

1 Post-translational modifications (PTMs) in priming the plant immune
2 system: ripe for exploitation?

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7 **Abstract**

8 Microbes constantly challenge plants and some can successfully infect their host and
9 ultimately cause disease. In order to cope against pathogen infection, plants must be ready to
10 “fight back”. Basal immunity in many cases, is not enough for survival and leads to disease
11 and ultimately a premature death of the host. However, the plant immune system can be
12 temporarily and even trans-generationally primed; this ‘primed state’ leads to changes in the
13 plant involving transcriptional, post-translational, metabolic, physiological and epigenetic
14 reprogramming, which enables the plant to fine-tuning its defence mechanisms for a rapid
15 and/or more robust response after abiotic and/or biotic stress. This can ultimately affect
16 pathogen infection speed and hence decrease its ability to overcome host resistance and the
17 final outcome of the host-pathogen interaction. The role of the three major PTMs (protein
18 ubiquitination, phosphorylation and SUMOylation) in plant immunity has been well-
19 established and new PTMs have emerged as plant cell signalling regulators such as S-
20 acylation. However, the role of PTMs on defence priming and how PTM machinery is
21 affected in primed plants and its connection to plant resistance against biotic/abiotic stress is
22 not well understood. This review highlights the current state of play of priming-mediated
23 post-translational reprogramming and explores new areas for future research.

24 **Key words:** basal immunity, gene-for-gene resistance, priming, defence, post-translational
25 modifications, SUMOylation, epigenetics

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29	Introduction
30	1. Plant innate immunity: basal resistance and post-translational modifications
31	2. Novel roles of post-translational modifications in defence priming
32	3. Priming via post-translational modifications as a key regulatory system for the onset,
33	speed and outcome of the plant defence response against biotic stress
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37 INTRODUCTION

38 1. Plant Innate immunity: basal resistance and post-translational modifications

39 Unlike animal cells, plants depend on their innate immunity due to their lack of somatic
40 adaptive defences [1]. However, plants are not unprotected against the pathogens and pests
41 that attack them. They have a developed and sophisticated immune system that must be able
42 to endure attacks from a wide variety of microorganisms, such as bacteria, oomycetes, fungi
43 and viruses. Despite the fact that pathogens have different host ranges depending on their
44 nature and specialization level; it is well-known they have coevolved with plants over
45 millions of years [2, 3] to develop a way to infect them, and at the same time plants have
46 developed more or less successful ways to resist infection and disease development. This co-
47 evolutionary development of the plant immune system has been generally accepted and
48 represented by a zig-zag model [1,4].

49 Many pathogens, such as oomycetes, aphids and fungi are able to penetrate directly their host
50 cell wall, unlike plant viruses and bacteria, which depend on natural openings, damaged
51 tissue or vectors [5]. In order to fight pathogen infection, plants have created a series of
52 resistance mechanisms. As a first physical defence, plants have a waxy layer on their leaf
53 surfaces beneath which are a series of cell-wall defences, such as lignin and callose
54 appositions, so-called papillae. If a pathogen attempts to infect and subsequently cause
55 disease in the plant it needs to first overcome these physical barriers. These callose-rich
56 papilla depositions are usually induced ubiquitously in plants upon pathogen attack, in
57 contrast with other types of defence pathways [6].

58 If a pathogen does manage to penetrate through these layers, the plant needs to be able to
59 combat it. As a primary defence response, plants have a wide range of specific cell-wall
60 surface receptor-type proteins called pattern-recognition receptors (PRRs) that respond to
61 microbes through the sensitive and quick recognition of conserved microbial features [7],
62 such as chitin, flagella, glycoproteins or lipopolysaccharides, called microbe-associated
63 molecular patterns (MAMPs) and pathogen-associated molecular patterns (PAMPs), or
64 molecules released on damaged tissue called damage-associated molecular patterns (DAMPs)
65 [5]. This recognition triggers a set of defence mechanisms in the plant that results in the
66 activation of PAMP-triggered immunity (PTI) which can prevent the pathogen from infecting
67 and colonising host tissues.

