Bulletin of Entomological Research

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Exogenous salicylic acid improves resistance of aphid-susceptible wheat to the grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae)

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Research Paper

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Cite this article: Feng J-L, Zhang J, Yang J, Zou L-P, Fang T-T, Xu H-L, Cai Q-N (2021). Exogenous salicylic acid improves resistance of aphid-susceptible wheat to the grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae). *Bulletin of Entomological Research* **111**, 544–552. https://doi.org/10.1017/S0007485321000237

Received: 6 November 2020
Revised: 7 February 2021
Accepted: 3 March 2021
First published online: 5 April 2021

Keywords:
Defence-related enzymes; phenolic acid; salicylic acid; *Sitobion avenae*; *Triticum aestivum*

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Abstract

Salicylic acid (SA), a phytohormone, has been considered to be a key regulator mediating plant defence against pathogens. It is still vague how SA activates plant defence against herbivores such as chewing and sucking pests. Here, we used an aphid-susceptible wheat variety to investigate *Sitobion avenae* response to SA-induced wheat plants, and the effects of exogenous SA on some defence enzymes and phenolics in the plant immune system. In SA-treated wheat seedlings, intrinsic rate of natural increase (r_m), fecundity and apterous rate of *S. avenae* were 0.25, 31.4 nymphs/female and 64.4%, respectively, and significantly lower than that in the controls ($P < 0.05$). Moreover, the increased activities of phenylalanine-ammonia-lyase, polyphenol oxidase (PPO) and peroxidase in the SA-induced seedlings obviously depended on the sampling time, whereas activities of catalase and 4-coumarate:CoA ligase were suppressed significantly at 24, 48 and 72 h in comparison with the control. Dynamic levels of *p*-coumaric acid at 96 h, caffeic acid at 24 and 72 h and chlorogenic acid at 24, 48 and 96 h in wheat plants were significantly upregulated by exogenous SA application. Nevertheless, only caffeic acid content was positively correlated with PPO activity in SA-treated wheat seedlings ($P = 0.031$). These findings indicate that exogenous SA significantly enhanced the defence of aphid-susceptible wheat variety against aphids by regulating the plant immune system, and may prove a potential application of SA in aphid control.

Introduction

The grain aphid, *Sitobion avenae*, is a major *Triticeae* pest, causing serious losses in grain yields through direct damage by sucking plant sap and mediating the global transmission of plant viruses (Glinwood *et al.*, 2014). The enhancement of host resistance (defence) to aphids has been considered as a safe, effective and environment-friendly method of aphid management through which the plant injury is largely alleviated (Cai *et al.*, 2004; Razmjou *et al.*, 2011). Some pre-existing phenolic compounds in *Triticeae* plants largely contribute to resistance to aphids (Hu *et al.*, 2008). The synthesis of these defence compounds in plants is often regulated through changing the defence enzymes involved in the phenylpropanoid pathway by some plant hormones (Dong *et al.*, 2010; Alvarez, 2014), which suggest the application of that phytohormone *in vitro* may improve the resistance of plants to pests.

Phytohormones, such as jasmonic acid (JA) and salicylic acid (SA), play crucial roles in regulating plant growth and development processes (Pieterse *et al.*, 2012) and mediating plant defence system against herbivores and pathogens (Smith *et al.*, 2009; Pyati *et al.*, 2011). The roles of phytohormones in inducing plant defence in signal-transduction pathways have been well documented (Smith *et al.*, 2009; Pyati *et al.*, 2011; Zhang *et al.*, 2013). In general, JA-mediated signalling pathways are implicated in the regulation of antiherbivore defence via the phenylpropanoid pathway which involves the biosynthesis of lignin, flavonoids and other aromatic metabolites (Kim and Jander, 2007; Vogt 2010; Campos *et al.*, 2014). The phenylpropanoid pathway starts with phenylalanine which is transformed by phenylalanine-ammonia-lyase (PAL) into cinnamic acid and *p*-coumaroyl-CoA, the general precursor of phenolic acids and flavonoids (Valiñas *et al.*, 2017). Whereas SA pathway is associated with defence responses against pathogens (Smith *et al.*, 2009). Nevertheless, recent reports showed that JA was also engaged in plant defence against necrotrophic pathogens (Ballaré, 2011) and SA-mediated responses affected the phloem feeders (Urbanek Krajnc *et al.*, 2011). These phenomena probably result from mutual interferences among phytohormones (Engelberth *et al.*, 2011).

