Phytochemistry 143 (2017) 160–169

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Benzoylsalicylic acid derivatives as defense activators in tobacco and Arabidopsis

Samuel Kamatham [a], Reddanna Pallu [b], Anil Kumar Pasupulati [a], Surya Satyanarayana Singh [c], Padmaja Gudipalli [d, *]

[a] Department of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad, 500046, Telangana, India
[b] Department of Animal Biology, School of Life Sciences, University of Hyderabad, Hyderabad, 500046, Telangana, India
[c] Department of Biochemistry, Osmania University, Hyderabad, 500007, Telangana, India
[d] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, 500046, Telangana, India

ARTICLE INFO

Article history:
Received 1 March 2017
Received in revised form
25 July 2017
Accepted 28 July 2017

Keywords:
Nicotiana tabacum
Arabidopsis thaliana
Benzoylsalicylic acid (BzSA)
BzSA derivatives
Systemic acquired resistance
Non-expressor of pathogenesis-related gene-1
Pathogenesis-related genes

ABSTRACT

Systemic acquired resistance (SAR) is a long lasting inducible whole plant immunity often induced by either pathogens or chemical elicitors. Salicylic acid (SA) is a known SAR signal against a broad spectrum of pathogens in plants. In a recent study, we have reported that benzoylsalicylic acid (BzSA) is a SAR inducer in tobacco and *Arabidopsis* plants. Here, we have synthesized BzSA derivatives using SA and benzoyl chlorides of various moieties as substrates. The chemical structures of BzSA derivatives were elucidated using Infrared spectroscopy (IR), Nuclear magnetic spectroscopy (NMR) and High-resolution mass spectrometer (HRMS) analysis. The bioefficacy of BzSA derivatives in inducing defense response against tobacco mosaic virus (TMV) was investigated in tobacco and SA abolished transgenic *NahG Arabidopsis* plants. Interestingly, pre-treatment of local leaves of tobacco with BzSA derivatives enhanced the expression of SAR genes such as *NPR1* [*Non-expressor of pathogenesis-related* (*PR*) *genes 1*], *PR* and other defense marker genes (*HSR203*, *SIPK*, *WIPK*) in systemic leaves. Pre-treatment of BzSA derivatives reduced the spread of TMV infection to uninfected areas by restricting lesion number and diameter both in local and systemic leaves of tobacco in a dose-dependent manner. Furthermore, pre-treatment of BzSA derivatives in local leaves of SA deficient *Arabidopsis NahG* plants induced SAR through *AtPR1* and *AtPR5* gene expression and reduced leaf necrosis and curling symptoms in systemic leaves as compared to BzSA. These results suggest that BzSA derivatives are potent *SAR* inducers against TMV in tobacco and *Arabidopsis*.

1. Introduction

Plants have evolved both innate and acquired immunity to counter microbial pathogens by activating a variety of inducible defense mechanisms (van Loon et al., 2006). Plants develop disease resistance by two branched innate immune system. The first branch recognizes pathogen (microbe)-associated molecular patterns (PAMPs/MAMPs) through plant pattern-recognition receptors (PRR) resulting in PAMP/MAMP-triggered immunity (PTI) that can halt further colonization (Boller and Felix, 2009; Jones and Dangl, 2006). The second branch innate immune system responds to pathogen effectors either directly or through their effects on the host cellular targets. Recognition of pathogen effectors by nucleotide-binding leucine rich repeat (NBLRR) proteins encoded by disease resistance (R) genes, activate effector-triggered immunity (ETI) that accelerates and amplifies PTI response leading to induction of HR (hypersensitive response) and SAR (systemic acquired resistance) in the host (Jones and Dangl, 2006; Schwessinger and Zipfel, 2008; Zipfel, 2009). The plant genome encodes hundreds of resistance (R) proteins that allow the plants to recognize specific pathogen-derived molecules known as avirulence (avr) factors. The R-avr recognition between plants and pathogens often triggers a localized reaction at the site of infection known as hypersensitive response (HR) (Dangl and Jones, 2001; Durrant and Dong, 2004). It was demonstrated that during HR several events such as programmed cell death, production of reactive oxygen species (ROS) and synthesis of anti-microbial compounds can occur at the site of infection (Dangl and Jones, 2001; Hammond-Kosack

* Corresponding author.
E-mail address: gudipallipadmaja@gmail.com (P. Gudipalli).

http://dx.doi.org/10.1016/j.phytochem.2017.07.014
0031-9422/

and Jones, 1996). The signaling compounds generated at the primary site of infection elicit increased resistance to secondary infection in uninfected parts of the plants and this phenomenon is known as systemic acquired resistance (SAR) (Delaney et al., 1994; Ross, 1961; Ryals et al., 1994; Ward et al., 1991). It is largely considered that salicylic acid (SA) is a major contributor to the development of SAR. The development of SAR is mainly associated with the coordinate expression of a large number of defense genes in systemic parts of the plants (Ryals et al., 1995).

Salicylic acid (SA) plays a critical role in SAR induction by acting as an endogenous signal for the induction of SAR genes (Delaney et al., 1994; Dempsey and Klessig, 2012). It was also reported that the translocating, SAR-inducing signal is not SA (Vernooij et al., 1994). Reciprocal grafts demonstrated that the signal requires the presence of SA in tissues distant from the infection site to induce systemic resistance (Vernooij et al., 1994). The intermediates of SA biosynthesis and the molecular events in plants were detailed by several researchers (Dempsey et al., 2011; Lee et al., 1995; Ribnicky et al., 1998; Silverman et al., 1995; Wildermuth et al., 2001). During pathogen infection, the endogenously accumulated SA is rapidly metabolized to its conjugates such as SA-*O*-β-D-glucoside and methylsalicylate (MeSA) and subsequent studies have demonstrated that MeSA is a critical mobile signal for SAR in plants (Enyedi et al., 1992; Lee et al., 1995; Park et al., 2007). Recently, it is also reported that plants regulate SA levels by converting it to 2, 3-dihydroxybenzoic acid (2, 3-DHBA) to prevent SA over-accumulation (Zhang et al., 2013). The requirement of SA in SAR development was demonstrated in transgenic *NahG* plants that harbor a bacterial gene encoding salicylate hydroxylase, which converts SA into inactive catechol (Gaffney et al., 1993). In our previous study, we reported benzoylsalicylic acid (BzSA) as a new derivative of SA that induces SAR more potently than SA and its derivative acetyl salicylic acid (ASA) (Kamatham et al., 2016). Induction of SAR was blocked when SA methyltransferase that converts SA to MeSA was silenced in primary infected leaves, and therefore it was concluded that MeSA is a SAR signal in tobacco (Park et al., 2007).

Recently, several metabolites have been identified as SAR inducing signals that work either dependent or independent of SA accumulation (Chaturvedi et al., 2012; Dempsey and Klessig, 2012; Gao et al., 2015; Shah et al., 2014). Dehydroabietinal (DA), an abietane diterpenoid, purified from vascular sap of *Arabidopsis thaliana* leaves induced SAR through the accumulation of SA (Chaturvedi et al., 2012). The non-protein amino acid pipecolic acid (Pip) regulates SAR and basal immunity to bacterial pathogen infection and biosynthesis of Pip in systemic tissues contributing to SAR establishment (Ding et al., 2016; Hartmann et al., 2017). Azelaic acid (AzA) a putative SAR signal, mobilizes *Arabidopsis* immunity in a concentration-dependent manner (Jung et al., 2009; Wittek et al., 2014). The other SAR inducers such as Benzo (1, 2, 3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) (Gorlach et al., 1996; Lawton et al., 1996) and 2, 6-dichloroisonicotinic acid (INA) (Metraux et al., 1990; Uknes et al., 1992) induced SAR independent of SA accumulation. In contrast, probenazole (PBZ) and its active metabolite 1, 2-benzisothiazol-3 (2H)-one 1,1-dioxide (BIT) induced SAR *via* SA accumulation (Yoshioka et al., 2001). During SAR, SA activates *NPR1* gene a transcriptional coregulator that activates SA-dependent defense genes (Wu et al., 2012). The expression of *PR* genes leads to the enhancement of SAR in plants (Durrant and Dong, 2004; Ryals et al., 1996). Loss of function studies involving mutations in *NPR1* gene, showed compromised SAR and therefore unable to develop resistance to pathogen infection (Kinkema et al., 2000; Mou et al., 2003; Spoel et al., 2009). Previously, it has been reported that SA and INA derivatives are biologically active and some of them induced *PR-1* expression in tobacco plants (Conrath et al., 1995). In addition, benzothiadiazole, N-cyanomethyl-2-chloroisonicotinamide (NCI), 3-chloro-1-methyl-1H-pyrazole-5-carboxylic acid (CMPA), 3-acetonyl-3-hydroxyoxindole (AHO), glycerol-3-phosphate (G3P) induced SAR *via PR* gene expression (Chanda et al., 2011; Gorlach et al., 1996; Lawton et al., 1996; Li et al., 2008; Nakashita et al., 2002; Yasuda et al., 2003a, 2003b).

