# Chapter 4
# Plant Defense Activators: Application and Prospects in Cereal Crops

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**Abstract** Cereal grains are one of the primary sources of food products in the world. Increased productivity in crop yield, particularly for cereal crops, is absolutely essential for future food security, but is impeded by disease, with annual estimates ranging from 10 to 30 % crop loss due to disease alone. There have been remarkable advances in understanding pest and disease resistance in plants in the past three decades, with the application of chemical plant defense activators (PDAs) being of particular interest. The advances in recent years in understanding the molecular basis for systemic acquired resistance (SAR), induced systemic resistance (ISR), priming, and next-generation immunity portend a wider role for PDAs. These agrochemicals are gaining some acceptance in Europe where there is a strong interest in curtailing the use of more traditional fungicides and pesticides. Much work, however, is needed to understand the effects of nutrition, dose rates, timing of application, and genotypic effects in the application of PDAs. This review addresses the current understanding of plant immunity, particularly with respect to cereal crops and the potential for PDAs to enhance the potential yield and nutritional quality of cereal crops.

## 4.1 Introduction

As the world population increases from the current estimate of 7 billion to a projected 9 billion by 2050 [1] and as greater demand on land usage for activities other than agriculture increases, food supply will become an issue of even greater importance. Cereal grains are one of the primary sources of food products in the world today [2]; this is unlikely to change in the foreseeable future. Thus, increased productivity in crop yield, particularly for cereal crops, is absolutely essential for future

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D. R. Gang (ed.), *50 Years of Phytochemistry Research*,
Recent Advances in Phytochemistry 43, DOI 10.1007/978-3-319-00581-2_4,

food security. One of the major impediments to crop yield is disease, with annual estimates ranging from 10 to 30% crop loss due to disease alone [1]. In addition to yield loss, pathogens can also contaminate food crops with toxins, rendering them useless, when detected, and dangerous for human consumption when left undetected. Disease loss, however, can be mitigated by informed agricultural practices.

There have been remarkable advances in understanding pest and disease resistance in plants in the past three decades. Much of this research has been conducted on dicots, *Arabidopsis* and tobacco being the two principal model organisms. After the physical barrier of the leaf cuticle or outer cell wall of other tissues, the fundamental disease resistance mechanism in plants is its basal resistance. Our understanding of this phenomenon has undergone dramatic changes in the last two decades, leading to a much keener understanding of the molecular events and signaling mechanisms involved in plant "immunity." As a result, improved methods to elicit this response have come to fruition. One of these methods is the application of chemical plant defense activators (PDAs). This review addresses the current understanding of plant immunity, particularly with respect to cereal crops and the potential for PDAs to enhance the potential yield and nutritional quality of cereal crops.

## 4.2 Pathogen Recognition

The prevailing model in plant disease resistance for most of the past century has been the gene-for-gene theory, based on the pioneering genetic studies of Harold H. Flor [3]. This theory posits that plants recognize microbial pathogens by their avirulence factors and combat them through expression of resistance genes, termed "R" genes. More recently, a "zigzag" model (Fig. 4.1) of pathogen resistance has emerged [4]. This paradigm rationalizes that plants recognize a pathogen invader first by interaction of pathogen-associated molecular patterns (PAMPs) (sometimes called microbe-associated molecular patterns (MAMPs)). The presence of these molecules at the plant cell membrane suggests attack by a potential pathogen. Bacterial flagellin, certain lipopolysaccharides, and chitin (polymeric *N*-acetyl glucosamine, a component of many fungal species cell walls and, coincidentally, crustacean shells) are well-documented examples of MAMPs. Plant cell surface receptors called pattern recognition receptors (PRR) interact with MAMPs to trigger the initial stage of plant defense, termed PAMP-triggered immunity (PTI). PRRs are transmembrane proteins consisting of an extracellular leucine-rich repeat motif and, typically, an intracellular protein kinase. Only a few PRRs have been characterized for their specific binding mechanisms. Perhaps the most thoroughly studied examples are the *Arabidopsis* receptor for bacterial flagellin peptide [5] and the receptor for bacterial elongation factor-Tu [6, 7]. For the most part, these PRRs recognize highly conserved pathogen-derived molecules. MAMPs appear to be essential to the survivability and pathogenicity of the offending organism, thus not being readily adaptable to mutation. Equally important is the ability of the plant to discern these MAMPs from beneficial microbes and even its own molecular patterns, thus