The phenylpropanoid pathway is a well-known JA-mediated metabolic pathway, in which some metabolic enzymes are involved in secondary metabolism such as PAL and 4-coumarate: CoA ligase (4CL), PAL induction by SA has not been reported, 4CL is also not reported to be

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induced by both JA and SA (Reymond and Farmer, 1998). Recent evidence indicated that SA could fine-tune plant defence responses by modulating JA signalling pathways (Smith *et al.*, 2009; Thaler *et al.*, 2012), and mediating plant-herbivore–pathogen interactions (Hatcher *et al.*, 2004). For example, SA induced an increase of PAL activity in *Vitis vinifera* (Wen *et al.*, 2005), and activated the typical insect-elicitor-induced JA in SA-treated corn seedlings as well (Engelberth *et al.*, 2011). Polyphenol oxidase (PPO) is regarded as a marker of JA response (Thaler and Bostock, 2004). Peroxidase (POD) and catalase (CAT) are also pivotal antioxidases in plants to function against pathogens and herbivores (Kim and Jander, 2007). The activities of these enzymes mediate the biosynthesis of some defence compounds in plants (Smith and Boyko, 2007).

With regard to phytohormone-mediated resistance to phloem feeders, although JA-inducible resistance was considered to deter some chewing herbivores before (Thaler *et al.*, 2012; Lazebnik *et al.*, 2014), the current opinion is that SA-inducible resistance is involved in protection against some phloem-feeding insects via eliciting JA signalling pathway (Alba *et al.*, 2015; Jonathan Gogbeu *et al.*, 2015). For example, SA was confirmed to be necessary for resistance to *Macrosiphum euphorbiae* in the tomato (Bhattarai *et al.*, 2007). By contrast, SA activation increased whitefly infestation in *Arabidopsis thaliana* through downstream suppression of JA defences (Zhang *et al.*, 2013). This evidence further suggests that SA-inducible defence against sap-sucking feeders depends on different plant species and may be controlled through crosstalk with the JA-mediated pathway.

To determine whether SA engages in the activation of defence system (resistance to aphids, phenolic acids and the defensive enzymes) for wheat aphids, we investigated the effects of exogenous SA application in aphid-susceptible wheat variety on important performances of *S. avenae*, the activities of some defence-related enzymes and contents of major phenolic acids. These findings provide crucial information for further understanding the effect of exogenous application of SA on the improvement of wheat resistance against *S. avenae*.

Materials and methods

Plant and aphid culture

Seeds of winter wheat, *Triticum aestivum* cv. Lovin 10, which is susceptible to *S. avenae* (Cai *et al.*, 2004), were germinated at room temperature, and sowed in plastic pots (20 cm diameter) with nutritional soil. The plastic pots with wheat seeds were kept in a climate chamber at 24 ± 1°C, 60%–70% relative humidity and 14 h illumination. The grain aphid, *S. avenae*, were collected from a winter wheat field at the Shangzhuang experiment station of China Agricultural University, and reared on Lovin 10 in a climate chamber over ten generations.

Salicylic acid treatment

The wheat seedlings were treated by a SA solution following a previously described method (Ananieva *et al.*, 2004) with some modifications. Briefly, SA (Sinopharm Chemical Reagent Co., Ltd., Beijing, China) was dissolved in distilled water to 1.0 mM with 1.0% Triton X-80 (V/V) following Radwan methods (Radwan, 2012), the control contained distilled water with 1.0% Triton X-80 (V/V). The wheat seedlings at the third- or fourth-leaf stage (three plastic pots, 15–20 seedlings per pot) were sprayed at 8:00 am with SA or the control solution using a 10 ml portable ultra-low-volume sprayer until runoff at daytime in mesh room. The experiment was carried out in triplicate. Each pot containing sprayed wheat seedlings was immediately covered with a sealed glass box ($35 \times 35 \times 60\ cm^3$) for 24 h in a mesh room. Subsequently, the sealed glass box was removed and the seedlings were maintained for growth in the mesh room. Another management procedure was as previously described. To assay defence-related enzyme activities and contents of phenolic acids, the wheat leaves of SA treatment and control without aphids were randomly harvested at 24, 48, 72 and 96 h post-SA treatment (p.s.t.), wrapped in aluminium foil, snap-frozen in liquid nitrogen and stored at −80°C until use in the assays.

Aphid performance assay

To evaluate the effect of SA application on the performance of wheat plants against aphid, referring to Radwan's methods (Radwan, 2012), wheat seedlings at the second- or third-leaf stage were treated using SA solutions of 1.0 mM with 1.0% Triton X-80 and distilled water with 1.0% Triton X-80 for 24 h as described above.

