown IR, Bennett MHR, Mansfield JW. (1997) Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringae* pv *phaseolicola*. *Plant Cell* **9**:209–221.

Bolwell GP. (1993) Dynamic aspects of the plant extracellular matrix. *Int Rev Cytol* **146**:261–323.

Bray EA. (2002) Abscisic acid regulation of gene expression during water-deficit stress in the era of the *Arabidopsis* genome. *Plant Cell Environ* **25**:153–161.

Buchanan BB, Gruissem W, Jones R. *Biochemistry and Molecular Biology of Plants*. Rockfield, MD, USA, ASP, 2000.

Cahill DM, Ward EWB. (1989) Rapid localized changes in abscisic acid concentrations in soybean in interactions with *Phytophthora megasperma* f. sp. *glycinea* or after treatment with elicitors. *Physiol Mol Plant Pathol* **35**:483–493.

Cahill DM, Morris PF, Ward EWB. (1993) Influence on abscisic acid levels in soybean hypocotyls infected with *Phytophthora sojae*. *Physiol Mol Plant Pathol* **42**:109–121.

Chini A, Grant JJ, Seki M, Shinozaki K, Loake GJ. (2004) Drought tolerance established by enhanced expression of the *CC-NBS-LRR* gene, *ADR1*, requires salicylic acid, EDS1 and ABI1. *Plant J* **38**:810–822.

Collins NC, Thordal-Christensen H, Lipka V et al. (2003) SNARE-protein-mediated disease resistance at the plant cell wall. *Nature* **425**:973–977.

Desikan R, Griffiths J, Hancock J, Neill S. (2002) A new role for an old enzyme: nitrate reductase mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* **99**:16314–16318.

Donofrio NM, Delaney TP. (2001) Abnormal callose response phenotype and hypersusceptibility to *Peronospora parasitica* in defense-compromised *Arabidopsis* nim1-1 and salicylate hydroxylase expressing plants. *Mol Plant Microbe Interact* **14**:439–450.

Fraser RSS. (1982) Are 'pathogenesis-related' proteins involved in acquired systemic resistance of tobacco plants to tobacco mosaic virus? *J Gen Virol* **58**:305–313.

Heath MC. (2002) Cellular interactions between biotrophic fungal pathogens and host or nonhost plants. *Can J Plant Pathol* **24**:259–264.

Hoth S, Morgante M, Sanchez JP, Hanafey M, Tingey S, Chua N-H. (2002) Genome-wide gene expression profiling in *Arabidopsis thaliana* reveals new targets of abscisic acid and largely impaired gene regulation in the abi1-1 mutant. *J Cell Sci* **115**:4891–4900.

Jacobs A, Lipka V, Burton R et al. (2003) An *Arabidopsis* callose synthase, *GSL5*, is required for wound and papillary callose formation. *Plant Cell* **15**:2503–2513.

Jung EH, Jung HW, Lee SC, Han SW, Heu S, Hwang BK. (2004) Identification of a novel pathogen-induced gene encoding a leucine-rich repeat protein expressed in phloem cells of *Capsicum annuum*. *Biochim Biophys Acta* **1676**:211–222.

Kim ST, Kim SG, Hwang DH et al. (2004) Expression of a salt-induced protein (SALT) in suspension-cultured cells and leaves of rice following exposure to fungal elicitor and phytohormones. *Plant Cell Rep* **23**:256–262.

Klüsener B, Young JJ, Murata Y et al. (2002) Convergence of calcium signaling pathways of pathogenic elicitors and abscisic acid in *Arabidopsis* guard cells. *Plant Physiol* **130**:2152–2163.

Koornneef M, Karssen CM. Seed dormancy and germination. In: Somerville CR, Meyerowitz EM (eds), *Arabidopsis*, pp. 313–334, Cold Spring Harbor, NY, USA, Cold Spring Harbor Laboratory Press, 1994.

Kuc J. (1995) Phytoalexins, stress metabolism, and disease resistance in plants. *Annu Rev Phytopathol* **33**:275–297.