68 It has been discovered that successful pathogens have acquired host-specific molecules called
69 effectors [8] that they release to prevent host recognition of their PAMPs/MAMPs or by
70 directly suppressing PTI responses [9].

71 Peptide-based post-translational modifications (PTMs) are regulatory processes that can alter
72 the function, structure and activity of the proteome. Studies on the role of PTMs in plant
73 immunity and cell signalling have increased over the last decade [10]. Furthermore, the three
74 major PTMs, protein phosphorylation, ubiquitination and SUMOylation, are well-known to
75 mediate PTI and R gene-dependent signalling. PTI-induced mitogen-activated protein kinase
76 (MAPK) signalling regulates transcription factors through phosphorylation which are in turn
77 targets for the Small Ubiquitin-like Modifier (SUMO) protein [11]. Plasma membrane-related
78 proteins are also a target for lipid-based post-translational modifications, including S-
79 acylation, N-myristoylation, prenylation and glycosylphosphatidylinositol (GPI) anchors
80 [12]. This review briefly examines some key aspects of the three major post-translational
81 modifications (PTM) (ubiquitination, phosphorylation and SUMOylation) in plant immunity
82 and defence priming with an aim to provide new insights into current knowledge.

83 In a constant plant-pathogen arms race, plants acquired a second layer of immune response in
84 which they can recognise effectors with resistance (R) proteins and subsequently trigger so-
85 called effector-triggered- immunity (ETI) [13]. This coevolution between the pathogen and
86 the host, where the pathogen avirulence (Avr) gene evolves to avoid recognition and the host
87 resistance (R) gene changes in order to scan and recognize pathogen MAMPS/PAMPS is
88 accepted as the distinctive gene-for-gene model [3].

89 During this plant-pathogen interaction there is an onset of defence systems triggered by the
90 plant which leads to resistance or, if ineffective, disease development. Many different R and
91 Avr proteins have been characterized through the years providing a better understanding of
92 the plant-microbe interactions [14], including the tomato R protein Cf-4 mediating the
93 recognition of the *Cladosporium fulvum* effector protein Avr4 [15,16], the potato R protein
94 R3a that recognises Avr3a effector from *Phytophthora infestans* [17] and the recognition of
95 AvrPto from *Pseudomonas syringae* pv tomato by receptor kinase Pto in tomato [18].

96 R-mediated resistance is indirectly mediated by PTMs, where resistance (R)-type proteins,
97 such as SNC1, a TIR-NBS-LRR class disease resistance protein, interact with the SUMO
98 targets Topless-related 1 and HDA19, a transcriptional co-repressor and histone deacetylase
99 respectively [11]. Furthermore, SIZ1, a SUMO E3 ligase, negatively regulates salicylic acid
100 (SA) and PAD4-mediated R-mediated gene signalling and *siz1* mutant Arabidopsis plants
101 constitutively express systemic-acquired resistance (SAR) conferring resistance to the

102 bacterial pathogen *Pseudomonas syringae* pv. tomato (*Pst*) DC3000 expressing avrRps4 [19].
103 This clearly shows an involvement of PTMs, apart from basal resistance, in induced
104 resistance (IR) defence mechanism, which can potentially be further exploited to fine-tune
105 plant immune system in response to elicitor molecules.

