Chemical inducers of systemic immunity in plants

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Abstract
Systemic acquired resistance (SAR) is a highly desirable form of resistance that protects against a broad-spectrum of related or unrelated pathogens. SAR involves the generation of multiple signals at the site of primary infection, which arms distal portions against subsequent secondary infections. The last decade has witnessed considerable progress, and a number of chemical signals contributing to SAR have been isolated and characterized. The diverse chemical nature of these chemicals had led to the growing belief that SAR might involve interplay of multiple diverse and independent signals. However, recent results suggest that coordinated signalling from diverse signalling components facilitates SAR in plants. This review mainly discusses organized signalling by two such chemicals, glycerol-3-phosphate and azelaic acid, and the role of basal salicylic acid levels in G3P-conferred SAR.

Key words: Chemical signals, defence, plant metabolites, signalling, systemic.

Introduction
Plants, like all other organisms, are constantly challenged by microbial pathogens. Their static nature and the absence of an active circulatory immune system pose added disadvantages while dealing with such stresses. Thus, plants have developed some unique mechanisms to defend themselves from biotic stresses. In addition to preformed physical barriers such as the cuticle and cell wall and the production of antimicrobial metabolites, plants also utilize induced defence-signalling mechanisms against microbes (Eulgem, 2005; Jones and Dangl, 2006). These include nonhost resistance, pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI), and effector-triggered immunity (ETI). In PTI, general pathogen elicitors (e.g. flagellin from Gram-negative bacteria) are recognized by extracellular pattern-recognition receptors in the plant (Thordal-Christensen, 2003). PTI can in turn be suppressed by specialized pathogen elicitors, also designated avirulence (avr) factors, which often either physically interact with or alter one or more components essential for the induction of PTI (Shang et al., 2006; Shabab et al., 2008; Shan et al., 2008; Zhou and Chai, 2008; Zipfel and Rathjen, 2008). To counteract this interference, plants evolved to 'recognize' Avr proteins via specialized proteins termed resistance (R) proteins. The direct or indirect recognition of pathogen Avr by a plant R protein usually results in the induction of ETI, and this recognition is highly species specific (Flor, 1971; Jones and Dangl, 2006).

In addition to local defence responses, plants can also induce systemic responses such as systemic acquired resistance (SAR), and induced systemic resistance (ISR). ISR is generally induced in response to root colonization by beneficial bacteria (Van Wees et al., 2008), whereas SAR is triggered in response to pathogen infection (Dempsey and Klessig, 2012; Spoel and Dong, 2012; Fu and Dong, 2013; Kachroo and Robin, 2013). During SAR, localized infection by primary pathogen results in the induction of a broad-spectrum and long-lasting resistance to secondary pathogens in the systemic tissues of the plant. Moreover, SAR can be transferred to the immediate next generation of progeny via modifications in the chromatin structure (Luna et al., 2012; Slaughter et al., 2012). Due to its unique benefits, SAR is highly desirable in crop production. In the last two decades, SAR has been particularly intensely studied, resulting in the
elucidation of many crucial aspects of this form of defence. These results are well reviewed in several recent articles (Dempsey and Klessig, 2012; Fu and Dong, 2013; Kachroo and Robin, 2013; Lucas et al., 2013; Shah and Zeier, 2013). This review summarizes findings related to newly identified chemical inducers of SAR and highlights the convergent nature of the signalling events that eventually induce SAR.

### Chemical inducers of SAR

The onset of SAR involves the generation of mobile signal(s) at the site of local infection, and this occurs within 4–6 h of primary infection (Rasmussen et al., 1991; Smith-Becker et al., 1998; Chanda et al., 2011; Chaturvedi et al., 2012). The signal(s) then translocate presumably through the phloem to the systemic tissues, where it activates defence response. Several chemical inducers of SAR have been identified including, salicylic acid (SA) and its methylated derivative MeSA (Durrant and Dong, 2004; Park et al., 2007), jasmonic acid (JA; Truman et al., 2007), auxin (Truman et al., 2010), piperolic acid (Pip; Návarová et al., 2012), dehydroabietial (DA; Chaturvedi et al., 2012), azelaic acid (AzA; Jung et al., 2009), and glycerol-3-phosphate (G3P; Chanda et al., 2011; Mandal et al., 2011; Yu et al., 2013; Table 1). Among these chemicals, the SAR-associated role of JA remains contentious and will not be discussed here (Chaturvedi et al., 2008; Attaran et al., 2009; Xia et al., 2009).