Kunkel BN, Brooks DM. (2002) Cross talk between signaling pathways in pathogen defense. *Curr Opin Plant Biol* **4**:325–331.

Leung J, Giraudat J. (1998) Abscisic acid signal transduction. *Annu Rev Plant Physiol Plant Mol Biol* **49**:199–222.

Leyman B, Geelen D, Quintero FJ, Blatt MR. (1999) A tobacco syntaxin with a role in hormonal control of guard cell ion channels. *Science* **283**:537–540.

McDonald KL, Cahill DM. (1999) Influence of abscisic acid and the abscisic acid biosynthesis inhibitor, norfluorazon, on interactions between *Phytophthora sojae* and soybean (*Glycine max*). *Eur J Plant Pathol* **105**:651–658.

Mohr PG, Cahill DM. (2003) Abscisic acid influences the susceptibility of *Arabidopsis thaliana* to *Pseudomonas syringae* pv. *tomato* and *Peronospora parasitica*. *Funct Plant Biol* **30**:461–469.

Neill S, Desikan R, Clarke A, Hancok J. (2002) Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. *Plant Physiol* **128**:13–16.

Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H, Somerville S. (2003) Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science* **301**:969–972.

Penninckx IAMA, Thomma BPHJ, Buchala A, Métraux J-P, Broekaert WF. (1998) Concomitant activation of jasmonate and ethylene responses is required for induction of a plant defensin gene in *Arabidopsis*. *Plant Cell* **10**:2103–2114.

Pratelli R, Sutter J-U, Blatt MR. (2004) A new catch in the SNARE. *Trends Plant Sci* **9**:187–195.

Prats E, Mur LAJ, Sanderson R, Carver TLW. (2005) Nitric oxide contributes both to papilla-based resistance and the hypersensitive response in barley attacked by *Blumeria graminis* f. sp. *hordei*. *Mol Plant Pathol* **6**:65–78.

Rezzonico E, Flury N, Meins F Jr, Beffa R. (1998) Transcriptional down-regulation by abscisic acid of pathogenesis-related beta-1,3-glucanase genes in tobacco cell cultures. *Plant Physiol* **117**:585–592.

Rock C, Quatrano RS. The role of hormones during seed development. In: Davies P (ed.), *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, pp. 671–697, Dordrecht, The Netherlands, Kluwer Academic Publishers, 1995.

Roetschi A, Si-Ammour A, Belbahri L, Mauch F, Mauch-Mani B. (2001) Characterization of an *Arabidopsis-Phytophthora* pathosystem: resistance requires a functional PAD2 gene and is independent of salicylic acid, ethylene and jasmonic acid signaling. *Plant J* **3**:293–305.

Rojo E, Leon J, Sanchez-Serrano JJ. (1999) Cross-talk between wound signaling pathways determines local versus systemic gene expression in *Arabidopsis thaliana*. *Plant J* **20**:135–142.

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD. (1996) Systemic acquired resistance. *Plant Cell* **8**:1809–1819.

Ryerson E, Li A, Young JP, Heath MC. (1993) Changes in abscisic acid levels in bean leaves during the initial stages of host and nonhost reactions to rust fungi. *Physiol Mol Plant Pathol* **43**:265–273.

Shin R, Park CJ, An JM, Paek KH. (2003) A novel TMV-induced hot pepper cell wall protein gene (*CaTin2*) is associated with virus-specific hypersensitive response pathway. *Plant Mol Biol* **51**:687–701.

Smart MG, Aist JR, Israel HW. (1986) Structure and function of wall appositions: 1. General histochemistry of papillae in barley (*Hordeum vulgare*) coleoptiles attacked by *Erysiphe graminis* f. sp. *hordei*. *Can J Bot* **64**:793–801.

Stanghellini ME, Rasmussen SL, Vandemark GJ. (1993) Relationship of callose deposition to resistance of lettuce to *Plasmopara lactucae-radicis*. *Phytopathology* **83**:1498–1501.

Sticher L, Mauch-Mani B, Métraux JP. (1997) Systemic acquired resistance. *Annu Rev Phytopathol* **35**:235–270.