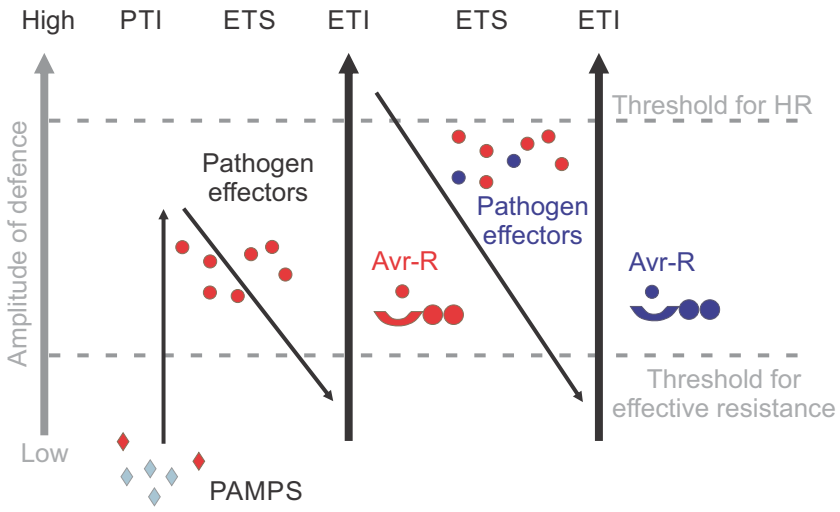


**Fig. 4.1** The "zigzag" model of pathogen resistance

avoiding complications arising from autoimmunity [8]. Plants do, however, have the ability to detect self-antigens in the form of damage-associated molecular patterns (DAMPs) typically resulting from herbivore damage or microbial pathogen-mediated lytic enzymes (see [9] for an excellent review of the biochemistry of plant PRRs).

Upon activation, the PRRs initiate a plethora of defense mechanisms, including callose deposition in the cell wall, increased ion flux, particularly the influx of $H^+$ and $Ca^{++}$ and efflux of $K^+$ ions, activation of plant mitogen-activated protein kinases (MAPKs) with concurrent phosphorylation of numerous signaling proteins, the generation of reactive oxygen species, biosynthesis of pathogenesis-related (PR) proteins,and the production of phytoalexins.

To overcome the basal immunity elicited through PTI, pathogenic microbes have evolved another strategy to circumvent the signaling mechanisms triggered by their MAMPs. This second phase of the zigzag model involves the delivery of effectors into the plant cell. Effectors are, to a large extent, what was previously termed virulence (or avirulence) factors in Flor's gene-for-gene theory of disease resistance. In essence, effectors are an array of pathogen-derived metabolites and proteins that interfere with host defense mechanisms. Pathogenic bacteria typically inject their effectors into the host cytoplasm through a type-three secretory system [10]. Introduction of effectors by other eukaryote pathogens is not as well understood; however, evidence points to RxLR-EER protein motifs, similar to those employed by *Plasmodium* (malarial) parasites in mammals [11]. This motif binds to phosphatidylinositol-3-phosphate moieties in the cell membrane [12], whereupon they are translocated into the cytoplasm. This likely represents a mechanism for both fungal and oomycete effectors [12, 13]. Effectors work through a wide range

of mechanisms. Small molecule effectors like coronatine, a non-host-specific phytotoxin produced by pathovars of the bacterial pathogen *Pseudomonas syringae*, appear to mimic the action of jasmonic acid (JA) [4, 14, 15], thus suppressing the effect of salicylic acid (SA) [16, 17]. Effectors also abrogate the defense response through other, largely unknown, mechanisms. Many clearly inhibit the host defense signaling pathways [18, 19].