DA is a C₉ diterpenoid, which requires SA and its receptor/signalling component NPR1 to confer SAR (Cao et al., 1997; Wu et al., 2012). DA and AzA appear to act in an additive manner since exogenous application of low concentrations of DA or AzA, which do not activate SAR, do so when applied together (Chaturvedi et al., 2012; Table 2). However, AzA and DA differ in their mechanism of SAR activation: DA increases SA levels in local (treated) and distal tissues whereas AzA induces G3P biosynthesis (Yu et al., 2013). Notably, DA levels are similar in petiole exudates from mock-inoculated primary infection (Rasmussen et al., 1991; Smith-Becker et al., 1998, 2011; Chanda et al., 2010, 2011; Smith-Becker et al., 2013; Shah and Zeier, 2013).

Pip does function as a true SAR inducer; localized application of Pip induces resistance in systemic untreated tissues (C Wang, A Kachroo, and P Kachroo, unpublished results). Besides its structural difference from DA, Pip also differs in the fact that it accumulates significantly in the petiole exudates of pathogen-infected leaves, although its ability to move systematically remains untested (Návarová et al., 2012). Nevertheless, Pip appears to also share many features with DA. Like DA, Pip treatment can induce SA biosynthesis in the absence of pathogen and Pip-induced SAR is dependent on both SA and its receptor and signalling component NPR1 (Návarová et al., 2012). The biosynthesis of Pip is dependent on ALD1 (AGD2-like Defence) that has in vitro aminotransferase activity (Song et al., 2004a, b). The aldl mutants are defective in PTI, ETI, and SAR, but root application of Pip can restore these responses to wild-type levels. ALD1 is also required for Pip-induced priming activity during SAR.

### Table 1. Attributes of SAR-defective mutants

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Gene ID</th>
<th>Ecotype</th>
<th>Basal immunity</th>
<th>ETI</th>
<th>SAR signal generation</th>
<th>SAR signal perception</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ald1</td>
<td>At2g13810</td>
<td>Col-0</td>
<td>Defective</td>
<td>Defective</td>
<td>Defective</td>
<td>NT</td>
<td>Song et al. (2004a, b); Navarova et al. (2012)</td>
</tr>
<tr>
<td>az1</td>
<td>At4g12470</td>
<td>Col-0</td>
<td>Normal</td>
<td>Normal</td>
<td>Defective</td>
<td>Defective</td>
<td>Jung et al. (2009); Yu et al. (2013)</td>
</tr>
<tr>
<td>bssmt1</td>
<td>At3g11480</td>
<td>Col-0</td>
<td>Normal</td>
<td>Normal</td>
<td>Defective</td>
<td>Normal</td>
<td>Liu et al. (2010, 2011)</td>
</tr>
<tr>
<td>dir1</td>
<td>At5g48485</td>
<td>Ws</td>
<td>Normal</td>
<td>Normal</td>
<td>Defective</td>
<td>Normal*</td>
<td>Maldonado et al. (2002); Champigny et al. (2011); Chanda et al. (2011); Champigny et al. (2013); Yu et al. (2013)</td>
</tr>
<tr>
<td>gfl1</td>
<td>At1g80460</td>
<td>Col-0</td>
<td>Defective</td>
<td>Normal</td>
<td>Defective</td>
<td>Normal</td>
<td>Chanda et al. (2008, 2011)</td>
</tr>
<tr>
<td>gfl2</td>
<td>At2g06090</td>
<td>Col-0</td>
<td>Defective</td>
<td>Normal</td>
<td>Defective</td>
<td>Normal</td>
<td>Chanda et al. (2008, 2011)</td>
</tr>
<tr>
<td>std1</td>
<td>At2g06090</td>
<td>N0</td>
<td>Normal</td>
<td>Normal</td>
<td>Defective</td>
<td>Normal</td>
<td>Nandi et al. (2004); Chaturvedi et al. (2008)</td>
</tr>
<tr>
<td>sid2</td>
<td>At1g74710</td>
<td>Col-0</td>
<td>Defective</td>
<td>Defective</td>
<td>Defective</td>
<td>Defective</td>
<td>Nawrath and Métraux (1999); Wildermuth et al. (2001)</td>
</tr>
</tbody>
</table>

*Based on PR-1 expression data and not pathogen resistance (Maldonado et al., 2002)
AzA-mediated systemic immunity

