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Priming in plant–pathogen interactions

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Plants can acquire enhanced resistance to pathogens after treatment with necrotizing attackers, nonpathogenic root-colonizing pseudomonads, salicylic acid, β-aminobutyric acid and many other natural or synthetic compounds. The induced resistance is often associated with an enhanced capacity to mobilize infection-induced cellular defence responses – a process called 'priming'. Although the phenomenon has been known for years, most progress in our understanding of priming has been made only recently. These studies show that priming often depends on the induced disease resistance key regulator NPR1 (also known as NIM1 or SAI1) and that priming has a major effect on the regulation of cellular plant defence responses.

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In addition to constitutive barriers, plants have evolved distinct inducible defence mechanisms to protect themselves against pathogen attack. For example, upon inoculation with NECROSIS-inducing pathogens (see Glossary) [1,2] or various nonpathogenic root-colonizing pseudomonads [3], or treatment with SALICYLIC ACID (SA) [1,2], β-AMINOBUTYRIC ACID (BABA) [4] or various other natural and synthetic compounds [5], plants acquire enhanced resistance to a broad spectrum of pathogens. The induced resistance occurs not only at the site of the initial treatment but also in distal, untreated plant parts. The various induced resistance phenomena are all associated with an enhanced capacity for the rapid and effective activation of cellular defence responses, which are induced only after contact with a (challenging) pathogen [4,6–10]. These responses include the HYPERSENSITIVE RESPONSE (HR) [11], cell-wall strengthening [12–14], the OXIDATIVE BURST [15] and the expression of various defence-related genes [1,2].

By analogy with a phenotypically similar phenomenon in mammalian monocytes and macrophages, the augmented capacity to mobilize cellular defence responses has been called the 'PRIMED' [16] (or 'sensitized' [6]) state of the plant. Although the priming phenomenon has been known for years as a part of induced-resistance phenomena [6,13,14], it has mostly been overlooked in studies dealing with induced disease resistance of plants, because it only becomes apparent after challenge of the primed tissue. Therefore, the molecular mechanism(s) and genetic basis of priming and its role in induced disease resistance have remained largely unclear. This article reviews recent findings supporting a crucial role for priming in induced plant disease resistance.

Priming and systemic acquired resistance

The systemic resistance response activated upon infection of plants with necrotizing pathogens is called systemic-acquired resistance (SAR) [1,2], but SAR can also be induced by exogenous application of salicylic acid or its functional analogues 2,6-DICHLOROISONICOTINIC ACID (INA) and BENZOTHIADIAZOLE (BTH) [1,2,17]. Establishment of SAR requires an endogenous increase in salicylic acid levels [1,2,17] and its onset is associated with the expression of *SAR* genes [1], some of which encode PATHOGENESIS-RELATED (PR) PROTEINS [1,2,17]. Some PR proteins display antimicrobial activity *in vivo* [18] but their actual role in SAR remains uncertain. Unfortunately, the availability of tools and markers for monitoring other cellular plant defence responses such as the HR or local cell-wall strengthening is limited. Therefore, it is important to

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Glossary

β-Aminobutyric acid (BABA)
Non-protein amino acid that potentiates plant responses and confers resistance to biotic and abiotic stresses.

Avirulent
A pathogen strain that carries an avirulence (*Avr*) gene and cannot multiply in a resistant host plant cultivar expressing a complementary resistance gene.

Benzothiadiazole (BTH)
Synthetic compound inducing SAR in various plants.

Callose
Plant 1,3-β-glucan contributing to cell wall strengthening beneath fungal penetration sites in the form of papillae.

2,6-Dichloroisonicotinic acid (INA)
Synthetic compound that causes SAR in certain plants.

Elicitor
Compound inducing defence responses in plants.

Ethylene
Gaseous plant hormone implicated in development and disease resistance.

Hypersensitive response (HR)
Rapid collapse (programmed death) of cells after attack by an avirulent pathogen.

Methyl jasmonate
Gaseous plant hormone implicated in development and disease resistance.

Necrosis
Development of brown, dry, collapsed tissue areas at the site of pathogen attack.

Oxidative burst
Rapid accumulation of reactive oxygen species (e.g. O_2^-, H_2O_2) with direct antimicrobial activity, but also implicated in plant signalling.