In the present study, we have chemically synthesized and characterized BzSA derivatives. Pre-treatment of BzSA derivatives (**1**–**14**) activated *NPR1* dependent SAR gene expression in systemic leaves of tobacco plants. Similarly, pre-treatment of SA- deficient *NahG* transgenic *Arabidopsis* plants with BzSA derivatives induced *PR* gene expression. Furthermore, pre-treatment of local leaves of tobacco plants with BzSA derivatives (**1**–**14**) prevented the spread of tobacco mosaic virus (TMV) infection to uninfected systemic leaves, thus reducing TMV lesion number and diameter more potently than known SAR inducers such as SA, ASA, and BzSA. Likewise, pre-treatment of these derivatives reduced leaf necrosis and curling in case of *Arabidopsis NahG* plants.

2. Results and discussion

2.1. Chemical synthesis of BzSA derivatives

In the previous study, we have purified BzSA from the seed coats of *Givotia rottleriformis* and reported as an efficient SAR inducer as compared to SA and ASA (Kamatham et al., 2016). In the present study, we have synthesized BzSA and its derivatives (**1**–**14**) with different chemical modifications (Fig. 1a and b) according to previously described method (Cheong et al., 2008). The chemical synthesis of BzSA derivatives was performed in two steps, in the first step benzoic acid with different moieties were converted into corresponding benzoyl chlorides. The commercially available various substituted precursors such as (3, 4-dimethoxy), (4-methoxy), (3, 4, 5 − tri methoxy), 4-nicotinyloxy, (4-fluoro), (6-chloronicotinolyoxy), (4-chloro), (4-bromo), (4-ido), (3-chloro), (4-trifluoro), (4-nitro), (3-bromo), (thiophenyl-2 carbonyloxy) benzoyl chlorides were purchased from Avera chemicals, India. In the second step, the acid chloride was conjugated to SA and the resultant BzSA derivatives were named according to their moieties as 2-(4-methoxy) BzSA [**1**], 2-(4-iodo) BzSA [**2**], 2- (3-chloro) BzSA [**3**], 2-(3, 4-dimethoxy) BzSA [**4**], 2- (3, 4, 5-trimethoxy) BzSA [**5**], 2-(6-chloro nicotinoyloxy) BzSA [**6**], 2-(4-nitro) BzSA [**7**], 2-(4-chloro) BzSA [**8**], 2-(4-fluoro) BzSA [**9**], 2-(4-bromo) BzSA [**10**], 2-(4-nicotinoyloxy) BzSA [**11**], 2-(thiophenyl-2-carbonyloxy) BzSA [**12**], 2-(4-trifluoromethyl) BzSA [**13**] and 2-(3-bromo) BzSA [**14**]. The unreacted substrates were removed by open silica column chromatography and the purity of BzSA derivatives (**1**–**14**) was verified using thin layer chromatography (TLC). The chemical structural analysis of purified BzSA derivatives (**1**–**14**) was performed using IR, ^{1}H and ^{13}C NMR analysis and the molecular mass of each derivative was determined by HRMS (Supplementary Figs. S1–S30). Recently, we have reported that purified BzSA from the seed coats of *Givotia rottleriformis* induced SAR in tobacco and *Arabidopsis* (Kamatham et al., 2016). None of the BzSA derivatives (**1**–**14**) are reported as SAR inducers in plants so far.

2.2. BzSA derivatives induced SAR genes expression in tobacco plants

The establishment of SAR is mainly associated with the accumulation of a large number of defense genes such as *NPR1*, *PR*, *HR* and *MAPK* genes upon pathogen infection or by exogenous application of chemical elicitors (Chaturvedi et al., 2012; Dempsey and Klessig, 2012; Kinkema et al., 2000; Mou et al., 2003; Spoel et al., 2009).

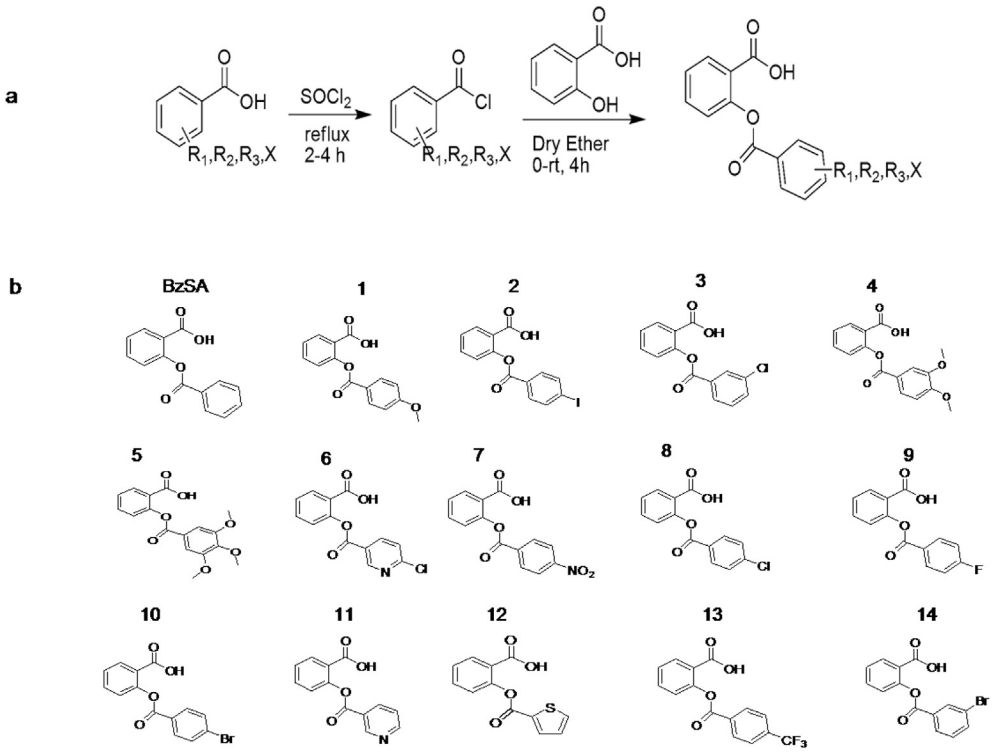


Fig. 1. Chemical synthesis of benzoylsalicylic acid derivatives. (a) Schematic representation of chemical synthesis of benzoylsalicylic acid (BzSA) derivatives. (b) Chemical structures of BzSA derivatives (**1**–**14**).

2.2.1. *NPR1 gene*

In order to determine the SAR activity by BzSA derivatives, we first studied the expression pattern of *NPR1* gene, which is a key player of SAR. The turnover of nuclear NPR1 protein plays an important role in modulating transcription of its target genes (Kinkema et al., 2000; Mou et al., 2003; Spoel et al., 2009). Foliar pre-treatment of **1**–**14** BzSA derivatives (5.0 μM) on local leaves of tobacco plants triggered the expression of *NPR1* gene higher than BzSA, SA and ASA in systemic leaves except derivative 6 (Fig. 2a). Interestingly, derivatives **9**, **11** and **13** induced a pronounced increase in *NPR1* gene expression (65 folds higher than BzSA) in systemic leaves (Fig. 2a) than the remaining BzSA derivatives. Previous studies have demonstrated that SA analog INA exhibits a long-lasting defense response by continuous expression of *NPR1* gene, while 3, 5-dichloroanthranilic acid (DCA) partially influenced the expression of *NPR1* gene during SAR development (Knoth et al., 2009; Vernooij et al., 1995). Probenazole (PBZ; 3-allyloxy-1,2-benzisothiazole-1,1-dioxide) and its active metabolite, BIT, stimulated SA/NPR1-mediated defense signaling upstream of SA pathway (Yoshioka et al., 2001).

2.2.2. *PR genes*

We have also assessed the expression of *PR* genes in systemic leaves upon exposure of local tobacco leaves with BzSA derivatives in a dose-dependent manner. Exogenous pre-treatment of local tobacco leaves with BzSA derivatives **1**–**14** (5.0 μM) induced the expression of *PR1a* (acidic) transcripts in systemic leaves of tobacco plants however, no significant difference was noticed as compared to SA, ASA and BzSA pre-treatments (Fig. 2b). Except **6**, **10** and **12** derivatives, remaining all derivatives induced the expression of *PR1b* (basic) transcripts at higher levels than SA, ASA and BzSA pre-treatments. Previous studies have suggested that the elevated expression of *PR1a* and *PR1b* genes was associated with the development of disease resistance in plants (Cutt et al., 1988, 1989; Wang et al., 2016).