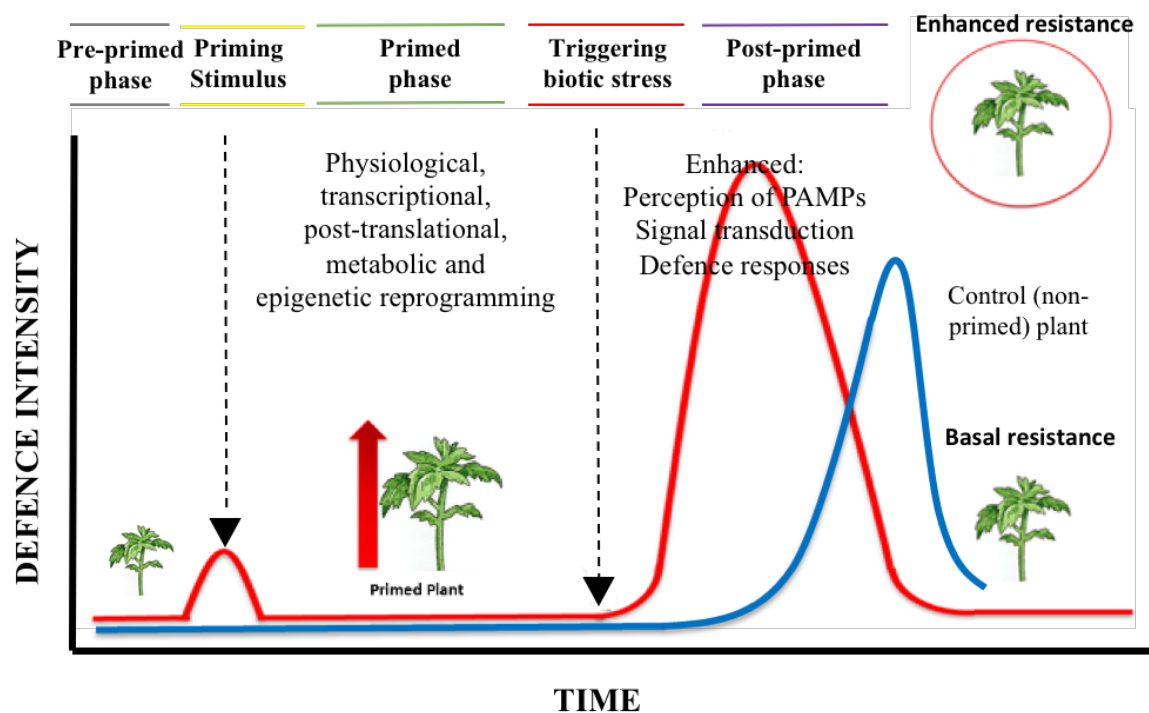
106 2. Novel roles of post-translational modifications in defence priming

107 Until recently, plant defence mechanisms were explained based on basal immune responses
108 after pathogen challenge. As stated above, basal resistance, in many cases, is not enough for
109 survival and leads to disease and ultimately a premature death of the host. However, plants
110 are capable of defending themselves and fight off pathogen attack through constitutive and
111 inducible defence mechanisms [20].

112 Elicitor molecules can induce resistance in plants, and subsequently can enhance the plant
113 basal resistance after perception of elicitor signals against pathogen attack [21]. One of the
114 main mechanisms of induced resistance is priming [22, 23], which enables the plant to fine-
115 tuning its defences for a more rapid and/or more robust response to abiotic and/or biotic
116 stress [24, 25] and implies activation of systemic responses only when the pathogen reaches
117 the infection site [24].