Pathogenesis-related (PR) protein
Plant proteins, some of which display antimicrobial activity. Accumulation of some PR proteins is associated with the onset of SAR.

Phytoalexins
Pathogen-induced low molecular weight, antimicrobial plant secondary metabolites.

Potentiated
Augmented induction of pathogen- or elicitor-induced plant defence responses.

Primed
State of enhanced ability to mobilize pathogen- or elicitor-induced cellular defence responses.

Salicylic acid (SA)
Secondary metabolite with important roles in plants. Induces *SAR* gene expression, potentiates systemic-acquired resistance (SAR)-related cellular defence responses and induces SAR in plants.

study further defence-associated cellular events whose induction is stronger in SAR-protected plants. Such events include the activation of various defence-related genes and the deposition of CALLOSE.

Cell culture model system

In the 1990s, it was reported that pretreating parsley (*Petroselinum crispum*) cell cultures with the SAR inducers salicylic acid, INA or BTH did not directly induce various assayed cellular defence responses [16,19–22]. Interestingly, the preincubation with SAR inducers primed the cells for POTENTIATED activation (increased induction) of various cellular defence responses, which were subsequently induced by otherwise non-inducing doses of a cell wall ELICITOR from *Phytophthora sojae* [16,19–22]. These potentiated responses include the early oxidative burst [21], the incorporation of various phenolic compounds and a lignin-like polymer into the cell wall [20], and the secretion of antimicrobial PHYTOALEXINS (coumarins) [16,19]. The potentiated phytoalexin response was associated with enhanced activity of coumarin biosynthetic enzymes [19] and augmented expression of genes encoding enzymes involved in coumarin biosynthesis [16,19,20,22]. In soybean cell cultures, physiological concentrations of salicylic acid strongly enhanced the induction of defence gene transcripts, H_2O_2 accumulation and the HR caused by AVIRULENT pseudomonads [23]. As the salicylic acid-mediated potentiation of defence responses in the soybean cells was independent of prolonged preincubation with salicylic acid, this mechanism of regulation obviously differs from the priming in cultured parsley cells. However, the observations made with parsley and soybean cell suspensions revealed that plant cell cultures can be suitable model systems for studying the potentiation of cellular plant defence responses.

Dual role for SAR inducers

While elucidating the influence of salicylic acid and BTH on the activation of defence genes in the parsley cell culture, it became clear that the inducer's effect on defence gene activation depends on the gene that is being monitored [16,22]. One class of genes, including those encoding anionic peroxidase and mannitol dehydrogenase, was found to be directly responsive to relatively low concentrations of the two inducers tested [16,22]. A second class of parsley defence genes [including those encoding phenylalanine–ammonia lyase (PAL), 4-coumarate–CoA ligase, PR-10 proteins and a hydroxyproline-rich glycoprotein] was only slightly responsive to the treatment with these relatively low concentrations of salicylic acid or BTH. Yet, even at low inducer concentrations, these genes displayed salicylic acid- and BTH-dependent potentiation of their expression following treatment with a low dose of the elicitor [16,22]. For example, >500 μM salicylic acid was needed to activate *PAL* using only salicylic acid, whereas as little as 10 μM salicylic acid greatly potentiated the elicitor activation of the *PAL* gene [24]. These results revealed a dual role for SAR inducers in the activation of plant defence responses: low doses of salicylic acid primed for potentiated induction of certain defence genes, whereas higher doses were directly inductive for another set of defence genes. Because the potentiation by salicylic acid and BTH of both elicited *PAL* gene expression and phytoalexin secretion depended strongly on an extended preincubation period, the SAR inducers were proposed to mediate a time-dependent

response that shifts the cells when alerted [16,22]. However, whether this response includes the proposed synthesis of cellular factors with important roles in the coordination and expression of cellular defence remained unclear.

Similar observations to those in parsley have been reported from cowpea (*Vigna unguiculata*) seedlings induced with BTH [24]. The enhanced disease resistance of cowpea was associated with rapid and transient increases in the activity of PAL and chalcone isomerase (CHI) followed by accelerated accumulation of the isoflavonoid phytoalexins kievitone and phaseollidin in infected hypocotyls. These responses were not observed in induced uninoculated tissues, suggesting that the protection of cowpea seedlings by BTH is mediated by the potentiation of early defence mechanisms [24]. In systemically resistant cucumber hypocotyls [25] and wounded soybean tissue [26], augmentation was also seen for the development of elicitation competency. It is unclear whether the augmented induction of elicitation competency is based on a similar priming mechanism to the one described above.