The expression of *PR1*, *PR2*, *PR3* and *PR5* genes was also examined in BzSA pre-treated systemic leaves using RT-PCR (Fig. 3a and b). Previous reports have suggested that the expression of these genes was required for the establishment of SAR in plants during pathogen infection (Jia et al., 2016; Oide et al., 2013; Ryals et al., 1996). Foliar pre-treatment of local leaves with BzSA derivatives resulted in induction of *PR1* gene although the expression levels remained lower than that of BzSA and ASA in systemic leaves of tobacco plants (Fig. 3a and b). Nevertheless, **1**, **2**, **11** and **14** BzSA derivatives showed significant *PR1* gene expression as compared to SA pre-treatment (Fig. 3a and b). It is interesting to note that none of the BzSA derivatives induced *PR2* expression, however, only derivative number **1** induced *PR3* gene expression greater than SA and BzSA pre-treatments (Fig. 3a and b). Pre-treatment of local tobacco leaves with low dose (5.0 μM) of BzSA derivatives enhanced *PR5* gene expression in systemic leaves of tobacco plants of which **1** and **9** derivatives induced significant *PR5* gene expression (Fig. 3a and b). Previously, it was reported that SA and its chlorinated derivatives such as 4CSA, 5CSA, 3,5-dichloro-SA, INA and three of its derivatives induced PR proteins, and thus enhanced disease resistance to TMV infection (Conrath et al., 1995; Vernooij et al., 1995). Recently, 4-phenyl-2-{[3-(tri-fluoromethyl) anilino] methylidene}cyclohexane-1,3-dione (PAMD) and its derivatives were reported to inhibit the signaling of SA and suppressed the expression of *PR* genes (Jiang et al., 2015; Seo et al., 2012). Probenazole and its active metabolite BIT also induced SAR by elevating the expression of PR proteins (Yoshioka et al., 2001). Although several derivatives of SA and INA have been shown to induce SAR in plants (Conrath et al., 1995), our newly synthesized BzSA derivatives induced SAR efficiently even at low concentrations (5.0 μM) triggering of *PR* genes. These results suggest that BzSA derivatives are much more potent SAR inducers than SA, ASA and BzSA.

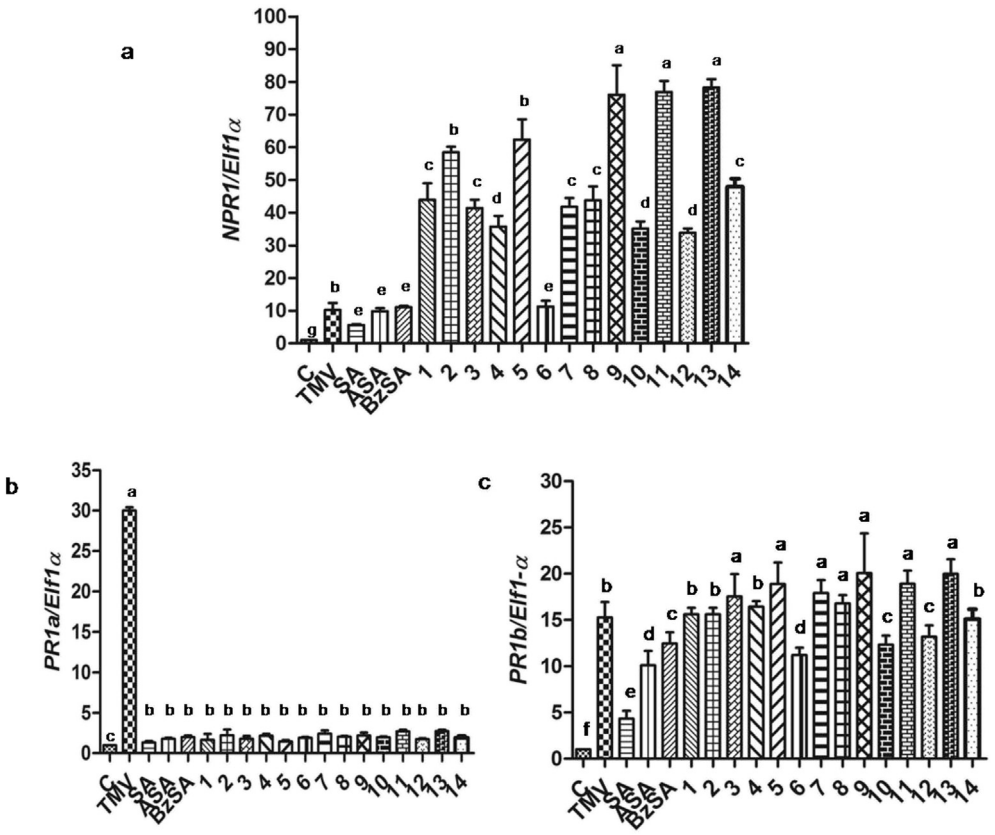


Fig. 2. RT-q-PCR analysis of SAR genes in systemic leaves after 24 h in pre-treated local leaves of tobacco plants with **1**–**14** benzoylsalicylic acid (BzSA) derivatives, benzoylsalicylic acid (BzSA), salicylic acid (SA) and acetylsalicylic acid (ASA). (a) Accumulation of *NPR1* transcript levels after 24 h pre-treatment of **1**–**14** BzSA derivatives (b) Accumulation of *PR1a* transcript levels after 24 h pre-treatment of **1**–**14** BzSA derivatives **1**–**14** (c) Accumulation of *PR1b* transcript levels after 24 h pre-treatment of **1**–**14** BzSA derivatives. Tobacco plants that were sprayed with 0.1% dimethylsulphoxide (DMSO) served as controls (C). The data were expressed as means ± SD of 3 independent experiments. The bars with the same letter are not significantly different ($p < 0.05$) by Newman-Keul's multiple comparisons test. The expression of the genes was normalized with constitutively expressed *Elf1- α* gene.

2.2.3. HR and MAPK genes

The *HIN1* gene is one of the *HR* markers; however, its expression is not induced by SA (Gorlach et al., 1996; Pontier et al., 1999). Exogenous pre-treatment of local leaves by foliar spray with **1**, **9**, **11** and **14** BzSA derivatives enhanced the expression of *HIN1* gene more than SA and BzSA in systemic leaves of tobacco plants (Fig. 4a and b). As stated in previous studies, *HIN1* gene is insensitive to SA pre-treatment (Gopalan et al., 1996). *HIN1* is known to be highly expressed during incompatible plant-pathogen interactions, and *NbHIN1* transcripts were significantly increased in *NbAOX1a* gene-silenced plants when infected with TMV (Takahashi et al., 2004; Zhu et al., 2015). The *HSR203*, also a *HR* marker gene, encodes a serine hydrolase enzyme displays esterase activity, controls cell death and detoxifies reactive oxygen species (ROS) levels (Tronchet et al., 2001). Among all the BzSA derivatives, only **1**, **2**, **5**, **9**, **11**, **13** and **14** induced higher *HSR203* expression whereas it remained similar or lower for other derivatives as compared to BzSA pre-treatment in systemic leaves of tobacco plants (Fig. 4a and b).

Mitogen-activated protein kinases (MAPK) that are associated with plant signaling cascades are involved in the multiple defense responses (Meng and Zhang, 2013). In plants, two MAPKs, *SIPK* and *WIPK* are activated in a disease resistance specific manner and thus helps in the transcriptional activation of defence-related genes and accumulation of defensive metabolites (Hettenhausen et al., 2015). Exogenous pre-treatment of BzSA derivatives by foliar spray in local leaves of tobacco for 24 h induced *SIPK* gene expression in systemic leaves of tobacco plants and the overall *SIPK* gene expression was found to be higher than SA, ASA and BzSA pre-treatments (Fig. 4a and b). Similarly, the expression of *WIPK* gene induced by BzSA derivatives was higher than SA, ASA and BzSA (Fig. 4a and b). Previously, it has been reported that the expression of *SIPK*/*WIPK* was required for the induction of *PR* and other defense genes (Kim and Zhang, 2004). However, SA activated only *SIPK* but not *WIPK* gene expression (Kumar and Klessig, 2000). It was also demonstrated that in the absence of pathogen, the activation of both *SIPK* and *WIPK*, or *SIPK* alone is sufficient to induce *HR*-like cell death; however the activation of both are required for pathogen-induced cell death (Zhang and Klessig, 2001). Interestingly, the expression of both *SIPK* and *WIPK* genes was induced by BzSA derivatives pre-treated tobacco plants as compared to SA, ASA and BzSA pre-treatments indicating that these derivatives are potential SAR inducers (Fig. 4a and b). Increased expression of *WIPK* gene in BzSA pre-treated tobacco systemic leaves demonstrated that these derivatives are potent inducers of SAR in tobacco plants (Fig. 4a and b).