## 4.3 Salicylic Acid Signaling

Recognition of pathogen or herbivore invasion results in what is now considered two separate signaling pathways that elicit an enhanced resistance response at locations distal to the site of infection. The first of these pathways was described by Ross in 1961 in which he demonstrated that tobacco leaves inoculated with tobacco mosaic virus (TMV) produced a lasting immunity in other portions of the plant against this virus as well as other viral pathogens. He termed this response systemic acquired resistance (SAR) [20]. This phenomenon has subsequently been demonstrated in numerous host–pathogen relationships and appears to be a characteristic of most, if not all, terrestrial plants [21]. The nature of the mobile signaling molecule(s) has been a subject of intense research and not inconsiderable controversy since SAR was initially proposed. Acetylsalicylic acid along with salicylic and benzoic acid were demonstrated to induce resistance against TMV in tobacco plants in 1979 [22]. Subsequently, SA and its methyl ester have been presented as the likely candidate as the mobile messenger in SAR [23, 24] and, in fact, a chemical analog for SA, 2,6-dichloroisonicotinic acid (INA), can replace SA in eliciting SAR in *Arabidopsis* and tobacco plants deficient in SA biosynthesis [25]. At present, SA is generally accepted as a key molecular component in SAR signaling [26]. Indeed, a chemical rationale for SA activation of defense responses has been demonstrated. Xinnian Dong and her colleagues' pioneering work has revealed important relationships between SA accumulation, PR protein expression, and activation of the nonexpressor of the PR (NPR1) protein [27]. NPR1 is so named because *Arabidopsis* mutants deficient in this protein do not respond to the normal signaling mechanisms for PR gene expression as well as a number of other pathogen defense-related genes [28]. This phenotype has also been called NIM1 and SAI1 [29]. NPR1 is now recognized as an essential regulator of plant defense mechanisms that normally resides in the cytosol of plant cells as a multimeric complex. This complex is maintained through redox-sensitive disulfide bonds [30, 31] that, under reducing conditions resulting from, for example, high concentrations of SA, partially disassociate into monomers [32]. The monomeric form of NPR1 is subsequently transported to the cell nucleus where it serves as a gene transcription coactivator [31]. Interestingly (and almost paradoxically), Spoel et al. [31] have recently shown that the full expression of SAR requires that NPR1 be imported into the nucleus and then be ubiquitinylated and degraded by nuclear proteasomes. This process is hypothesized to facilitate clearance of the NPR1-transcription factor (TF)/polymerase complex to allow fresh NPR1-TF