118 The priming process goes under three phases, which are 1) a pre-priming stimulus or ‘naïve’
119 phase, followed by 2) a post-priming stimulus or ‘primed phase’ (Figure 2) [26, 27, 28]
120 which leads to transcriptional, post-translational, metabolic, physiological and epigenetic re-
121 programming [29], such as DNA methylation and histone modification changes; these
122 changes in chromatin can be mediated by PTMs of histones (H), such as trimethylation of
123 histone 3 at lysine 4 (H3K4me3). The elicitor Benzo(1,2,3)-thiadiazole-7-carbothioic acid S-
124 methyl ester (BTH)-induced histones 3 and 4 methylation and acetylation of WRKY29,
125 WRKY53 and WRKY6 promoters [28]; histone variants in mammalian cells, such as
126 phosphorylation and ubiquitination [30] and the histone variant H2A.Z is subject to a variety
127 of post-translational modifications, including acetylation, ubiquitination, and SUMOylation
128 [31]; interestingly in *Arabidopsis* the accumulation of histone H2A substitute H2A.Z has
129 been proposed to be involved in priming suppressed SA-responsive loci (SArlc), such as PR-
130 1, to be ‘ready’ for transcription [32]. This may provide a link to a ‘post-primed phase’ where
131 the plant shows an enhanced resistance to pathogen challenge, mainly by a faster and/or
132 stronger defence response [27, 29]. However, the molecular-basis of the linkage between
133 some of the previous changes, in particular post-translational modifications (PTMs), such as
134 protein phosphorylation, ubiquitination, SUMOylation and the more recent lipid-based PTMs
135 and defence priming still remains unclear, however some evidence has been shown such as in

136 *Arabidopsis* the *ots1-ots2* double mutant and *siz1* mutant show constitutive SAR and
 137 resistance against *Pst.* DC3000 [19, 33]. Finally, 3) the ‘post-primed state’ has been related to
 138 an increased, more efficient activation of the plant defence response against pathogen attack
 139 (Figure 2) with minimal plant fitness costs [34, 35]. Moreover, the ‘post-primed state’ of the
 140 plant results from an amplified sensitization or perception (increased ‘alertness’) of
 141 immunity-inducing signals, rather than from direct gene induction [24, 36], which reinforces
 142 the importance of PTMs in the primed cell proteome to “fight back” against biotic and/or
 143 abiotic stresses.
 144



145
 146 Figure 1. Model of a general priming process with an elicitor or ‘priming agent’ (adapted from Martinez-
 147 Medina et al. 2016). The priming stimulus (e.g. chemical priming agent such as BABA, JA or chitosan) acts on
 148 a pre-primed organism which leads to a ‘primed phase’ and precedes the stress response induced by a triggering
 149 stimulus, such as pathogen infection. After the stress trigger (e.g. pathogen attack), the ‘post-primed’ plant
 150 shows a stronger and more rapid defence response which leads to an enhanced resistance against different
 151 stresses. The amplitude of defence is shown on the y axis and the time on the x axis.

152 As stated above, the implications of defence priming are numerous; including long-lasting
 153 resistance, changes in transcriptional, post-translational, metabolic and physiological
 154 regulation and even transgenerational primed progeny [34]. Some examples include the non-
 155 protein amino acid priming elicitor β -aminobutyric acid (BABA), which can induce
 156 resistance even 28 days after treatment, termed long-lasting resistance, in *Arabidopsis*

157 *thaliana* against *Hyaloperonospora arabidopsidis* (Hpa) and its priming effect can still be
158 detected in the next generation, which requires the central transcriptional regulator of basal
159 and systemic acquired resistance (SAR) protein NPR1 [37, 38]. The phytohormone jasmonic
160 acid (JA), together with BABA, applied as a seed treatment in tomato, is also able to induce
161 long-lasting priming against herbivores and powdery mildew (*Oidium neolycopersici*) at 8-9
162 weeks after treatment [34] or against *B. cinerea* [21].

163 However, both priming agents, JA and BABA also have an impact on plant growth at high
164 concentrations which must be taken into consideration in order to not over-stress the plant.
165 Even though priming rarely provides complete resistance in the host against biotic stress and
166 it is associated with plant fitness costs and trades-off [22, 39, 40, 41], its benefit relies on the
167 activation of MAMP/DAMP-mediated multi-genic defence response [37] that cannot be
168 easily overcome by the pathogen.

169 Thus, to achieve a more efficient defence strategy that is less costly in terms of plant fitness,
170 it is important, when using priming agents, to assess the effect of the concentration not only
171 on the activation of plant endogenous defences, but also on the growth and stress tolerance of
172 the plant.