2.3. BzSA derivatives reduced TMV infection in tobacco plants

In order to determine the potential of the BzSA derivatives in induction of disease resistance, tobacco plants (VT1158 resistant variety) were pre-treated with BzSA derivatives (**1**–**14**) and subsequently inoculated with TMV. According to previous studies, the SAR is characterized by the development of disease resistance to TMV in tobacco plants based on reduction in lesion size and number (Enyedi et al., 1992; Ross, 1961). The exogenous pre-treatment of BzSA derivatives in local leaves of tobacco plants reduced lesion number and diameter in local and systemic leaves of

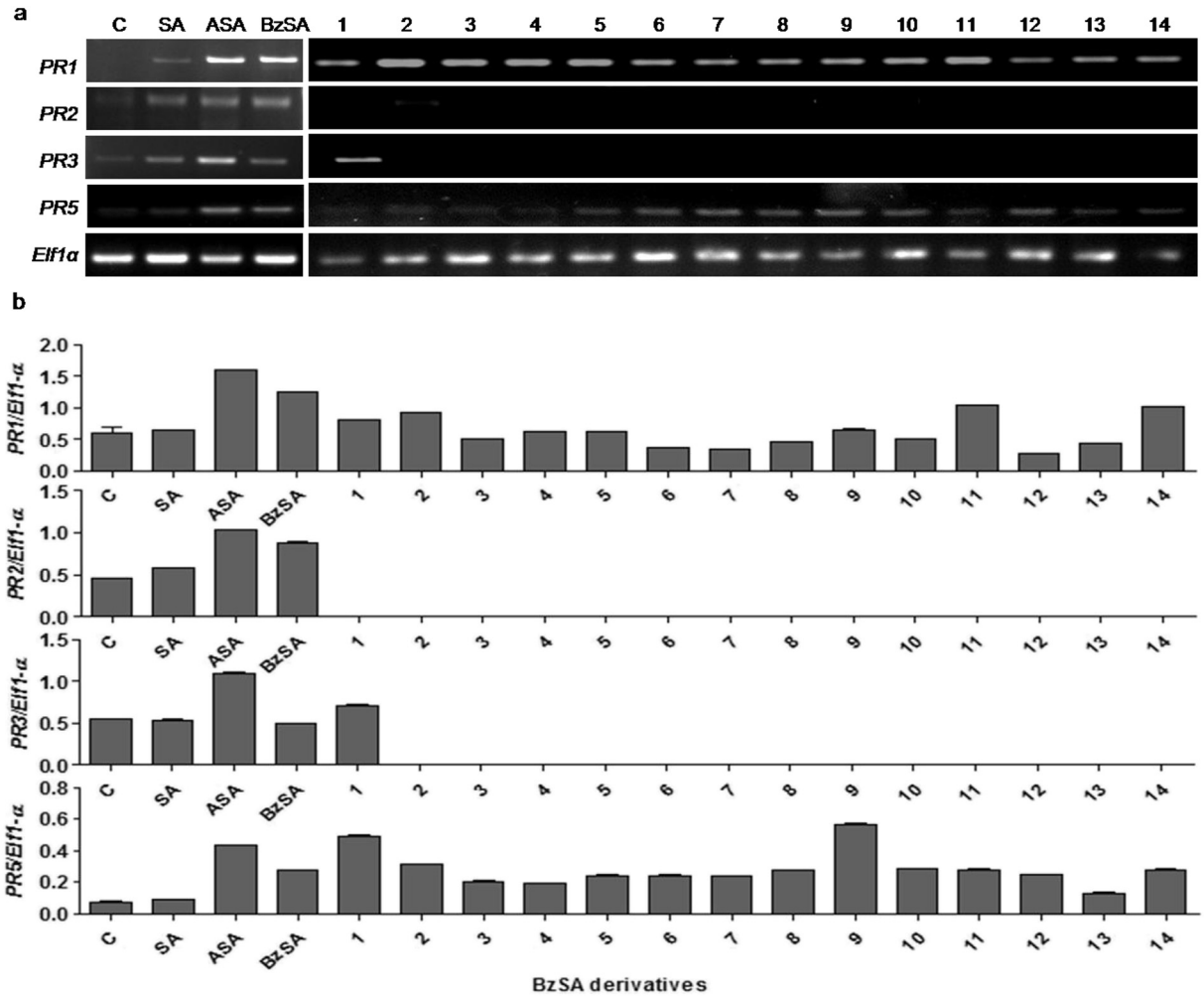


Fig. 3. RT-PCR analysis showing expression of *PR1, PR2, PR3* and *PR5* genes in **1**–**14** BzSA derivatives, salicylic acid (SA), acetylsalicylic acid (ASA) and benzoylsalicylic acid (BzSA) pre-treated tobacco plants. (a) Expression of *PR1*, *PR2*, *PR3* and *PR5* genes in systemic leaves after pre-treatment of local leaves of tobacco plants with **1**–**14** BzSA derivatives. (b) Densitometric analysis of *PR1, PR2*, *PR3* and *PR5* genes expression levels after normalizing with the internal control *Elf1-α*. Tobacco plants that were sprayed with 0.1% dimethylsulphoxide (DMSO) served as controls (C). The expression of the genes was normalized with constitutively expressed *Elf1- α* gene. The data presented are the means ± SD values of three independent experiments.

tobacco plants in a dose-dependent manner (Fig. 5a and b; Table 1). Pre-treatment of local tobacco leaves with BzSA derivatives reduced lesion number and diameter even at lower concentrations (5.0 μM) except derivative **6** in local and systemic tobacco leaves (Fig. 5a and b). A significant reduction in lesion number and diameter was noticed in local and systemic leaves of tobacco plants that were pre-treated with 5.0 μM of **3**, **4** and **5** BzSA derivatives as compared to SA, ASA and BzSA pre-treatments (Fig. 5a and b). An increased dose of BzSA derivatives (500 μM) further reduced TMV lesion number and diameter in both local and systemic tobacco leaves (Fig. 5c and d; Table 1). Notably, pre-treatment with 500 μM of **9**, **10**, **11** and **13** BzSA derivatives abolished TMV lesions in local and systemic leaves of tobacco plants (Fig. 5c and d). Previous studies have demonstrated that the exogenous application of chemical SAR inducers enhanced disease resistance to a broad spectrum of pathogens (Dempsey and Klessig, 2012; Gao et al., 2015). It was also reported that application of SA, INA and BTH derivatives reduced TMV lesions and thus conferred disease resistance in plants (Du et al., 2011). It is noteworthy that BzSA derivatives offered protection at low concentrations (5.0 μM), therefore, we suggest that these BzSA derivatives are potential SAR inducers in tobacco plants.

2.4. BzSA derivatives induced SAR genes in Arabidopsis NahG plants

In order to determine that BzSA derivatives induced SAR was independent of SA, we used SA abolished Arabidopsis *NahG* transgenic plants harboring a bacterial gene that encodes salicylate hydroxylase which converts SA into catechol (Gaffney et al., 1993). Interestingly, exogenous pre-treatment of local leaves with **2**, **3, 5**, **6**, **7**, **9** and **10** BzSA derivatives induced *AtPR1* and *AtPR5* gene expression in systemic leaves of Arabidopsis *NahG* plants (Fig. 6a). However, a pronounced increase in *AtPR5* gene expression was observed in **2**, **5**, **9** and **10** BzSA derivatives pre-treated systemic leaves of Arabidopsis *NahG* plants (Fig. 6a). Previously, it was suggested that treatment with AHO could not induce TMV resistance and *PR1* gene expression in Arabidopsis *NahG* plants, suggesting that AHO acts upstream of SA in SAR signaling pathway (Li et al., 2008; Yoshioka et al., 2001). Our results demonstrate that BzSA derivatives induced SAR genes in SA deficient *NahG* plants suggesting that BzSA derivatives induced SAR is independent of SA.