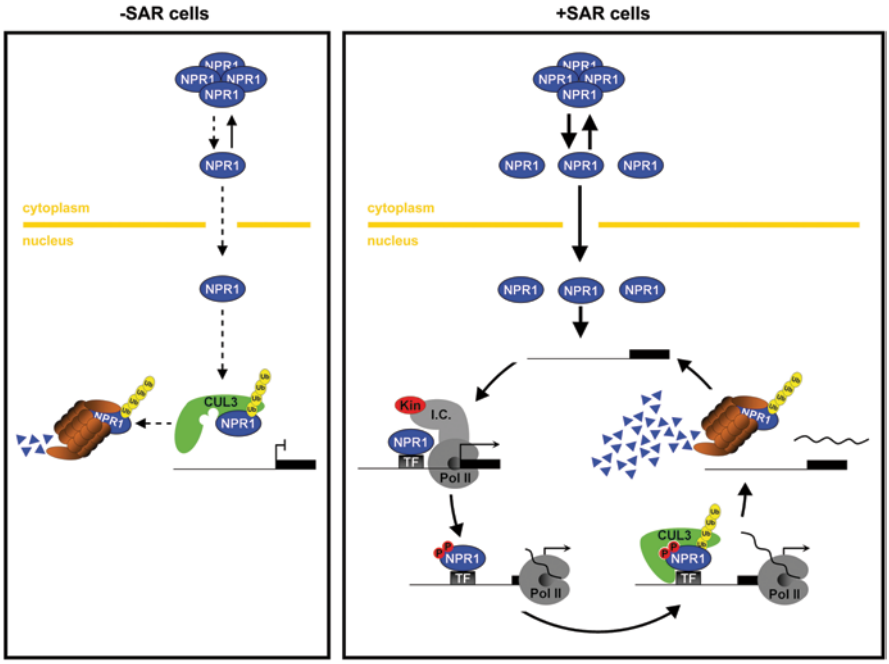


**Fig. 4.2** The role and activation of NPR1 in SAR + and SAR − cells

access to the promoter region of affected genes in order to maintain transcription activity. Ubiquitinylation appears to rely on phosphorylation of specific serine residues in the NPR1 protein (Fig. 4.2).

Additional mobile signals are being recognized as mediators of SAR. Azelaic acid [33] and glycerol-3-phosphate [34] are two small molecules recently associated with SAR. Both of these also require expression of DIR1 (defective in induced resistance) protein [35]. There appears to be additional, as yet undetermined, mobile signals in the phloem exudates from *Arabidopsis* associated with azelaic acid signaling [34].

Airborne signals can also contribute to plant defense against pests and pathogens [36]. Notably, JA and related jasmonates are known for their role in systemic responses to wounding by insect herbivores [37]. The methyl ester of JA and certain other jasmonates are also quite volatile and have been implicated in airborne signaling to nearby plants [36]. Other volatile metabolites, such as short-chain oxylipins and terpenoids, also function in plant-to-plant defense signaling as well as complex tritrophic interactions involving plant pests' predators [38].

The second of the two major defense pathways is termed induced systemic resistance (ISR)and is a systemic immune response elicited primarily by plant growth-stimulating rhizobacteria [39] and certain rhizosphere-associated fungi [40]. Similar to SAR in many respects, ISR, however, does not require SA; it is

more dependent on JA and ethylene signaling. ISR does not typically result in PR accumulation, although both pathways depend on NPR1 activation [41, 42].

Thus, plants have evolved intricate systems to defend themselves against herbivorous pests and microbial pathogens. These defenses involve signaling mechanisms to alert distal parts of the plant, or even other plants, of impending attack. Because plants must not only respond to invading pest and pathogens but also avoid autoimmune responses, or unnecessarily responding to a plethora of non-harmful (or even beneficial) microorganisms, multiple and complex signaling mechanisms should be expected.

## 4.4 Priming in Plant Defense

Another defense mechanism related to induced resistance is "priming." This is defined as a condition in which plants respond faster or more strongly than unprimed plants in the activation of defense responses when subsequently challenged by microbial pathogens, herbivorous insects, or abiotic stresses [43]. This phenomenon occurs after an initial encounter with a pathogen or chemical elicitor but without a display of the typical phenotypes of induced resistance such as upregulation of PR proteins or phytoalexins. Because the molecular mechanisms of priming are just now being determined (phenotypic analysis of priming has relied on tedious post-challenge defense responses [44]), this phenomenon has likely been overlooked in many SAR studies. Uwe Conrath and coworkers demonstrated that cultured parsley cells, pretreated with low concentrations of INA, responded to subsequent elicitation with a known fungal MAMP with dramatically higher levels of coumarins and phenylalanine ammonia lyase (PAL) activity, SAR biomarkers [45]. Similar results were observed using benzo (1,2,3) thiadiazole (BTH), another synthetic SA analog [46]. It is important to note that this "priming" was dependent on the dose rate of INA or BTH, with relatively low doses resulting in priming, and higher doses resulting in elicitation of SAR [43]. Thus, priming appears to potentiate the plant for subsequent pathogen or pest challenge. This phenotypic difference is important because direct activation of plant defense mechanisms appears to extract a fitness cost that may, for example, reduce seed set [47] (discussed later).