173 3. Priming via post-translational modifications as a key regulatory system for the 174 onset, speed and outcome of the plant defence response against biotic stress

175 As described above, the three major PTMs, protein phosphorylation, ubiquitination and
176 SUMOylation have been well-established as being key in plant signalling. It has recently
177 been showed that PTMs are essential regulatory mechanisms that enable host cells to deploy
178 defence responses quickly upon pathogen challenge and they can also be targeted by
179 pathogen effectors [10]. Even though the molecular basis of PTMs role in plant defence
180 priming is still largely unknown, several studies have acknowledged the importance of
181 histone acetylation and methylation and transcription factor phosphorylation for the cell to
182 acquire memory by storing information of PTM-induced changes and thus respond faster and
183 more robustly towards the same type of stress subsequently [22, 25, 28, 36].

184 It has also been hypothesized [24] and recently demonstrated [28] that some priming agents,
185 such as BABA, BTH and arbuscular mycorrhiza fungi (AMF), are able to transiently and/or
186 constitutively induce accumulation of cellular molecules, such as mRNAs, reactive oxygen
187 species (ROS), secondary metabolites and hence induce the increase in protein levels, which
188 in turn enhances the signalling component of the cellular immunity mechanisms. This process
189 leads to a more rapid and stronger defence response when the pathogen reaches the primed

190 cells [23]. It has been hypothesised that the increased abundance of “inactive” immune
191 signalling regulators in primed cells can be linked to PTMs [32], such as protein
192 phosphorylation, ubiquitination and SUMOylation. For example, it has been previously stated
193 that priming agents, such as the SA functional analogue and SAR activator
194 benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) has been reported to prime
195 *A. thaliana* cells by increasing the amount of mitogen-activated protein kinases (MAPK)
196 [25].

197 MAPK-mediated phosphorylation is a good example of the PTM machinery, as they are both
198 a target and a product for PTM. As noted in Section 1, phosphorylation dynamics are pivotal
199 for MAMP/PAMP perception and PTI and thus for rapid alterations of signalling pathways.
200 However, they can be pathogen targets to deploy infection also, such as the bacterial type III
201 effector proteins from *Pst* DC3000 that targets ROS and MAPK phosphorylation cascades
202 [42]. Interestingly, this suggests a potential link between priming and phosphorylation, as
203 after PAMP perception, the immune signalling cascade is transduced by MPK target
204 phosphorylation. Therefore, there is potential for manipulating the phosphorylation status of
205 MPKs as well as their substrates for defence priming.

206 In this case, PAMP/MAMP-based priming elicitors, such as *flg22* and chitin-based elicitors,
207 could have an impact on phosphorylation dynamics by activating the expression of defence-
208 related regulatory gene cascades, such as mitogen-activated protein kinases (MPKs) and
209 subsequent MPK kinases (MEKKs), which are involved in signal transduction and promoters
210 of transcription co-activator genes such as the WRKY domain proteins [25], thus
211 significantly increasing the speed of the defence response and improving plant-pathogen
212 interaction outcomes in favour of the host.

213 Ubiquitination has been commonly associated with protein degradation, protein function
214 regulation and modulation of plant responses to biotic stress [43]. The plant ubiquitin-
215 proteasome system (UPS) is involved in plant growth, development, abiotic stress responses
216 and ultimately plant immunity [44]. Ubiquitin E3 ligases are triggered in response to PAMP-
217 based elicitors and effectors [44] and ubiquitination of defence-related genes is essential for
218 their function, such as the SAR regulatory protein, NPR1, which is translocated into the
219 nucleus via the UPS [44].