2.5. BzSA derivatives induced resistance in NahG Arabidopsis plants against TMV

In order to further validate that BzSA derivatives induce defense

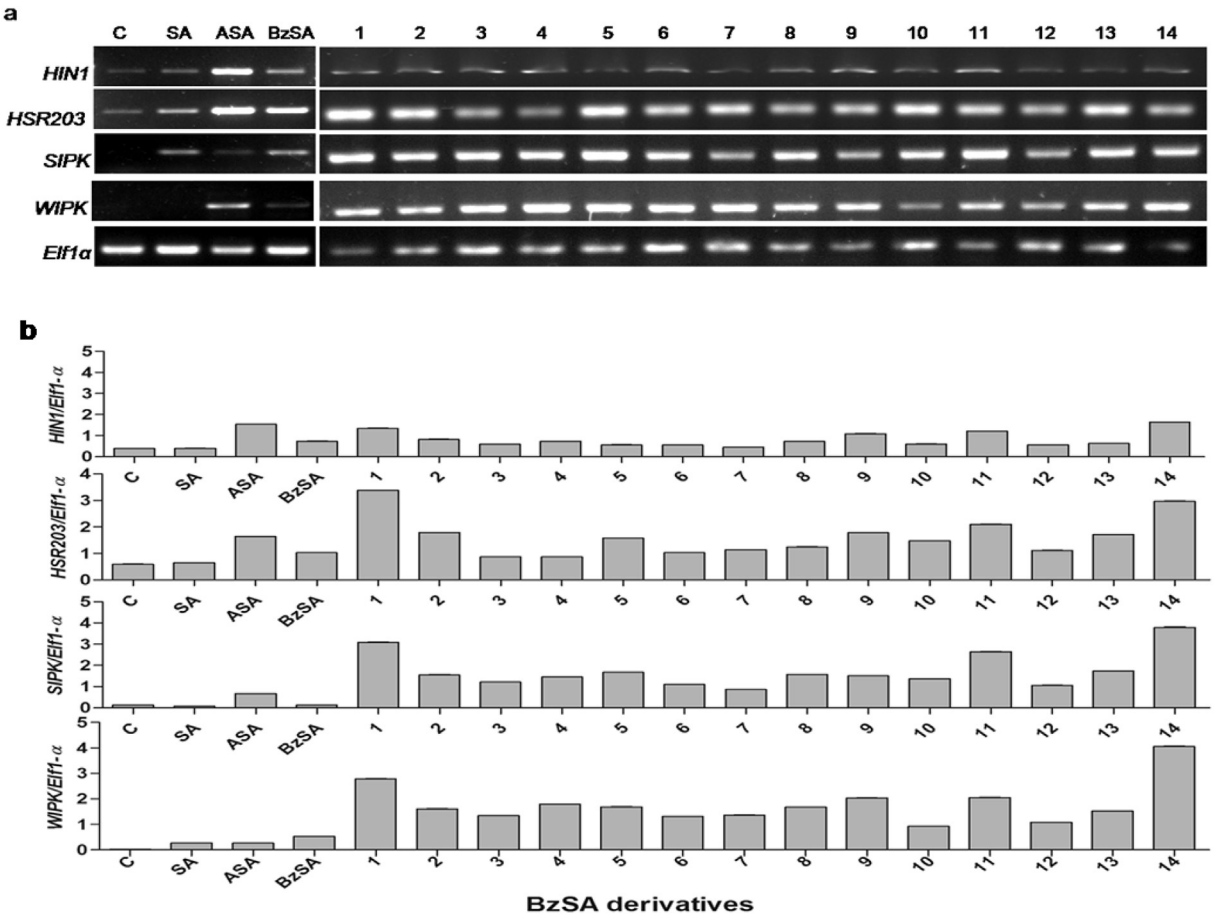


Fig. 4. RT-PCR analysis showing the expression of defense marker genes such as *HR* (*HIN1* and *HSR203*) and *MAPK* (*SIPK* and *WIPK*) in systemic leaves after 24 h pre-treatment of local tobacco leaves with **1**–**14** BzSA derivatives, salicylic acid (SA), acetylsalicylic acid (ASA) and benzoylsalicylic acid (BzSA). (a) Expression of *HIN1*, *HSR203*, *SIPK* and *WIPK* genes in systemic leaves after pre-treatment with **1**–**14** BzSA derivatives on local leaves of tobacco plants. (b) Densitometric analysis of *HIN1*, *HSR203*, *SIPK* and *WIPK* expression levels after normalizing with the *Elf1-α* used as internal control. Tobacco plants that were sprayed with 0.1% dimethylsulphoxide (DMSO) served as controls (C). The expression of the genes was normalized with constitutively expressed *Elf1- α* gene. The data presented are means ± SD of three independent experiments.

response independent of SA, we examined the defense response against TMV in BzSA pre-treated *Wt-Col* and *NahG* plants (Golem and Culver, 2003; Jia et al., 2016). The requirement of SA was demonstrated in transgenic tobacco plants that expressed a bacterial gene salicylate hydroxylase, which converts SA to catechol and accumulated little or no SA and were defective in their ability to induce SAR against TMV(Gaffney et al., 1993). For example, if BzSA derivatives undergo deacylation the resultant SA is converted to inactive catechol, and therefore, these *NahG* plants are unable to develop disease resistance and would exhibit susceptibility to TMV infection. Interestingly, exogenous pre-treatment of local leaves with BzSA derivatives (5.0 μM) enhanced disease resistance to TMV in *Wt-Col* and *NahG* Arabidopsis plants by reducing leaf necrosis and curling in systemic leaves (Fig. 6b and Supplementary Fig. S31a and b). Particularly, **2**, **5**, **9** and **10** BzSA derivatives showed higher response and reduced leaf necrosis as compared to **3**, **6** and **7** BzSA derivatives (Fig. 6b). These results showed the capability of BzSA derivatives in inducing disease resistance response independent of SA. The importance of SA signaling in dehydroabietinal (DA) induced resistance was demonstrated in Arabidopsis plants as DA was a poor inducer of SAR in SA synthesis/signaling-deficient plants in comparision with the wild-type (Chaturvedi et al., 2012). Recent studies by our group have showed that purified BzSA from the seed coats of *Givotia rottleriformis* reduced leaf necrosis in SA deficient *NahG Arabidopsis* in comparison with the WT plants (Kamatham et al., 2016). Literature survey also provided several lines of evidence of chemical SAR inducers that work either dependent and independent of endogenous SA synthesis/signaling (Dempsey and Klessig, 2012).

3. Conclusions

In the present study we have synthesized BzSA derivatives (**1**–**14**) and evaluated their potential in inducing the expression of SAR genes in tobacco plants. BzSA derivatives induced SAR genes and resistance to TMV in SA-deficient *NahG* Arabidopsis plants. Exogenous pre-treatment of BzSA derivatives by foliar spray in local leaves of tobacco and Arabidopsis *NahG* plants induced SAR gene expression in systemic leaves. Furthermore, pre-treatment of BzSA derivatives significantly decreased TMV lesion number and diameter in tobacco plants whereas leaf necrosis and curling symptoms were reduced in Arabidopsis plants. In conclusion, our studies have demonstrated that BzSA derivatives are highly potent SAR inducers as compared to known SAR inducers such as SA, ASA and BzSA.

4. Experimental procedure

4.1. Synthesis of BzSA derivatives

Chemical synthesis and structural characterization of BzSA

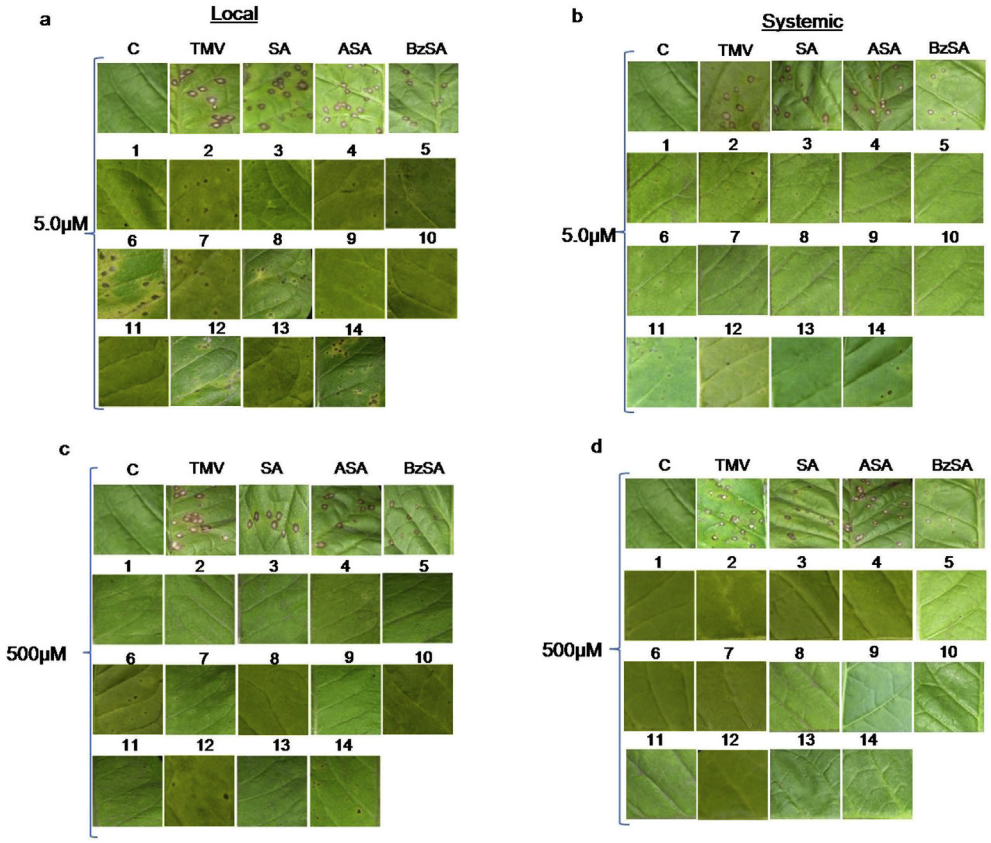


Fig. 5. Dose-dependent inhibition of TMV lesion number and diameter in **1**–**14** benzoylsalicylic acid derivatives pre-treated local and systemic tobacco leaves as compared to salicylic acid (SA), acetylsalicylic acid (ASA) and benzoylsalicylic acid (BzSA). (a) Reduced lesion number and diameter in 5.0 μM **1**–**14** BzSA derivatives pre-treated local leaves. (b) Reduced lesion number and diameter in 5.0 μM **1**–**14** BzSA derivatives pre-treated systemic leaves. (c) Increased reduction of lesion number and diameter in local leaves with increased dose of BzSA derivatives to 500 μM. (d) Reduction in lesion number and diameter in systemic leaves of tobacco plants. Tobacco plants that were sprayed with 0.1% dimethylsulphoxide (DMSO) and abraded with carborundum (CARB) served as controls (C). Tobacco plants inoculated with TMV served as infection controls (TMV).

derivatives have been described in detail in supplementary information.