The life table of *S. avenae* was established on the wheat seedlings of SA treatment and control with the methods of Aziz *et al.* (2013). One newborn nymph was inoculated on the leaves treated with SA treatment or control plants, respectively, and covered with a clip leaf cage (1 nymph per cage) to prevent the nymphs from escaping. Thirty-replicate assays were performed for the treatment and the control. All leaves with aphids were moved to the growth chamber and reared at 18–25°C and 50–70% RH under 16 h illumination. Ecdysis, stadium and the numbers of aphids (survived, died and newborn) were recorded every day until adult aphids started reproduction. Some biological parameters were calculated using the following formulae:

$$\text{Net reproductive rate: } R_0 = \sum lx.mx \quad (1)$$

$$\text{Mean length of generation: } T = \sum x(lx.mx)/(\sum (lx.mx) \quad (2)$$

$$\text{Intrinsic rate of natural increase: } r_m = \ln R_0/T \quad (3)$$

Here, x: age of the insects in days; lx: survival rate at the beginning of each interval; mx: the number of newborn nymphs produced per female from time x–1 to time x (days).

Aphid growth and development

Wheat seedlings were treated following the methods described above. Twenty weighed newborn nymphs were transported to SA-treated and control wheat seedlings by using a soft brush and reared in a growth chamber. Aphid-inoculated seedlings were covered with nylon mesh to prevent the cross escape of aphids among pots of treatments and controls. Subsequently, these aphids were weighed again on a Poton electronic balance (Mettler-Toledo, Germany) at 1, 3, 5 and 7 days post-inoculation, and the mean aphid weight was calculated to evaluate the effect of SA treatment on aphid growth and development. The experiments were repeated three times.

https://doi.org/10.1017/S0007485321000237 Published online by Cambridge University Press

Assay of defence-related enzyme activity

Plant tissue (300 mg) from each sample was ground in liquid nitrogen and homogenized in 1.5 ml of potassium phosphate buffer (0.1 M; pH 7.0) containing 1% polyvinylpyrrolidone, 1.0 mM Na_2-ethylene diamine tetraacetic acid and 7.0% glycerin. The resulting mixture was centrifuged using a Himac CR22E centrifuge (Hitachi Koki Co., Ltd., Japan) at 10,000 × *g* for 30 min at 4°C. The supernatant was used for the analyses of total protein content and enzymatic activity.

The Bradford method (Bradford, 1976) was used to determine total protein contents in samples. Three aliquots (20 µl) of each sample were mixed with 180 µl of the Bio-Rad reagent (Bio-Rad, Richmond, CA, USA). The absorbance of the reaction mixture was measured at 595 nm, and the protein content was determined using a standard curve, plotted using known quantities of bovine serum albumin (Sigma Chemical Co., USA).

Phenylalanine-ammonia-lyase

PAL activity was assayed using the method of Kováčik and Klejdus (2012) with slight modifications. In a cold mortar and pestle, 0.3 g of fresh leaves was homogenized in 2 ml of sodium borate buffer (pH 8.8) with quartz sand, and the resulting homogenates were immediately centrifuged (10,000 × *g*, 30 min, 4°C). The reaction mixtures, consisting of 800 µl of sodium borate buffer and 200 µl of homogenates, were preincubated at 37°C (3 min), and the reaction was started by adding 400 µl of L-phenylalanine (Sigma-Aldrich, Germany). After incubation at 37°C for 1 h, the reaction was stopped by adding 6 M HCl. Controls were prepared using the buffer instead of enzyme extract. The absorbance of the mixtures was monitored through spectrophotometry at 290 nm (extinction coefficient 9630 M^{-1} cm^{-1}). Assays were conducted in three replicated samples. The activity of PAL was expressed as micromole per minute per milligram of protein.

4-Coumarate:CoA ligase

4CL activity was tested following a previously described method (Knobloch and Hahlbrock, 1977; Saballos *et al.*, 2012). The reaction mixture contained 5.0 mM *p*-coumaric acid, 50 mM ATP, 1.0 mM CoA-SH, 15 mM $MgSO_4.7H_2O$ and 0.4 ml of enzyme extract. The reaction was carried out at 40°C for 10 min and then stopped. Controls were prepared using the buffer instead of enzyme extract. Assays were conducted in three replicated samples. 4CL activity was estimated on the basis of the increase in absorbance at 333 nm (extinction coefficient 21,000 M^{-1} cm^{-1}). 4CL activity was expressed as micromole per minute per milligram of protein.