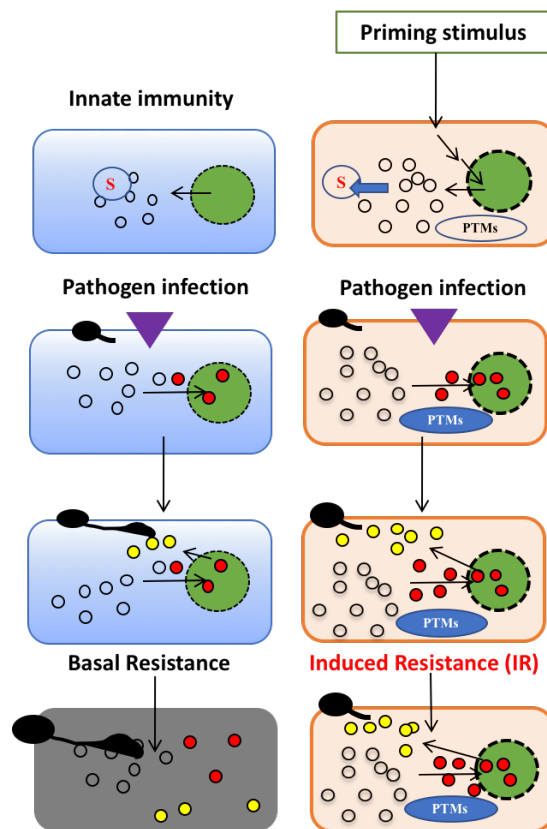
220 Signalling-based genes, such as some *Avr9/Cf-9* rapidly elicited (ACRE) genes encode
221 components of signalling cascades, including transcription factors, protein kinases, and
222 ubiquitination pathway-related proteins, such as, E3 ligases, F-box and U-box proteins [43].
223 Thus, targeting priming plant ubiquitination/UPS opens new possibilities to increase the

224 speed and efficacy of the plant signalling upon pathogen attack. However, the challenge is to
 225 prime the ubiquitin system towards immunity without having an impact in other ubiquitin-
 226 related processes, such as plant growth and development.

227 The role of SUMOylation in disease resistance is an emerging area of importance (Figure 2)
 228 where Arabidopsis SUMO E3 ligase (SIZ1) acts as a negative regulator of SA- and PAD4-
 229 mediated signalling in plants against Pst DC3000 expressing avrRps4 [19]. Moreover, the
 230 importance of SUMO conjugation in plant survival under abiotic stress has been described
 231 recently [45]. SUMO conjugation has also been shown to be required to suppress defence
 232 signalling in the absence of infection [42]. The question then remains as whether
 233 SUMOylation and other PTMs can be primed in order to facilitate a rapid immune response
 234 to prevent a lethal outcome from disease and lead to resistance.

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238 Figure 2. Model of the molecular basis of defence priming in plant cells and the connection to PTMs. In the
 239 non-primed (left) cell, the plant cell through the nucleus (green circles) remains with basal expression of
 240 defence-related genes and SUMO conjugation (S) represses signalling in pathogen absence. On the primed cell
 241 (right) the priming stimulus induces the nuclear-mediated transcription of mRNA, cleavage of SUMO proteins
 242 and accumulation of inactive post-translationally modified (PTMs) defence-related proteins. After pathogen
 243 challenge both cells trigger expression of signalling cascades and defence-related proteins, however only primed
 244 cells are able to quickly translate and activate the defence-related proteins (red circles) that were modified post-

245 translationally and hence ultimately express a fine-tuned faster defence response that enables the plant to display
246 antimicrobial proteins (yellow circles) that reduce and/or stop pathogen expansion, whereas non-primed cells
247 are not able to display quick defence response which leads into infection expansion and disease.

248

249 Interestingly, it has been shown that NPR1 is a SUMO protein target upon salicylic acid (SA)
250 induction and that NPR1 SUMOylation by SUMO3 is required for its immune activity and
251 degradation [38]. This clearly shows the potential implications and connection of PTMs and
252 priming, which is yet to be exploited. It may be possible to find novel PTMs targets, such as
253 JA-dependent transcription factors, e.g. JAZ and MYC2 are well -known PTM targets, which
254 would open up multiple implications for PTMs and long-lasting priming against necrotrophic
255 pathogens.