4.2. Plant materials and growth conditions

Seeds of *Nicotiana tabacum* (VT-1158 *NN* gene type resistant to TMV) were procured from Central Tobacco Research Institute (CTRI), Rajahmundry, Andhra Pradesh. The tobacco seeds were germinated and allowed to grow for 6 weeks in greenhouse and used for experiments. *Arabidopsis thaliana* seeds of both wild-type (Col-1) and *NahG* seeds were soaked in sterile distilled water for 2 days and seeded in plastic cups containing sterile vermiculite. The plastic cups were kept at 4 °C in the dark for 2 days and then transferred to a growth chamber and provided 8 h light by cool white fluorescent lamps (75 μE m^{-2} sec^{-1}) at 22 to 23 °C temperature.

4.3. Chemical induction and TMV inoculation

BzSA derivatives were dissolved in 0.1% dimethylsulfoxide (DMSO) and the lower leaves (local) of 6 week-old tobacco plants were pre-treated with increasing doses of BzSA derivatives (5.0 μM and 500 μM) by foliar spray along with known SAR inducers such as SA, ASA and BzSA. Plants sprayed with 0.1% DMSO and abarded with carborundum (CARB) served as controls. Treated and untreated plants were moved to 24 °C and maintained under standard conditions as described previously (Enyedi et al., 1992). After 24 h, the plants were inoculated with purified TMV (25 μg/ml) by gently rubbing the adaxial leaf surface with the help of wet carborundum. The lesion number and diameter was measured after 72 h of TMV inoculation as described previously (Enyedi et al., 1992). In case of Arabidopsis *NahG* plants (30 days old after transfer to the growth chamber), the symptoms of TMV infection such as leaf curling and necrosis were observed after 7 days. Pre-treatment and TMV inoculation of plants were repeated three times with 3 plants for each treatment.

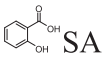
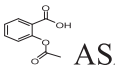
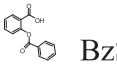
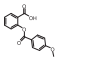
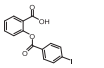
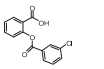
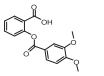
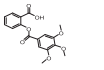
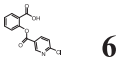
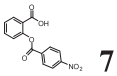
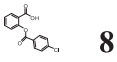
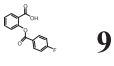
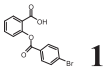
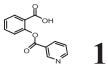
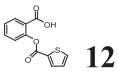
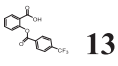
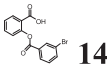
4.4. RNA isolation and cDNA synthesis

Lower leaves (local) of tobacco plants were pre-treated with individual BzSA derivatives and SA, ASA and BzSA. After 24 h, 100 mg of leaf tissues from each treatment were collected from systemic leaves of tobacco or Arabidopsis plants along with untreated controls and TMV infection controls in liquid N_2 and used for RNA isolation using RNA isolation kit (Qiagen, Germany). The purity and the concentration of RNA were checked using nanodrop method (Thermo Scientifics, USA). From each sample, 1 μg of total RNA was converted to first strand cDNA using cDNA synthesis kit (Takara) and the cDNA was diluted (1:20) using RNase-free water and stored at −20 °C for further use.

4.4.1. RT-qPCR

Real-time PCR was performed using the SYBR *Premix EXTaq*™ (Takara, USA) kit. Gene-specific primer sets were designed

Table 1
Reduction in tobacco mosaic virus (TMV) induced lesion number and diameter in systemic leaves of tobacco plants that were pretreated with benzoylsalicylic acid derivatives, BzSA, salicylic acid (SA) and acetylsalicylic acid (ASA). Treatments were given on local leaves of tobacco plants for 24 h and the lesion number and diameter were determined in systemic leaves of tobacco plants after 72 h of TMV inoculation. Tobacco leaves inoculated with TMV served as infection controls (TMV). Values are means ± SD of three experiments and each experiment consisted of 3 leaf replicates per treatment. The average lesion size in systemic leaves of each experiment with different pre-treatments was obtained from 3 individual plants (replicates). The experiments were repeated 3 times and the values are represented as means ± SD of 3 experiments.

Treatment	Average No. of lesions				Average diameter of lesions			
	5.0 μM	% Reduction	500 μM	% Reduction	5.0 μM	% Reduction	500 μM	% Reduction
TMV alone	70.0 ± 2.0	00.00	73.0 ± 2.0	00.00	2.9 ± 0.1	0.00	2.9 ± 0.1	00.00
 SA	57.0 ± 1.0	18.57	42.6 ± 2.5	41.64	2.4 ± 0.1	17.24	1.9 ± 0.1	34.48
 ASA	52.3 ± 2.5	25.28	38.0 ± 2.0	47.94	1.7 ± 0.2	41.37	1.6 ± 0.1	44.82
 BzSA	30.0 ± 1.0	57.14	22.3 ± 2.0	69.45	1.5 ± 0.1	48.27	1.0 ± 0.2	65.51
 1	25.0 ± 1.0	64.28	20.0 ± 1.0	72.60	0.1 ± 0.0	96.55	0.1 ± 0.0	96.55
 2	41.0 ± 3.6	41.42	9.7 ± 0.6	86.71	0.2 ± 0.1	93.10	0.1 ± 0.0	96.55
 3	1.7 ± 0.56	97.57	1.3 ± 0.6	98.21	0.1 ± 0.0	96.55	0.0 ± 0.0	100.00
 4	30.3 ± 2.5	56.71	6.3 ± 0.6	91.36	0.6 ± 0.1	79.31	0.3 ± 0.1	89.65
 5	22.3 ± 2.1	68.14	11.0 ± 1.0	84.93	0.1 ± 0.0	96.55	0.1 ± 0.0	96.55
 6	13.0 ± 2.0	81.42	11.7 ± 1.5	83.97	1.1 ± 0.1	62.06	0.1 ± 0.0	96.55
 7	50.0 ± 2.0	28.57	10.0 ± 1.7	86.30	0.1 ± 0.1	96.55	0.1 ± 0.0	96.55
 8	24.7 ± 1.5	64.71	15.0 ± 1.0	79.45	0.6 ± 0.1	79.31	0.3 ± 0.1	89.65
 9	22.7 ± 1.5	67.57	4.7 ± 0.6	93.56	0.1 ± 0.0	96.55	0.1 ± 0.0	96.55
 10	42.0 ± 3.0	40.00	11.7 ± 1.5	83.97	0.1 ± 0.0	99.66	0.1 ± 0.0	96.55
 11	33.0 ± 3.0	52.85	3.7 ± 0.6	94.93	0.1 ± 0.0	96.55	0.1 ± 0.0	96.55
 12	44.7 ± 2.2	36.14	6.3 ± 1.5	91.36	0.9 ± 0.1	68.96	0.6 ± 0.1	79.31
 13	24.0 ± 1.0	65.71	1.6 ± 1.5	97.83	0.1 ± 0.0	96.55	0.0 ± 0.0	100.00
 14	50.3 ± 4.5	28.14	22.3 ± 2.0	69.45	0.6 ± 0.1	79.31	0.1 ± 0.0	96.55

using Primer 3 software. The total cDNA was diluted (1:40) and then 2 μl of each sample was used for quantification of target genes using gene-specific primers (Supplementary Table S1) with the following conditions, 95 °C for 10 min, 95° for 15 s, 50° for 20 s and 60 °C for 1 min for 40 cycles in a light cycler 7500 RT-q-PCR (ABI applied systems) with a final melting curve at 72 °C for 20 min.