Over the past 3–5 years, inroads have been achieved in determining the molecular mechanisms responsible for priming. *Arabidopsis* plants treated with BTH under priming conditions were shown to upregulate the levels of two mitogen-activated protein kinases, MPK3 and MPK6. These are cytosolic elements that transmit and amplify extracellular stimuli from external receptors into intracellular responses through a series of protein phosphorylation reactions. This study convincingly demonstrated that *Arabidopsis* plants primed with BTH responded far more strongly to biotic and abiotic stress in producing SAR biomarkers such as PR proteins and PAL. The primed plants also proved significantly more refractive to bacterial infection. Use of mpk3- and mpk6-deficient mutants conclusively demonstrated the involvement of these two enzymes in priming [48, 49].

Priming was also shown to modify chromatin associated with the promoter regions of certain WRKY genes in *Arabidopsis*. Methylation and acetylation of histones are instrumental in gene regulation [50, 51] and can result in long-term activation (or suppression) of the associated gene [52, 53]. WRKYs are transcription factors closely allied with many defense-related genes in plants [54–56]. Using chromatin immunoprecipitation, Jaskiewicz et al. [57] recently demonstrated that histones bound to certain WRKY promoter regions are methylated and or acetylated in response to priming by either BTH treatment or exposure to a pathogen. Thus, chromatin modification appears to be involved in priming.

Epigenetic control of plant immunity can also be manifested through DNA methylation, which, in turn, can even result in enhanced pathogen resistance in progeny plants. This was recently demonstrated in *Arabidopsis* which the investigators termed "next-generation SAR" [58]. These investigators found that when *Arabidopsis* plants were repeatedly inoculated with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000) their first-and even second-generation progeny were more resistant to infection with the oomycete pathogen *Hyaloperonospora arabidopsidis*. Use of *Arabidopsis* mutant lines further demonstrated that this transgenerational resistance was dependent on functional NPR1 activation. Additionally, triple mutants deficient in DNA methylation were constitutively more resistant to *Pst*DC3000 infection, leading these investigators to suggest that hypomethylation plays a role in next-generation resistance [58]. Hypomethylation has been observed previously in *Arabidopsis* in response to *P. syringae* attack [59] and was specifically associated with defense-related gene expression in tobacco infected with TMV [60]. Similar transgenerational enhancement of SAR response, as determined by PR-1 biosynthesis and resistance to bacterial and oomycete challenge, was observed in *Arabidopsis* treated with β-aminobutyric acid (BABA), another of the chemical SA mimics [61]. Although in this case the next-generation priming only appeared to last through one generation with respect to PR-1 expression, second-generation BABA-treated plants did retain some resistance to pathogen challenge.

## 4.5 Commercial Plant Defense Activators

Advances in our understanding of the plant immune response have resulted in, and in some cases resulted from, the development of numerous synthetic compounds that appear to mimic the effect of SA in stimulating plant immunity. Some of these are currently marketed for commercial field application. Interestingly, the first of these commercial products was probenazole (3-allyoxy-1,2-benzisothiazole-1,1-dioxide) marketed under the trade name Oryzemate®, which proved effective in reducing rice blast infection. Developed in the mid-1970s, it was almost 20 years later that this compound was recognized as being effective against certain bacterial pathogens as well. Only due to advances in understanding SAR and ISR was the mechanism of resistance revealed [62, 63]. A systematic investigation of synthetic

chemical inducers of SAR by investigators at Ciba-Geigy in the early 1990s resulted in the discovery of INA and derivatives [64]. Although highly effective in some plants, INA was not well tolerated by others and was never developed commercially [65]. Subsequently, benzothiadiazole (BTH) derivatives were found to be just as effective in eliciting SAR but better tolerated in a broader range of crops. Formulations of these compounds have been marketed under the trade names BION® in Europe and Actigard® in the USA [62]. A more recent addition among the thiadiazole derivatives is 3′-chloro-4,4′-dimethyl-1,2,3-thiadiazole-5-carboxanilide, common name tiadinil, marketed by Nihon Nohyaku Co, Led with the trade name V-GET® for use against rice blast [66]. A bacterial pathogen effector protein, harpin, has been used to successfully combat blue mold in apples [67]. Fragments of the harpin protein also stimulated plant growth and disease resistance in rice in field trials [68]. A commercial formulation of harpin was originally marketed by Enden Bioscience as Messenger®; it has recently been replaced by Employ® from Plant Health Products. A de-acetylated form of chitin (chitosan) has been produced as a commercial product named Elexa® in a 4% chitosan formulation. Greenhouse as well as field trials of Elexa® on pearl millet (*Pennisetum glaucum*) administered either as a seed treatment or as a foliar spray or in combination showed it to be highly effective at inducing resistance to downy mildew disease caused by *Sclerospora graminicola* [69]. Although chitin and chitosan are well-known elicitors of SAR, chitosan also shows some fungicidal activity [70]. BABA is a nonprotein amino acid that also induces systemic resistance in plants. The amino acid has been used extensively in experimental systems to induce SAR as well as priming. Its commercial application has not been realized.