Stone BA, Clarke AE. *Chemistry and Biology of (1 → 3)-b-D-Glucans*. Victoria, Australia, La Trobe University Press, 1992.

Stone BA, Evans NA, Bonig I, Clarke AE. (1985) The application of Sirofluor, a chemically defined fluorochrome from aniline blue for the histochemical detection of callose. *Protoplasma* **122**:191–195.

Thaler JS, Bostock RM. (2004) Interactions between abscisic acid-mediated responses and plant resistance to pathogens and insects. *Ecology* **85**:48–58.

Thaler JS, Owen B, Higgins VJ. (2004) The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. *Plant Physiol* **135**:530–538.

Thomma BPHJ, Eggermont K, Penninckx IAMA et al. (1998) Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proc Natl Acad Sci U S A* **95**:15107–15111.

Thomma BP, Penninckx IA, Broekaert WF, Cammue BP. (2001) The complexity of disease signaling in *Arabidopsis*. *Curr Opin Immunol* **13**:63–68.

Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB. (1997) Subcellular localization of H_2O_2 in plants: H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *Plant J* **11**:1187–1194.

Ton J, Mauch-Mani B. (2004) β-Aminobutyric acid induced resistance against necrotrophic pathogens is based on ABA-dependent priming of callose. *Plant J* **38**:119–130.

Ton J, Jakab G, Toquin V et al. (2005) Dissecting the β-aminobutyric acid-induced priming pathways in *Arabidopsis*. *Plant Cell* **17**:987–999.

Van Loon LC, Van Strien EA. (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol Mol Plant Pathol* **55**:85–97.

Verma DPS. (2001) Cytokinesis and building of the cell plate in plants. *Annu Rev Plant Physiol Plant Mol Biol* **52**:751–784.

Verma DP, Hong Z. (2001) Plant callose synthase complexes. *Plant Mol Biol* **47**:693–701.

Veronese P, Ruiz MT, Coca MA et al. (2003) In defense against pathogens. Both plant sentinels and foot soldiers need to know the enemy. *Plant Physiol* **131**:1580–1590.

Ward EWB, Cahill DM, Bhattacharyya M. (1989) Abscisic acid suppression of phenylalanine ammonia lyase activity and mRNA, and resistance of soybeans to *Phytophthora megasperma* f. sp. *glycinea*. *Plant Physiol* **91**:23–27.

Wolter M, Hollricher K, Salamini F, Schulze-Lefert P. (1993) The *mlo* resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defense mimic phenotype. *Mol Gen Genet* **239**:122–128.

Xiong L, Yang Y. (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell* **15**:745–759.

Zeevaart JAD, Creelman RA. (1988) Metabolism and physiology of abscisic acid. *Annu Rev Plant Physiol Plant Mol Biol* **39**:439–473.

Zeier J, Delledonne M, Mishina T, Severi E, Sonoda M, Lamb C. (2004) Genetic elucidation of nitric oxide signaling in incompatible plant-pathogen interactions. *Plant Physiol* **136**:1–12.

Zeyen RJ, Carver TLW, Lyngkjær MF. The formation and role of papillae. In: Bélanger RR, Bushnell WR, Dik AJ, Timothy LW (eds), *The Powdery Mildews: A Comprehensive Treatise*, pp. 107–125, Carver APS Press, St. Paul, Minnesota, 2002.

Zhu JK. (2002) Salt and drought stress signal transduction in plants. *Annu Rev Plant Biol* **53**:247–273.

Zhu J, Zhizhong G, Zhang C et al. (2001) A syntaxin protein in *Arabidopsis* controls abscisic acid-mediated and non-abscisic acid-mediated responses to abiotic stress. *Plant Cell* **14**:3009–3028.

Zimmerli L, Jakab G, Metraux JP, Mauch-Mani B. (2000) Potentiation of pathogen-specific defense mechanisms in *Arabidopsis* by beta-aminobutyric acid. *Proc Natl Acad Sci U S A* **97**:12920–12925.