4.4.2. RT-PCR

RT-PCR reaction was set using 1X Red Dye PCR master mix (Merck) and the target genes were amplified using gene specific primers (Supplementary Table S2). The PCR (Eppendorf) reaction conditions used were as follows: 95 °C for 5 min, 95 °C for 15 s, 55 °C for 1 min, 72 °C for 1 min for 29 cycles followed by final

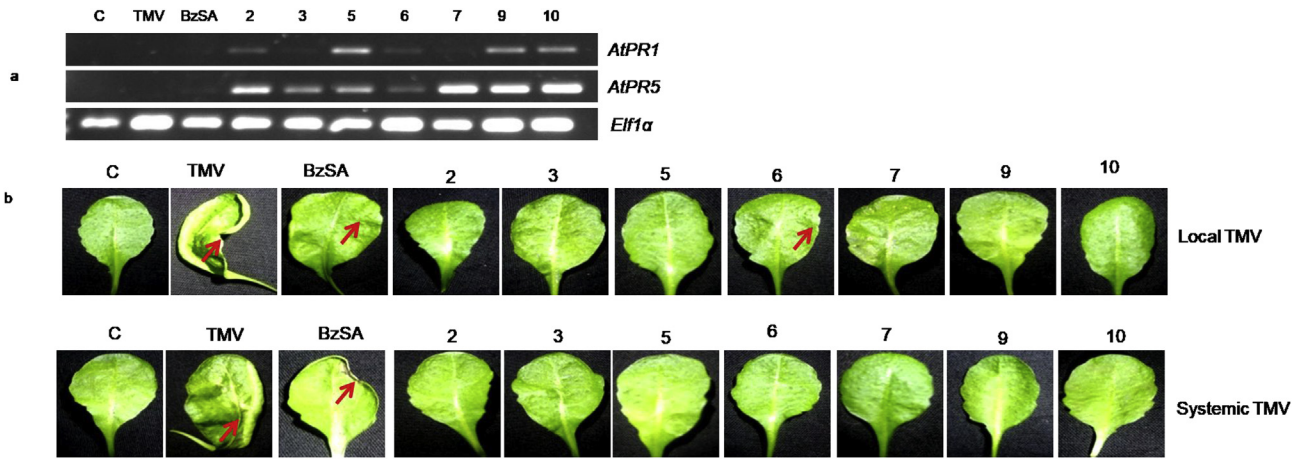


Fig. 6. Benzoylsalicylic acid derivatives (**2**, **3**, **5**, **6**, **7**, **9**, **10**) induced SAR gene expression and protection against TMV in salicylic acid (SA) deficient transgenic *NahG* Arabidopsis plants. (a) RT-PCR analysis showing the expression of *AtPR1* and *AtPR5* genes in systemic leaves of Arabidopsis *NahG* plants. (b) Reduction in leaf curling in TMV-inoculated local and systemic leaves of Arabidopsis *NahG* plants. *NahG* transgenic Arabidopsis plants that were pre-treated with 0.1% dimethylsulphoxide (DMSO) and abraded with carborundum (CARB) served as controls (C). Arabidopsis *NahG* plants inoculated with TMV served as infection controls (TMV). Constitutively expressed *actin-8* gene was used as an internal control.

extension for 10 min. The gene specific amplicons were resolved on 1.2% agarose gel (Sigma-Aldrich, USA), visualized under UV light and recorded using a gel documentation system (UVItec, UK). The relative quantification of band intensity was done using ImageJ software.

Acknowledgements

KS gratefully acknowledges the fellowship received from Young Scientist grant from SERB (SB/YS/LS-206/2013), UGC, CSIR, India. The research grants provided to GP by UGC, CSIR, UPE-Phase-2 and DST-PURSE is gratefully acknowledged. We are grateful to Dr. Naresh Babu V Sepuri, Department of Biochemistry, University of Hyderabad, Telangana, India for scientific suggestions and providing lab facilities to carry out the present work. We thank Dr. Jyoti Shah, Department of Biological Sciences, University of North Texas, Texas, USA, and Dr. Nandi Ashish, Jawaharlal Nehru University, New Delhi, India for providing the *Arabidopsis NahG* seeds. We acknowledge the infrastructural facilities provided by UGC-SAP, DST-FIST and DBT-CREBB at School of Life Sciences, University of Hyderabad.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.phytochem.2017.07.014.

References

Boller, T., Felix, G., 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60, 379–406.
Chanda, B., Xia, Y., Mandal, M.K., Yu, K., Sekine, K.T., Gao, Q.M., Selote, D., Hu, Y., Stromberg, A., Navarre, D., Kachroo, A., Kachroo, P., 2011. Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. Nat. Genet. 43, 421–427.
Chaturvedi, R., Venables, B., Petros, R.A., Nalam, V., Li, M., Wang, X., Takemoto, L.J., Shah, J., 2012. An abietane diterpenoid is a potent activator of systemic acquired resistance. Plant J. 71, 161–172.
Cheong, Y.K., Duncanson, Philip, Griffiths, D.V., 2008. Cyclisation reactions of 2-substituted benzoylphosphonates with trialkyl phosphites via nucleophilic attack on a carbonyl-containing ortho substituent. Tetrahedron 64, 2329–2338.
Conrath, U., Chen, Z., Ricigliano, J.R., Klessig, D.F., 1995. Two inducers of plant defense responses, 2,6-dichloroisonicotinic acid and salicylic acid, inhibit catalase activity in tobacco. Proc. Natl. Acad. Sci. U. S. A. 92, 7143–7147.
Cutt, J.R., Dixon, D.C., Carr, J.P., Klessig, D.F., 1988. Isolation and nucleotide sequence of cDNA clones for the pathogenesis-related proteins PR1a, PR1b and PR1c of *Nicotiana tabacum* cv. Xanthi nc induced by TMV infection. Nucleic Acids Res. 16, 9861.
Cutt, J.R., Harpster, M.H., Dixon, D.C., Carr, J.P., Dunsmuir, P., Klessig, D.F., 1989. Disease response to tobacco mosaic virus in transgenic tobacco plants that constitutively express the pathogenesis-related *PR1b gene*. Virology 173, 89–97.
Dangl, J.L., Jones, J.D., 2001. Plant pathogens and integrated defence responses to infection. Nature 411, 826–833.
Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E., Ryals, J., 1994. A central role of salicylic acid in plant disease resistance. Science 266, 1247–1250.
Dempsey, D.A., Klessig, D.F., 2012. SOS - too many signals for systemic acquired resistance? Trends Plant Sci. 17, 538–545.
Dempsey, D.A., Vlot, A.C., Wildermuth, M.C., Klessig, D.F., 2011. Salicylic acid biosynthesis and metabolism. In: Arabidopsis Book, vol. 9, p. e0156.
Ding, P., Rekhter, D., Ding, Y., Feussner, K., Busta, L., Haroth, S., Xu, S., Li, X., Jetter, R., Feussner, I., Zhang, Y., 2016. Characterization of a pipecolic acid biosynthesis pathway required for systemic acquired resistance. Plant Cell 28, 2603–2615.
Du, Q., Zhu, W., Zhao, Z., Qian, X., Xu, Y., 2011. Novel benzo-1,2,3-thiadiazole-7-carboxylate derivatives as plant activators and the development of their agricultural applications. J. Agric. Food Chem. 60, 346–353.
Durrant, W.E., Dong, X., 2004. Systemic acquired resistance. Annu. Rev. Phytopathol. 42, 185–209.
Enyedi, A.J., Yalpani, N., Silverman, P., Raskin, I., 1992. Localization, conjugation, and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. Proc. Natl. Acad. Sci. U. S. A. 89, 2480–2484.
Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., Ryals, J., 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261, 754–756.
Gao, Q.M., Zhu, S., Kachroo, P., Kachroo, A., 2015. Signal regulators of systemic acquired resistance. Front. Plant Sci. 6, 228.
Golem, S., Culver, J.N., 2003. Tobacco mosaic virus induced alterations in the gene expression profile of *Arabidopsis thaliana*. Mol. Plant Microbe Interact. 16, 681–688.
Gopalan, S., Wei, W., He, S.Y., 1996. *hrp* gene-dependent induction of *hin*1: a plant gene activated rapidly by both harpins and the *avr Pto* gene-mediated signal. Plant J. 10, 591–600.
Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., Ryals, J., 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8, 629–643.
Hammond-Kosack, K.E., Jones, J.D., 1996. Resistance gene-dependent plant defense responses. Plant Cell 8, 1773–1791.
Hartmann, M., Kim, D., Bernsdorff, F., Ajami-Rashidi, Z., Scholten, N., Schreiber, S., Zeier, T., Schuck, S., Reichel-Deland, V., Zeier, J., 2017. Biochemical principles and functional aspects of pipecolic acid biosynthesis in plant immunity. Plant Physiol. 174, 124–153.
Hettenhausen, C., Schuman, M.C., Wu, J., 2015. MAPK signaling: a key element in plant defense response to insects. Insect Sci. 22, 157–164.
Jia, X., Meng, Q., Zeng, H., Wang, W., Yin, H., 2016. Chitosan oligosaccharide induces resistance to Tobacco mosaic virus in Arabidopsis via the salicylic acid-mediated signalling pathway. Sci. Rep. 6, 26144.
Jiang, K., Kurimoto, T., Seo, E.K., Miyazaki, S., Nakajima, M., Nakamura, H., Asami, T., 2015. Development of inhibitors of salicylic acid signaling. J. Agric. Food Chem. 63, 7124–7133.