## 4.6 Induced Resistance in Cereal Crops

The bulk of the research on induced resistance has been performed on dicots, particularly the model plants *Arabidopsis* and tobacco. Nevertheless, monocots are capable of generating SAR and ISR and thus likely possess all the requisite signaling and defense activators found in dicots [71–74]. Indeed, BTH was originally developed to protect wheat from a variety of fungal pathogens. It proved effective in both growth chamber and field experiments [65]. These results were somewhat telling because although an important role of SA in dicot innate immunity is well established [26], an analogous role in monocots was not obvious at the time of these experiments. Rice (*Oryza sativa*) had been shown to constitutively produce dramatically higher levels of SA than healthy tobacco plants and these levels did not appear to be affected by infection with avirulent or virulent pathogens [75]. Thus, early investigations into the mechanism of SAR in cereals cast doubt on the role of SA. In addition, while INA and BTH proved effective in eliciting SAR-like responses in cereals [65, 76], the suite of defense-related gene expression appears to differ from dicots. Specifically, PR-1-related protein expression was not observed in wheat treated with INA or BTH [77]. Thus, some investigators did not consider PR gene expression a particularly reliable biomarker for SAR in monocots, although other

"chemically inducible" genes have been described in wheat, rice, and barley [78]. Nevertheless, as more research on defense mechanisms in monocots is published, the similarity to dicots becomes more apparent [72–74, 79]. Rice, for example, has now been shown to have an SA/NPR1-mediated defense network similar to *Arabidopsis* [80]. Thus, employment of PDAs on cereal crops appears to be perfectly feasible, including those that mimic SA, at least in terms of eliciting a defense response. The method of PDA application on cereals, however, can be a critical factor. Initial efforts to induce resistance to *Fusarium* head blight (FHB) in wheat, through the application of BTH as a foliar spray, proved ineffective [81]. More recently, both SA and BTH proved highly effective in protecting wheat against the same pathogen (*Fusarium graminearum*) in greenhouse trials when applied as a root soak [82].

Another aspect of PDA application that warrants consideration is their ability to elicit phytoalexin biosynthesis. Oat (*Avena sativa*) plants treated with SA or BTH were recently shown to dramatically increase their production of avenanthramides. Avenanthramides are phenolic alkaloid compounds produced, among food crops, exclusively by oats. They are known to function as phytoalexins in the vegetative tissue in response to crown rust (*Puccinia coronata*) infection [83, 84]. These metabolites are also potent antioxidants that, in laboratory trials, show potential as phytonutrients [85]. Unfortunately, the levels of avenanthramides found in the grain are highly variable and subject to strong environmental influence [86], and there appears to be an association between crown rust infection and avenanthramide content in the mature grain under field conditions [87]. The means of enhancing the levels of grain avenanthramides is of interest. BTH treatment in the form of Actigard® was recently shown to strongly induce avenanthramide biosynthesis in vegetative tissue of oat seedlings when administered as a root soak in greenhouse experiments [88]. RNA hybridization (Northern) analysis also showed elicitation of an RNA message hybridizing with a barley PR-1 probe, suggesting that the avenanthramide production was part of a SAR response. Moreover, when mature plants were treated with BTH just prior to heading, certain cultivars showed a statistically significant increase in avenanthramide content in the filling grain [89]. Indeed, all of the treated oat cultivars were higher in grain avenanthramide content than the untreated controls. However, since oat constitutively produces avenanthramides in their grain, and these levels show high variability, it can be difficult to establish a statistically significant difference. Nevertheless, these findings portend the utility of PDAs to upregulate the biosynthesis of avenanthramides in oat and, by extension, may be of use in other crops whose phytonutrient content is augmented by natural phytoalexins. Harpins, for example, were shown, in field trails, to increase yield and catechol levels in green tea [90].