256 Few studies have examined this in detail in crop systems. [21] Luna et al., 2016 showed that
257 a soil drench of BABA at high concentrations (10 mM) and JA (1 mM) on 1-week-old
258 tomato seedlings abolished plant growth and had lethal effects. The importance of the SUMO
259 proteases OTS1 and OTS2 has been shown in promoting plant growth under salt stress and
260 that SUMO1 over-expression has a repressive effect on plant development [46]. Thus, it
261 would be interesting to investigate further the molecular basis of this common phenotype of
262 BABA/JA-induced and SUMO1-overexpression related repression of plant development.
263 Furthermore, *ots1 ots2* double mutant has been shown to be more resistant against *Pst*
264 DC3000, hence there may be opportunities to exploit these putative common pathways to
265 boost defence priming and promote growth under stress.

266 It is well-known that a previous stress stimulus can induce epigenetic changes in the plant
267 and subsequently enhance its defence mechanisms [28]. Moreover, the link between post-
268 translational modifications (PTMs) and priming has been demonstrated through post-
269 translational modifications of histones at promoter regions of primed defence genes [37]; it
270 has also been shown that RNA Polymerase V mutants were enriched in H3K4me3 at the
271 promoter of PR-1 and PDF1.2 defence-related genes, which lead to an enhanced resistance to
272 *Pst* [47]. Furthermore, application of the hormone salicylic acid (SA) and *Pst* DC3000
273 infection has been linked to the accumulation of the acetylated and methylated versions of
274 histones H3 (H3Ac), H4 (H4Ac) and H3K4me2, H3K4me3 and HDA19 at the promoter
275 region of PR-1, WRKY38 and WRKY62. It is postulated that this remodelling of chromatin
276 of these SA-responsive loci may be repressed by SUMO but not shown [32] and therefore it
277 is likely that SUMO will have a critical role in defence priming.

278 Conclusions and Perspectives

279 In a world where human population has increased exponentially in recent decades reaching
280 7.6 billion in 2017 and projected to reach 8.6 billion by 2030 (United Nations, The 2017
281 Revision of World Population Prospects), a major challenge in the fight against pathogen
282 damage to crop yields worldwide is the ineffectiveness of conventional crop protectants due
283 to pathogen resistance and the fast evolution of pathogens towards their hosts to overcome
284 resistance and promote disease.

285 Priming has emerged over the last decade as a promising wide-ranging inducible defence
286 mechanism with minimal costs in plant development. Multiple examples have shown the
287 ability of certain molecules to potentiate the plant 'alertness' to perceive and subsequently
288 respond to pathogen attack. The three major post-translational modifications, including
289 phosphorylation, ubiquitination and SUMOylation are key components of the plant immune
290 system, cell signalling and they are inter-linked but their roles in defence priming have yet to
291 be deciphered. Other PTMs such as S-nitrosylation of proteins, irreversible tyrosine
292 nitration, acetylation and methylation have emerged as pivotal mechanisms in the plant
293 immune system with the potential to be primed. Furthermore, there are still many questions
294 as to how these signals are transmitted intra- and even inter-cellularly? How do primed cells
295 regulate post-translational modifications? Are PTMs essential for the establishment of
296 elicitor-induced resistance? What are the molecular mechanisms underlying the priming-
297 related PTMs linked to the fine-tuning and accelerating plant defence responses after
298 pathogen challenge?

299 Thus, the potential exploitation of PTMs as priming targets has become a 'hotspot' in the
300 race to find new insights in plant immune responses against biotic/abiotic stresses [32] and
301 the current availability of appropriate molecular tools will facilitate deciphering the PTM
302 code for defence priming.

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