AzA is a C₉ dicarboxylic acid. It was identified as a compound that accumulates up to 6-fold more in the petiole exudates of pathogen-infected plants (Jung et al., 2009). At least some (~7%) of this AzA translocates to the distal tissues, largely as derivatized conjugates (Yu et al., 2013). Recent studies have shown that pathogen infection releases free fatty acids (FAs) from membrane lipids, and these can be hydrolysed to generate AzA (Yu et al., 2013). For example, the C18 unsaturated FAs, oleic (18:1), linoleic (18:2), and linolenic (18:3) acids, all of which accumulate in response to pathogen infection, can serve as precursors for AzA (Zoeller et al., 2012; Yu et al., 2013). Thus, there appears to be some redundancy in the substrates for biosynthesis of AzA. Notably, and consistent with the in planta and in vitro studies demonstrating generation of AzA from free C₁₈ unsaturated FAs, genetic mutations affecting further desaturation of C₁₈ FAs on membrane lipids do not affect AzA levels or compromise SAR. This includes the fad7 (mutation in FA desaturase 7, which desaturates 18:2 to 18:3 on the chloroplastic membrane) mutant whose SAR defect was initially assigned to a defect in glycerolipid biosynthesis (Chaturvedi et al., 2008). However, a recent study showed that the SAR defect in the fad7-1 mutant was associated with a second site mutation in the GLABRA gene, which contributes to trichome and cuticle biogenesis (Xia et al., 2010). A number of other cuticular components contributing to SAR have been identified and characterized and these contribute to perception and/or generation of SAR signal(s) (Xia et al., 2009, 2012).

AzA was proposed to induce SAR by priming plants to accumulate higher levels of SA and SA-mediated responses but only in the presence of virulent pathogen (Jung et al., 2009). However, recent results suggest that AzA induces SAR by promoting G3P accumulation (Yu et al., 2013). This correlates well with the observation that exogenous AzA upregulates expression of the G3P biosynthetic genes GLY1 (G3P dehydrogenase, G3PDH) and GLII (glycerokinase, GK) and that plants defective in GLY1 or GLII are unable to respond to AzA. Likewise, plants defective in the biosynthesis of proteins AZI1 and DIR1 are also unable to induce SAR in response to AzA.

Transport studies using isotope-labelled AzA have shown that only ~7% of AzA moves from local to distal tissues and that this movement is independent of pathogen infection (Best et al., 2012; Yu et al., 2013). AzA transport is also independent of DIR1, AZI1, GLY1, GLII1, or exogenous G3P, suggesting that transport of AzA may not be associated with the onset of SAR. Interestingly, nearly all of the AzA in distal tissues is present as a derivatized product, suggesting that AzA may be derivatized before transport or rapidly converted to a derivative upon transport (Yu et al., 2013). The importance of AzA transport and/or derivatization during the onset of SAR needs further rigorous investigation.

G3P-derived SAR signalling

G3P, a phosphorylated sugar derivative, is a conserved metabolite and the obligatory precursor of all glycerolipid biosynthesis in diverse organisms. Increased cellular G3P levels, or exogenous G3P application, correlate with resistance to the hemibiotrophic pathogen Colletotrichum higginsianum in Arabidopsis and the induction of SAR in Arabidopsis and soybean (Chanda et al., 2008, 2011). Conversely, reduction in G3P levels compromises SAR and increases susceptibility to C. higginsianum. More recently, G3P was shown to contribute to SAR in monocots as well (Yang et al., 2013). In plants, G3P is generated through glycerol via GK (GLII; Kang et al., 2003) or the reduction of dihydroxyacetone phosphate (DHAP) via G3PDH (Browse and Somerville, 1991; Kachroo et al., 2004). Arabidopsis encodes multiple G3PDH isoforms located in different cellular compartments, several of which contribute to SAR (Wei et al., 2001; Shen et al., 2003; Shen et al., 2006; Quettier et al., 2008; Chanda et al., 2011; Lorenc-Kukula et al., 2012). It is possible that specific pools of G3P may be channeled into glycerolipid biosynthesis versus SAR, since both Arabidopsis plastidal G3PDH isoforms (At5g40610 and GLY1-At2g40690) are essential for SAR, whereas only GLY1 contributes to glycerolipid biosynthesis (Chanda et al., 2011). Alternatively the various G3PDH activities may be tightly and differentially regulated to generate G3P for specific cellular processes. Besides the two plastidal isoforms, a mutation in a cytosolic G3PDH isoform (At3g07690) and the GLII-encoded GK also compromise SAR. This suggests that the different G3P biosynthetic activities contribute nonredundantly to the overall G3P pool and that their activities may be interregulated. Indeed, pathogen-infected g3pdh or gk (gl1) mutants contain near basal levels of G3P, and overexpression of Arabidopsis G3PDH isoforms (which are feedback regulated) does not increase basal G3P levels (Chanda et al., 2008). On the other hand, overexpression of a nonfeedback-regulated bacterial G3PDH did result in G3P accumulation in Arabidopsis (Shen et al., 2010).