Priming during SAR in Arabidopsis

In *Arabidopsis*, BTH was found to activate *PR-1* directly and to prime the plants for potentiated *PAL* expression in response to subsequent infection by phytopathogenic *Pseudomonas syringae* pv. *tomato* (*Pst*) [7]. BTH-induced priming also enhanced both *PAL* activation and callose deposition when these responses were induced by either mechanically wounding the leaves with forceps or infiltrating them with water [7,8]. These observations with *Arabidopsis* not only confirmed the above-described dual role for SAR inducers in the activation of cellular plant defence responses, they also suggested that priming might be a common component that mediates cross-talk between pathogen defence and wound or osmotic stress responses [27].

Intriguingly, when *Arabidopsis* SAR was biologically induced by previous infection with an avirulent strain of *Pst*, there was potentiated activation of both *PAL* and *PR-1* upon challenge infection with virulent *Pst* [7,28]. Thus, it is likely that priming plays an important role not only in chemically induced but also in pathogen-activated SAR of plants. The same conclusion was drawn from studies with salicylic acid-primed transgenic tobacco plants that displayed potentiated expression of chimeric *Asparagus officinalis PR-1::GUS* and *PAL-3::GUS* defence genes after pathogen attack or wounding [29]. The *Arabidopsis* mutant *edr1* shows constitutive enhanced resistance to the DC3000 strain of *Pst* and to the fungal pathogen *Erisyphe cichoracearum* [30]. Interestingly, this mutant is different from other enhanced resistance mutants because it shows no constitutive induction of *PR-1* and *BGL2* even though transcripts of both genes accumulate after infection. This, and the fact that *edr1* displays stronger induction of defence responses such as HR and callose deposition after infection, strongly suggest that EDR1 is involved in priming. EDR1 is a putative mitogen-activated protein kinase kinase kinase (MAPKKK) and mediates resistance via salicylic acid-inducible defences [31]. Future mutational approaches in *Arabidopsis* are likely to yield more genes that play roles in the establishment of priming.

Arabidopsis npr1 mutants (also known as *nim1* or *sai1*) accumulate wild-type salicylic acid levels in response to treatment with avirulent pathogens. However, they are unable to express biologically or chemically induced SAR [32–34]. Intriguingly, the potentiation by BTH priming of both *Pst*-induced *PAL* expression and wound- or water-infiltration-induced *PAL* activation and callose production are absent in the *npr1* mutant [7,8].

The *cpr1* and *cpr5* mutants of *Arabidopsis* have a constitutive SAR in the absence of pretreatment with SAR inducers [35,36]. In these mutants, there was constitutive priming without BTH pretreatment, potentiated PAL activation by *Pst* infection and increased induction of both *PAL* expression and callose deposition upon wounding or infiltrating the leaves with water [7,8]. Constitutive priming in the *cpr1* and *cpr5* mutants might still be due to the expression of a large repertoire of defence genes in these plants or to the activation of other stress response mechanisms in addition to the SAR pathway [37,38], but it is likely that the enhanced levels of salicylic acid in *cpr1* and *cpr5* [37,38] mean that these mutants are permanently in the primed (alarm) state. Because of constitutive priming, *cpr1* and *cpr5* might be able rapidly and effectively to induce their various cellular defence responses, leading to enhanced defence responses to pathogens, wounding or infiltration of water [7,8]. In this context, it is noteworthy that the constitutively enhanced pathogen resistance of another *Arabidopsis* mutant, *cpr5-2*, has been ascribed to the potentiated induction of *PR-1* [37]. There is evidence that a null *eds1* mutation suppresses the disease resistance of both *cpr1* and *cpr6* but only partially represses that of *cpr5*, pointing to a different dependency of *CPR* genes from EDS1 [38]. EDS1 is also likely to play a role in priming in connexion with PAD4 [39]. Although both proteins operate upstream of pathogen-induced salicylic acid accumulation, their expression can be potentiated by previous salicylic acid treatment of the plants [23]. It has been proposed that EDS1 takes part in the amplification of defence responses, possibly by associating with PAD4 [40].