## 4.7 Fitness Costs

The evolutionary rationale for induced resistance holds that plants cannot afford to biosynthesize the pest and pathogen defense metabolites on a constitutive basis because of either detrimental allocation of nutrient resources, production of autotoxic

metabolites, or negative effects on beneficial microorganisms [67, 91]. Thus, it is better to resort to these biosynthetic pathways only when they are in dire need. The application of PDAs circumvents this "just-in-time" approach evolved in plants. In most cases, field studies have focused on the efficacy of PDAs to reduce disease pressure. One study specifically aimed at determining the "fitness" cost of BTH application was conducted with spring wheat (*Triticum aestivum* cv 'Hanno') by treating them with BION® under a variety of treatment regimens and growing them either hydroponically with carefully controlled nutrient conditions or in a field environment with added fertilizer and fungicide treatment to ensure no disease pressure [47]. The investigators reported a statistically significant reduction in seed production and growth rates in the BTH-treated plants relative to uninduced control plants when nitrogen availability was limited. They suggested that this likely represented an allocation cost to chemically induced resistance in the absence of disease pressure. It should be noted, however, that in those plants treated late in their growth cycle or provided adequate nitrogen no significant differences in the measured parameters were observed between the BION®-treated and the untreated controls [47]. Several studies comparing the yield of various cereals treated with BTH versus standard pesticides provide little evidence for increased yield from BTH treatment (summarized in [42]). Treatment of plants with PDAs mimicking the SA elicitation pathway can also prove antagonistic to JA signaling. A study conducted with two barley (*Hordeum vulgare*) cultivars, 'Celler' and 'Optic', treated with a combination of BION®, BABA, and *cis*-jasmone under field conditions resulted in a marked decrease in infection levels by the biotrophic pathogen *Blumeria graminis* and the hemibiotroph *Rhynchosporium secalis*, etiological agents of powdery mildew and leaf scald, respectively. Infection by *Ramularia collo-cygni* (*Ramularia* leaf spot), another hemibiotroph, however, was significantly higher in the treated plants. Analysis of PR-1 and lipoxygenase (LOX2, an enzyme involved in JA biosynthesis) showed that elicitor treatment upregulated PR-1 expression, whereas LOX2 expression was downregulated. The combination of PDAs was used to specifically target *R. secalis*, making the interpretation of the results somewhat complicated. Note, also, that the grain yield from the elicitor-treated cultivars was slightly higher than the controls in both years of this study, although the authors speculated that the mixed result in protection might be due to suppression of the JA signaling, this pathway possibly being more important in defense against *R. collo-cygni* infection [92]. BTH-treated tomato plants have shown enhanced resistance to *Pseudomonas syringae* pv. *tomato* but compromised resistance to herbivore attack by *Spodoptera exigua* and, conversely, treatment with JA enhanced herbivore resistance at the expense of bacterial infection [93]. Thus, antagonism between SA and JA signaling may result in tradeoffs in the protective effects of PDAs in some situations [67].

In contrast to PDA-induced resistance, priming seems to have minimal allocation costs. Laboratory trials using *Arabidopsis* treatment with BABA in a range of concentrations resulted in induced direct defense response (as determined by PR-1 biosynthesis) at higher concentrations and priming of the plants at lower treatment levels. The primed plants were only slightly less resistant to subsequent challenge with

either the bacterial pathogen *P. syringae* or the fungal pathogen *Hyaloperonospora parasitica* but did not demonstrably increase PR-1 levels prior to the challenge [94]. All the PDA-treated plants, including control plants fully induced with BTH, were significantly more resistant to infection than the mock-induced controls. Moreover, fitness costs, evaluated in terms of seed set and relative growth rates, were only marginally affected in the BABA-primed plants; plants induced to direct defense levels demonstrated significantly lower fitness levels.