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**Table 2. Interactions between SAR signals**

<table>
<thead>
<tr>
<th>AzA</th>
<th>DA</th>
<th>G3P</th>
<th>MeSA</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZA</td>
<td>Coinfiltration enhance</td>
<td>–</td>
<td>Requires G3P biosynthesis</td>
<td>Induces local MeSA level</td>
</tr>
<tr>
<td>DA-inducing activity</td>
<td>–</td>
<td>–</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>G3P</td>
<td>Biosynthesis induced by AzA</td>
<td>NT</td>
<td>–</td>
<td>Repress AtBSMT in distal tissues</td>
</tr>
<tr>
<td>MeSA</td>
<td>NT</td>
<td>–</td>
<td>–</td>
<td>NT</td>
</tr>
<tr>
<td>PA</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>–</td>
</tr>
</tbody>
</table>

AzA, azelaic acid; DA, dehydroabietinal; G3P, glycerol-3-phosphate; MeSA, methylated ester of SA; PA, pimelic acid; NT, not tested.
Like AzA, G3P-induced SAR requires DIR1 and AZI1. Notably, G3P and DIR1 are interdependent on each other for systemic translocation, although direct association between G3P and DIR1 has not been detected (Chanda et al., 2011). The movement and/or induction of DIR1 in local and distal tissues in response to pathogen infection or G3P treatment was also demonstrated in two subsequent studies (Champion et al., 2013; Yu et al., 2013). However, like AzA, G3P is also modified to derivatives upon/during translocation, and one of these derivatives (identity unknown) is biologically active in inducing SAR (C Wang and P Kachroo, unpublished results). Thus, it is possible that DIR1 directly associates with this bioactive G3P-derivative and that, upon translocation to the distal tissues, this complex then induces SAR. Interestingly, AZI1 can compensate for the lack of DIR1 when overexpressed, and vice versa. Importantly, AZI1 and DIR1 overexpression can restore pathogen-responsive G3P accumulation in the dir1 and azl1 mutants, respectively (Yu et al., 2013). The inability of the azl1 and dir1 mutants to accumulate pathogen-responsive G3P, together with the inability of gly1/gli1 mutants to accumulate DIR1/AZI1 proteins even when expressed as transgenes via the constitutive cauliflower mosaic virus 35S promoter, suggests that G3P, DIR1, and AZI1 might operate in a feedback regulatory loop. Notably, dir1 and azl1 mutants are not defective in AzA accumulation. Thus, the inability of dir1 and azl1 mutants to induce AzA-mediated SAR is related to their impaired G3P accumulation, not AzA biosynthesis. The interdependence of G3P, DIR1, and AZI1 for inducing SAR, the colocalization of the DIR1 and AZI1 proteins to the endoplasmic reticulum, and their interaction with each other all suggest that a complex comprising the G3P derivative, DIR1, and AZI1 regulates SAR (Yu et al., 2013). Interestingly, a recent study showed that AZI1 also interacts with the mitogen-activated protein kinase 3 (MPK3) and is phosphorylated by MPK3 in vitro (Pitzschke et al., 2013). Moreover, this study also showed a role for AZI1 in salt stress; the azl1 mutant and AZI1 overexpression leads to hypersensitivity or tolerance towards salt stress, respectively. This suggests that AZI1 either participates in several different cellular processes or that high-salinity stress response overlaps with SAR. Notably, AZI1 transcripts were less stable in both G3P-deficient gly1/gli1 as well as mpk3 mutant backgrounds (Pitzschke et al., 2013; Yu et al., 2013) and, just like gly1 and gli1, a mutation in mpk3 also compromised SAR (Beckers et al., 2009). In view of these results it is tempting to speculate an overlap between G3P- and MPK3-mediated signalling pathways leading to SAR.