The strong correlation between SAR and priming suggests that priming is a crucial mechanism in SAR of plants. This assumption is further supported by the close correlation between the ability of

various chemicals to induce SAR against tobacco mosaic virus in tobacco [41] and to prime for potentiated *PAL* expression induced by either elicitor treatment in parsley cells [16,22] or *Pst* infection, wounding or water infiltration in *Arabidopsis* plants [7,8]. Moreover, a reduction in priming and the accompanying loss of potentiated induction of the oxidative burst have recently been associated with a lack of resistance to avirulent bacterial pathogens in tobacco [42]. Furthermore, overexpression in tomato of the disease resistance-associated gene *PTI5* potentiates pathogen-induced defence gene expression and enhances the resistance to *Pst* [43].

Priming and rhizobacteria

Priming of defence responses is not solely confined to the SAR response. Priming of defence responses has also been demonstrated in rhizobacterium-mediated induced systemic resistance (ISR). Rhizobacterium-mediated ISR is a plant-mediated, broad-spectrum resistance response that is activated by selected strains of saprophytic rhizosphere bacteria [3]. The first evidence that potentiation of plant defence responses is involved in ISR came from experiments with carnation (*Dianthus caryophyllus*). Carnation plants develop an enhanced defensive capacity against *Fusarium oxysporum* f.sp. *dianthi* after colonization of the roots by the non-pathogenic rhizobacterial strain *Pseudomonas fluorescens* WCS417. Before challenge inoculation, no increase in phytoalexin levels could be detected in induced and uninduced plants but, upon subsequent inoculation with *F. oxysporum* f.sp. *dianthi*, phytoalexin levels in ISR-expressing plants rose significantly faster than in uninduced plants [44]. Evidence for rhizobacterium-induced potentiation of host cell wall strengthening has been described as well. In bean (*Phaseolus vulgaris*), the rhizobacterium *Bacillus pumilus* SE34 induces ISR against the root-rot fungus *F. oxysporum* f.sp. *pisi*. By itself, colonization of the roots by the rhizobacteria did not induce morphological alterations of root tissue. However, upon challenge with *F. oxysporum*, root cell walls of ISR-expressing plants were rapidly strengthened at sites of attempted fungal penetration by appositions containing large amounts of callose and phenolic materials, thereby effectively preventing fungal ingress [45].

Priming during ISR in Arabidopsis

In *Arabidopsis*, ISR triggered by the nonpathogenic root-colonizing bacterium *P. fluorescens* WCS417 is independent of salicylic acid and of PR-gene activation, and instead requires an intact response to the plant defence signals jasmonic acid and ETHYLENE [46,47]. Analysis of local and systemic levels of jasmonic acid and ethylene revealed that ISR is not associated with changes in the production of these signal molecules [48]. This suggests that the induced resistance is based on an enhanced sensitivity to these plant hormones rather than on an increase in their production. If this is the case, ISR-expressing plants would be expected to be primed to react faster or more strongly to jasmonic acid and ethylene produced after pathogen infection. The hypothesis that induced resistance is based on enhanced sensitivity to jasmonic acid and ethylene is supported by the finding that the expression of the jasmonic acid-inducible gene *AtVSP* was potentiated in ISR-expressing leaves after challenge with *Pst* DC3000 [10]. Several other jasmonic acid-responsive genes were also tested in the same study but these failed to show any enhancement of the pathogen-induced expression level in ISR-expressing leaves, suggesting that ISR in *Arabidopsis* is associated with the potentiation of a specific set of jasmonic acid-responsive genes. Potentiation of jasmonic acid-dependent responses has been reported in other systems as well. For instance, pretreatment with METHYL JASMONATE potentiates the elicitation of various phenylpropanoid defence responses in parsley suspension cell cultures and primes them for enhanced induction of the early oxidative burst [49] and various phenylpropanoid defence responses [50]. Moreover, in rice, jasmonic acid potentiates the expression of *PR-1* and the level of resistance against the fungus *Magnaporthe grisea* induced by low doses of INA [51].