Saccharin is a metabolic by-product of probenazole (Oryzamate®) that can induce priming in barley [95]. In a study on fitness costs of saccharin-induced priming in barley (*H. vulgare* cv 'Celler'), Walters et al. found that, in greenhouse experiments, priming did not incur significant costs under low disease pressure by the hemibiotrophic fungal pathogen *R. secalis* and that it provided significant benefits under high disease pressure [96]. Use of saccharin-primed barley, in a field environment subject to low disease pressure from three fungal pathogens, *R. secalis, B. graminis, and R collo-cygni*, similarly showed little or no fitness costs, although the application of commercial fungicide resulted in significantly higher grain yield in adjacent plots [96].

## 4.8 Plant Defense Activators, Prospects for Cereal Crops

The commercial application of PDAs in cereal crops has been met with limited enthusiasm [67, 97]. Probenazole, as Oryzemate®, has been used for over three decades and remains one of the major fungicides used for seedling box treatment of rice [98]. It is noteworthy that no resistance to this product has yet developed [62]. Other commercial formulations such as BION®/Actigard®, Messenger®, and Elexa® have found use primarily on vegetable crops [62]. Some of the major drawbacks to PDAs are their unreliability under field conditions [99]. Abiotic and biotic factors affecting induced resistance in commercial/field application are still poorly understood. Numerous parameters must be further investigated. For example, the timing of application may be critical [99, 100]. Recent evidence has even shown that plant immune responses, particularly those associated with SA, are sensitive to light intensity and circadian rhythms [101]. Genotypic effects have also received little attention. Oat cultivars, for example, show dramatically different responses in both the magnitude and kinetics of avenanthramide biosynthesis in response to BTH and INA treatment [89]. When seven cultivars of spring barley were assessed for the efficacy of induced resistance to *R. secalis* and powdery mildew (*B. graminis*) induced by treatment with a suite of PDAs (BION®, BABA and *cis*-jasmone), significant differences were observed between cultivars [102]. A few additional examples are outlined by Walters and Fountaine [67].

PDAs are not curative; they must be administered prior to pathogen or pest invasion; thus, any fitness costs incurred may, in fact, be as detrimental to crop yield as the pathogen itself. However, an integrated crop management approach where PDAs are used in concert with more traditional fungicides/pesticides or other

agents such as biocontrol or plant growth promoting rhizobacteria might have some merit, particularly in reducing fungicide treatment levels [103]. Certainly more research on fertilizer augmentation to ameliorate allocation costs is warranted. PDAs can elicit volatile signals yielding protective effects against bacterial infection on neighboring plants as was recently demonstrated in lima bean (*Phaseolus lunatus*) [104]. This suggests the possibility of treating border rows, for example, to enhance resistance in the larger field. Maize is well known to produce volatile organic compounds (VOCs) in response to herbivore attack [105, 106]. There is evidence that these airborne signals can prime neighboring plants to respond to subsequent herbivore attack [107, 108]. Cereal crops also release VOCs in response to fungal infection [109], although the chemical ecology of these emissions is poorly understood. Indeed, in spite of an extensive literature on the generation of VOCs in response to herbivore attack, where cereal crops such as corn and rice are well represented [110], there is little research on the chemical ecology of VOC emission resulting from pathogen infection, especially in cereal crops.

The advances in recent years in understanding the molecular basis for SAR, ISR, priming, and next-generation immunity portend a wider role for PDAs. These agrochemicals are gaining some acceptance in Europe where there is a strong interest in curtailing the use of more traditional fungicides and pesticides. Much work, however, is needed to understand the effects of nutrition, dose rates, timing of application, and genotypic effects in the application of PDAs. Exploitation of plant immunity can and should be a useful tool in our collective arsenal for combating plant disease.

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