Jones, J.D., Dangl, J.L., 2006. The plant immune system. Nature 444, 323–329.
Jung, H.W., Tschaplinski, T.J., Wang, L., Glazebrook, J., Greenberg, J.T., 2009. Priming in systemic plant immunity. Science 324, 89–91.
Kamatham, S., Neela, K.B., Pasupulati, A.K., Pallu, R., Singh, S.S., Gudipalli, P., 2016. Benzoylsalicylic acid isolated from seed coats of *Givotia rottleriformis* induces systemic acquired resistance in tobacco and Arabidopsis. Phytochemistry 126, 11–22.
Kim, C.Y., Zhang, S., 2004. Activation of a mitogen-activated protein kinase cascade induces WRKY family of transcription factors and defense genes in tobacco. Plant J. 38, 142–151.
Kinkema, M., Fan, W., Dong, X., 2000. Nuclear localization of NPR1 is required for activation of *PR* gene expression. Plant Cell 12, 2339–2350.
Knoth, C., Salus, M.S., Girke, T., Eulgem, T., 2009. The synthetic elicitor 3,5-dichloroanthranilic acid induces NPR1-dependent and NPR1-independent mechanisms of disease resistance in Arabidopsis. Plant Physiol. 150, 333–347.
Kumar, D., Klessig, D.F., 2000. Differential induction of tobacco MAP kinases by the defense signals nitric oxide, salicylic acid, ethylene, and jasmonic acid. Mol. Plant Microbe Interact. 13, 347–351.
Lawton, K.A., Friedrich, L., Hunt, M., Weymann, K., Delaney, T., Kessmann, H., Staub, T., Ryals, J., 1996. Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway. Plant J. 10, 71–82.
Lee, H.I., Leon, J., Raskin, I., 1995. Biosynthesis and metabolism of salicylic acid. Proc. Natl. Acad. Sci. U. S. A. 92, 4076–4079.
Li, Y., Zhang, Z., Jia, Y., Shen, Y., He, H., Fang, R., Chen, X., Hao, X., 2008. 3-Acetonyl-3-hydroxyoxindole: a new inducer of systemic acquired resistance in plants. Plant Biotechnol. J. 6, 301–308.
Meng, X., Zhang, S., 2013. MAPK cascades in plant disease resistance signaling. Annu. Rev. Phytopathol. 51, 245–266.
Metraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., Inverardi, B., 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science 250, 1004–1006.
Mou, Z., Fan, W., Dong, X., 2003. Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell 113, 935–944.
Nakashita, H., Yasuda, M., Nishioka, M., Hasegawa, S., Arai, Y., Uramoto, M., Yoshida, S., Yamaguchi, I., 2002. Chloroisonicotinamide derivative induces a broad range of disease resistance in rice and tobacco. Plant Cell Physiol. 43, 823–831.
Oide, S., Bejai, S., Staal, J., Guan, N., Kaliff, M., Dixelius, C., 2013. A novel role of PR2 in abscisic acid (ABA) mediated, pathogen-induced callose deposition in *Arabidopsis thaliana*. New Phytol. 200, 1187–1199.
Park, S.W., Kaimoyo, E., Kumar, D., Mosher, S., Klessig, D.F., 2007. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 318, 113–116.
Pontier, D., Gan, S., Amasino, R.M., Roby, D., Lam, E., 1999. Markers for hypersensitive response and senescence show distinct patterns of expression. Plant Mol. Biol. 39, 1243–1255.
Ribnicky, D.M., Shulaev, V.V., Raskin, I.I., 1998. Intermediates of salicylic acid biosynthesis in tobacco. Plant Physiol. 118, 565–572.
Ross, A.F., 1961. Systamic acquired resistance induced by localized virus infections in plants. Virology 14, 340–358.
Ryals, J., Uknes, S., Ward, E., 1994. Systemic acquired resistance. Plant Physiol. 104, 1109–1112.
Ryals, J., Lawton, K.A., Delaney, T.P., Friedrich, L., Kessmann, H., Neuenschwander, U., Uknes, S., Vernooij, B., Weymann, K., 1995. Signal transduction in systemic acquired resistance. Proc. Natl. Acad. Sci. U. S. A. 92, 4202–4205.
Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y., Hunt, M.D., 1996. Systemic acquired resistance. Plant Cell 8, 1809–1819.
Schwessinger, B., Zipfel, C., 2008. News from the frontline: recent insights into PAMP-triggered immunity in plants. Curr. Opin. Plant Biol. 11, 389–395.
Seo, E.K., Nakamura, H., Mori, M., Asami, T., 2012. Screening and characterization of a chemical regulator for plant disease resistance. Bioorg Med. Chem. Lett. 22, 1761–1765.
Shah, J., Chaturvedi, R., Chowdhury, Z., Venables, B., Petros, R.A., 2014. Signaling by small metabolites in systemic acquired resistance. Plant J. 79, 645–658.
Silverman, P., Seskar, M., Kanter, D., Schweizer, P., Metraux, J.P., Raskin, I., 1995. Salicylic acid in rice (Biosynthesis, conjugation, and possible role). Plant Physiol. 108, 633–639.
Spoel, S.H., Mou, Z., Tada, Y., Spivey, N.W., Genschik, P., Dong, X., 2009. Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. Cell 137, 860–872.
Takahashi, Y., Berberich, T., Yamashita, K., Uehara, Y., Miyazaki, A., Kusano, T., 2004. Identification of tobacco HIN1 and two closely related genes as spermine-responsive genes and their differential expression during the Tobacco mosaic virus -induced hypersensitive response and during leaf- and flower-senescence. Plant Mol. Biol. 54, 613–622.
Tronchet, M., Ranty, B., Marco, Y., Roby, D., 2001. HSR203 antisense suppression in tobacco accelerates development of hypersensitive cell death. Plant J. 27, 115–127.
Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E., Ryals, J., 1992. Acquired resistance in arabidopsis. Plant Cell 4, 645–656.
van Loon, L.C., Rep, M., Pieterse, C.M., 2006. Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 44, 135–162.
Vernooij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H., Ryals, J., 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. Plant Cell 6, 959–965.
Vernooij, B., Friedrich, L., Ahl Goy, P., Staub, T., Kessmann, H., Ryals, J., 1995. 2,6-dichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. Mol. Plant-Microbe Interact. 8, 228–234.
Wang, N., Liu, M., Guo, L., Yang, X., Qiu, D., 2016. A novel protein elicitor (PeBA1) from *Bacillus amyloliquefaciens* NC6 induces systemic resistance in tobacco. Int. J. Biol. Sci. 12, 757–767.
Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Metraux, J.P., Ryals, J.A., 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell 3, 1085–1094.
Wildermuth, M.C., Dewdney, J., Wu, G., Ausubel, F.M., 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414, 562–565.
Wittek, F., Hoffmann, T., Kanawati, B., Bichlmeier, M., Knappe, C., Wenig, M., Schmitt-Kopplin, P., Parker, J.E., Schwab, W., Vlot, A.C., 2014. Arabidopsis enhanced disease susceptibility 1 promotes systemic acquired resistance *via* azelaic acid and its precursor 9-oxo nonanoic acid. J. Exp. Bot. 65, 5919–5931.
Wu, Y., Zhang, D., Chu, J.Y., Boyle, P., Wang, Y., Brindle, I.D., De Luca, V., Despres, C., 2012. The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Rep. 1, 639–647.
Yasuda, M., Nakashita, H., Hasegawa, S., Nishioka, M., Arai, Y., Uramoto, M., Yamaguchi, I., Yoshida, S., 2003a. N-cyanomethyl-2-chloroisonicotinamide induces systemic acquired resistance in arabidopsis without salicylic acid accumulation. Biosci. Biotechnol. Biochem. 67, 322–328.
Yasuda, M., Nishioka, M., Nakashita, H., Yamaguchi, I., Yoshida, S., 2003b. Pyrazolecarboxylic acid derivative induces systemic acquired resistance in tobacco. Biosci. Biotechnol. Biochem. 67, 2614–2620.
Yoshioka, K., Nakashita, H., Klessig, D.F., Yamaguchi, I., 2001. Probenazole induces systemic acquired resistance in Arabidopsis with a novel type of action. Plant J. 25, 149–157.
Zhang, S., Klessig, D.F., 2001. MAPK cascades in plant defense signaling. Trends Plant Sci. 6, 520–527.
Zhang, K., Halitschke, R., Yin, C., Liu, C.J., Gan, S.S., 2013. Salicylic acid 3-hydroxylase regulates Arabidopsis leaf longevity by mediating salicylic acid catabolism. Proc. Natl. Acad. Sci. U. S. A. 110, 14807–14812.
Zhu, F., Deng, X.G., Xu, F., Jian, W., Peng, X.J., Zhu, T., Xi, D.H., Lin, H.H., 2015. Mitochondrial alternative oxidase is involved in both compatible and incompatible host-virus combinations in *Nicotiana benthamiana*. Plant Sci. 239, 26–35.
Zipfel, C., 2009. Early molecular events in PAMP-triggered immunity. Curr. Opin. Plant Biol. 12, 414–420.