The role of ethylene in priming is more ambiguous. Although ethylene plays an important role in the ISR signalling pathway of *Arabidopsis*, ethylene production is not increased in ISR-expressing tissue [52]. However, after treatment with a saturating 1 mM dose of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC), ISR-expressing plants emit significantly more ethylene than ACC-treated control plants [48,52]. Evidently, the capacity to convert ACC to ethylene is increased in ISR-expressing plants. Because ACC levels increase rapidly in infected tissues as a result of pathogen-induced ACC synthase activity, the enhanced ACC-converting capacity observed in ISR-expressing plants might prime the plant for a faster or greater production of ethylene upon pathogen attack. Interestingly, exogenous application of ACC has been shown to induce resistance against *Pst* DC3000 in *Arabidopsis* [10,47,48]. Therefore, a faster or greater production of ethylene in the initial phase of infection might contribute to enhanced resistance against this pathogen. In this context, it is noteworthy that ethylene can strongly enhance the activation of *PR-1* by salicylic acid in *Arabidopsis* plants [53]. Moreover, ethylene and jasmonate were found to act together to regulate proteinase inhibitor gene expression during the wound response of tomato plants [54].

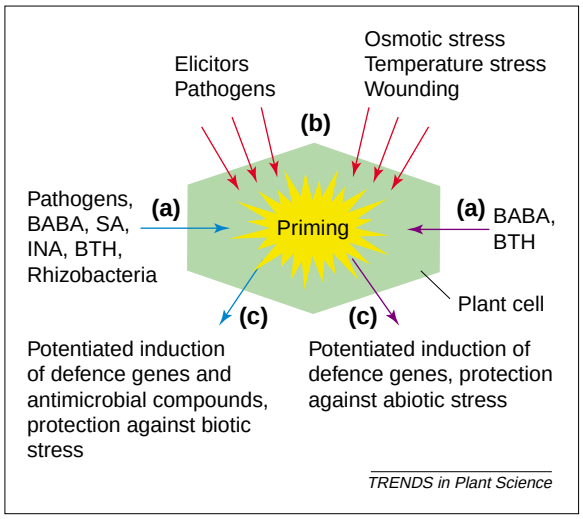

Fig. 1. In plants, a pretreatment with salicylic acid (SA), β-aminobutyric acid (BABA), dichloroisonicotinic acid (INA) or benzothiadiazole (BTH) primes the cells to react more quickly and efficiently to subsequent elicitor treatment or pathogen attack. The cells are also primed to protect themselves better against abiotic stresses such as wounding and infiltration of water into leaves (by BTH), and high salt, drought and cold (by BABA). (a) Priming step. (b) Challenge with biotic or abiotic stress. (c) Potentiated response.

Priming *Arabidopsis* with BABA

The non-protein amino acid BABA has been known for years to be an effective inducer of resistance in various crops [4]. However, it became apparent only recently that this substance exerts its effect on the defence capability of plants via priming [9,55]. In *Arabidopsis*, BABA pretreatment leads to a more rapid and stronger deposition of callose-containing papillae at the site of infection by *Peronospora parasitica* [9]. When BABA-pretreated *Arabidopsis* plants are challenged with a virulent strain of *Pst*, priming becomes apparent as a strong potentiation of *PR-1* expression [9]. The induction kinetic of *PR-1* is in this case an almost exact copy of the one observed in a resistance response with avirulent pseudomonads [9]. Interestingly, in the interaction of *Arabidopsis* with *Botrytis cinerea*, it is again *PR-1* that shows strongly potentiated expression [55] and not, as expected, *PDF1.2*, the gene that is typically used as a marker for involvement of the jasmonic acid or the ethylene defence pathway, which are thought to mediate resistance against *Botrytis* in *Arabidopsis* [56].

Interestingly, BABA treatment itself does not induce the expression of *SAR* genes in *Arabidopsis*, as opposed to salicylic acid, INA or BTH. This is reminiscent of observations made with different fungicides, although BABA itself has no direct fungicidal or antibacterial activity [9]. In *Arabidopsis* plants impaired in disease resistance signal transduction, such as *nahG* or *nim1*, the fungicides metalaxyl, fosetyl-Al and $Cu(OH)_2$ are much less effective [57] than they are in plants with an intact disease resistance signalling pathway, suggesting that these substances possess some resistance-inducing activity besides their fungicidal properties. The fungicide-mediated resistance could be based on priming because fungicide application alone does not lead to any obvious changes in gene induction in *Arabidopsis*. A role for NIM1 in priming of fungicide or chemical inducer action is also supported by the fact that *NIM1*-overexpressing *Arabidopsis* plants display both potentiated disease resistance and enhanced efficacy of fungicides [58].