Role of SA and MeSA in SAR

SA is not only essential for pathogen-induced SAR, but also none of the identified chemical inducers can induce SAR in SA-deficient backgrounds. Notably however, the importance of increased SA accumulation in the primary infected and/or systemic tissues in SAR needs further clarification. Clearly, SA accumulation alone is insufficient to induce SAR (Cameron et al., 1999). SA is synthesized via the shikimic acid pathway, which bifurcates into two branches after the biosynthesis of chorismic acid. Both branches contribute to SA biosynthesis and are required for SAR (Vernooij et al., 1994; Pallas et al., 1996; Wildermuth et al., 2001). In one branch, chorismic acid is converted to SA via phenylalanine and cinnamic acid intermediates via the key enzyme phenylalanine ammonia lyase (PAL). In the other branch, chorismic acid is converted to SA via isochorismic acid and this is catalysed by isochorismate synthase (ICS1/SID2; Wildermuth et al., 2001; Strawn et al., 2007). Pathogen inoculation increases SA levels, primarily in the local leaves, while the distal tissues show a nominal or insignificant increase (Rasmussen et al., 1991; Yalpani et al., 1991; Meuwly and Métraux, 1993; Mölders et al., 1994; Xia et al., 2010; Chanda et al., 2011). In fact, majority of the SA (~70%) in the systemic leaves is thought to be that which is transported from the primary infected leaves (Shulaev et al., 1995). However, radioisotope-based studies show that at least some SA is transported to distal tissues in a pathogen-independent manner (Mölders et al., 1996) and grafting studies indicate that transport of SA alone is insufficient to induce SAR (Vernooij et al., 1994; Pallas et al., 1996). Perhaps like SA, isolated transport of other chemical SAR inducers such as DA, AzA, and G3P might also be insufficient to induce SAR.

MeSA, a volatile and phloem mobile derivative of SA, which accumulates in infected and distal tissues in response to pathogen infection, is also important for SAR. For SAR development, MeSA must be converted to SA in the distal tissues in the 48–72-h period post primary infection. This time frame correlates with that of MeSA accumulation in infected and systemic tissues. The biosynthesis of MeSA is catalysed by SA methyltransferases (SAMT/BSMT), and the conversion of MeSA back to SA is mediated by methyl esterase (MES, SABP2) (Chen et al., 2003; Effmert et al., 2005; Koo et al., 2007). Grafting studies in tobacco plants silenced for SABP2 (SA-binding protein 2) have shown that SABP2 activity in scions, but not root stocks, is required for normal SAR (Kumar and Klessig, 2003; Park et al., 2007). Furthermore, the synthetic SA analogue, tetrAFA (2,2,2,2-tetra-fluorooctothophene), which inhibits the esterase activity of SABP2, inhibits SAR (Park et al., 2009). As in tobacco, Arabidopsis SABP2 (AtMES9) and AtBSMT1 are required for SAR in Arabidopsis (Attaran et al., 2009; Liu et al., 2011). However, prolonged light exposure post pathogen inoculation can eliminate the requirement for Atbsmt1 in SAR (Attaran et al., 2009; Liu et al., 2011). It is unclear whether MeSA merely delivers SA to the distal tissues or has other more specific function(s) in SAR, particularly since SA itself is transported to systemic tissues. The SAR defect of dir1 plants was proposed to be associated with increased AtBSMT1 expression and in turn the increased MeSA and reduced SA levels in this mutant (Liu et al., 2011). However, several other independent studies have reported normal SA levels in pathogen-infected dir1 plants (Maldonado et al., 2002; Chaturvedi et al., 2012; C Wang, D Navarre and P Kachroo, unpublished results). Notably, localized application of G3P in Arabidopsis induced a gene encoding SABP2-like protein and repressed AtBSMT1 expression (Chanda et al., 2011), suggesting that G3P application might
promote conversion of MeSA to SA in distal leaves. However, application of G3P alone or together with pathogen does not induce SA synthesis in either local or distal tissues (Chanda et al., 2011; Yu et al., 2013). This suggests that the differential expression of SABP2 and AtBSMT1 might be associated with maintaining sufficient SA levels in distal tissues, further reiterating a critical role for basal SA in SAR. Notably, G3P-induced SAR does require basal SA, although SA is unable to confer SAR in G3P-deficient backgrounds. This suggests that G3P and SA might function independently to induce SAR, possibly via parallel pathways (Fig. 1). Besides SA itself, many SA signalling components also contribute to SAR. The precise involvement of these components in SAR has been extensively reviewed in other recent articles (Fu and Dong, 2013; Lucas et al., 2013).

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