Priming against abiotic stresses

As described above for the potentiated response of systemic resistant *Arabidopsis* to stimulation by wounding or the infiltration of water into the leaves, plants are also known to display priming-like reactions to abiotic stresses. Pretreatment with cold can lead to acclimation, which manifests itself in the ability of a plant to survive much lower temperatures than without this pretreatment [59]. In *Arabidopsis*, similar results can be obtained upon pretreatment with BABA before subjecting the plants to temperatures of <0°C. Interestingly, BABA treatment also seems to prime *Arabidopsis* to react faster to other forms of abiotic stress, such as high concentrations of salt [4], elevated temperatures [4] or drought (G. Jakab *et al.*, unpublished). The efficacy of BABA in priming *Arabidopsis* to react faster and more effectively not only to biotic but also to abiotic stimuli further points to a connection between these two types of stresses at the molecular level. This conclusion is supported by the fact that plant-growth-promoting rhizobacteria induce alterations in plant gene expression that can be correlated to resistance against biotic and abiotic stresses [60].

The presence of priming in SAR, ISR and BABA-mediated resistance suggests that it has a crucial role in many, if not all, induced plant disease-resistance phenomena. Interestingly, preincubation with the wound-generated systemic peptide messenger systemin enhanced the early oxidative burst subsequently induced by oligogalacturonides or osmotic stress in cultured tomato cells [61]. Also, pretreatment with a strobilurin fungicide, pyraclostrobin, was found to prime tobacco plants for accelerated and augmented activation of *PR-1* upon infection with tobacco mosaic virus (S. Herms *et al.*, unpublished). Together with the findings of a possible role in abiotic stress responses, these observations indicate that plant-cell priming has a complex, multi-entrance nature (Fig. 1).

Cell priming in animals and humans

The priming for potentiated induction of pathogen defence responses in plants has phenotypic similarity to the administration of defence responses in animals and humans. For example, in adult female locusts, application of a subclinical dose of the active juvenile hormone analogue methoprene accelerates (potentiates) the appearance of the egg-yolk protein vitellogenin induced by normal doses of

juvenile hormone [62]. Furthermore, pretreatment with granulocyte–macrophage colony-stimulating factor or interferon-γ (IFN-γ) was reported to prime human monocytes and macrophages for increased lipopolysaccharide-induced production of various defence-related cytokines, including IFN-α and -β, tumour necrosis factor, and interleukin-12 [63–65]. Using tumour-necrosis-factor induction as a model, it has been shown that monocyte priming by IFN-γ requires a prolonged preincubation period [66]. In addition, IFN-γ-induced monocyte priming is primarily manifested at the level of tumour-necrosis-factor transcript accumulation, emphasizing the similarity to defence response administration in plant cells (Fig. 1).

Conclusions

Induced disease resistance of plants is a widely observed phenomenon and priming for potentiated induction of defence responses has emerged as being an important part. The mode of action of priming and the resulting potentiation of cellular defence responses, rather than the direct upregulation of defence signalling cascades, might prove to be of enormous advantage in terms of energy costs for the plant, because the defence responses are only expressed when they are really needed – upon pathogen attack. This is also advantageous from a medical point of view, because some *SAR* proteins have been reported to act as potent allergens in humans [67].

Although the molecular basis of cell priming is unclear, we hypothesize that, during priming, there might be an increase in the amount of cellular components with important roles in defence response signalling, possibly including certain transcription factors. The increased presence of cellular signalling components might then lead to an accelerated and enhanced response when the cells are challenged by a second stress stimulus. This mechanism of regulation would explain why priming leads to the potentiation of different subsequently applied stimuli, because promoters of various stress-responsive genes are known to harbour similar regulatory elements that are recognized by the same set of transcription factors. Alternatively, or in addition, priming might be mediated at the post-translational level by protein modification. The modified proteins themselves might not be sufficient for the activation of the actual defence response whose induction might require a second signal produced by a challenging stressor.

A better knowledge of the molecular mechanism(s) of priming will be instrumental in improving the plants' ability to perceive stress stimuli more rapidly, thereby coping with different forms of stress more efficiently and in a natural way. The emerging link between priming against biotic and abiotic stresses points to the possibility of improving the plants' natural defence potential against multiple forms of stress simultaneously.

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