Polyphenol oxidase

PPO activity was determined following a previously described method (Han *et al.*, 2009). Enzyme extract (100 µl) was mixed with a solution containing catechol (500 µl, 1.6% w/w), HEPES buffer (100 µl, 200 mM, pH 6.0) and deionized water (800 µl). Controls were prepared using the buffer instead of enzyme extract. Assays were conducted in three replicated samples. PPO activity was estimated from the increase at 470 nm. PPO activity was expressed as U (activity unit) per minute per milligram protein, that is, one unit of PPO activity was defined as the amount that caused a change of 0.001 in absorbance per minute (Aksoy, 2020).

Guaiacol peroxidase

Guaiacol POD activity was measured following a previously described method (Han *et al.*, 2009). Enzyme extract (10 µl) was mixed with 1 ml of substrate containing hydrogen peroxide (10 µl, 30%, w/w), guaiacol (1 µl, 20 mM) and HEPES (100 µl, 200 mM, pH 7.0) in deionized water. Controls were prepared using the buffer instead of enzyme extract. Assays were conducted in three replicated samples. POD activity was estimated from the increase in absorbance at 470 nm (extinction coefficient 26.6 mM^{-1} cm^{-1}). POD activity was expressed as millimole per minute per milligram of protein.

CAT activity was determined using previously described methods (Horváth *et al.*, 2002) with slight modifications. An aliquot (0.4 ml) of enzyme extract was added to 2 ml of a mixture containing 15 mM H_2O_2 in 0.15 mM potassium phosphate buffer (pH 7.0). The enzyme activity was measured as the decrease in absorbance at 240 nm (extinction coefficient of 36 mM^{-1} cm^{-1}). Assays were conducted in three replicated samples. The activity was expressed as millimole per minute per milligram of protein.

Quantification of phenolic compounds

SA-treated wheat leaves were dried in a freeze dryer (Biocool Co., Beijing, China) for 12 h and powdered. Phenolic compounds were extracted using the methods of Ruelas *et al.* (2006). Dry powder (100 mg) was mixed with 3 ml of extracting solution (4% NaOH: absolute ethyl alcohol = 2:1, v/v), in brown tubes containing 2 g l^{-1} benzaldehyde in triplicate. The resulting mixture was homogeneously agitated, crushed in a JY92 ultrasonic cell crusher (Ningbo Scientz Biotechnology Co., Hangzhou, China) for 30 min, homogeneously mixed with 500 µl of HCl (6.0 M) and centrifuged at 6700 × *g* for 20 min. The supernatant was collected and dried using a rotary evaporator. The resulting phenolic compounds were redissolved in 1.0 ml of mixed solvent (methanol: sodium acetate buffer = 1:1, v/v; pH 5.4) and filtered using a 0.45 µm filter membrane. The final extract was stored in brown tubes for HPLC analysis. Treatment or control assay was conducted in three replicated samples.

The Agilent 1100 HPLC system was used throughout this study. It consisted of a binary pump (G1312A), an autosampler (G1313A), a vacuum degasser (G1322A), a thermostatted column compartment (G1316A) and a diode array detector (G1315B). A Phenomenex column (250 × 4.6 mm, 5.0 µm, Phenomenex, CA, USA) was used for analysis. The column temperature was maintained at 30°C. Chromatographic separations were performed by following the methods of Ruelas *et al.* (2006) and Zhang *et al.* (2010). Solvent A (methanol) and solvent B (0.05 M acetic acid, pH 5.4) under gradient conditions were used at a flow rate of 1.0 ml min^{-1}, and the injection volume was 20 µl. A 22 min gradient program was used in this study. The initial percentages of solvents A and B in the solvent mixture were 12 and 88%, respectively, for 8 min, and these percentages were held for 12 min; subsequently, the percentage of solvent A was ramped up to 17% for 21 min and then ramped down to 12% for 22 min. The percentage of solvent B was adjusted accordingly to achieve a total of 100%. The detection wavelength was 316 nm.

Phenolic compounds were quantified using a ten-point regression curve constructed on the basis of *p*-coumaric acid, caffeic acid, ferulic acid and chlorogenic acid (Sigma, USA). The contents are expressed as milligrams per gram of tissue dry weight (mg g^{-1} dw).

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Statistical analysis

The statistical analysis of all data was performed using SPSS software (version 11.5). Based on the aphid life table, aphid parameters such as r_m, fecundity, wingless aphid rate, female reproduction time, time to onset of reproduction and life time were calculated as described above. The aphid biological parameter, aphid body mass, enzyme activities and phenolic contents of wheat plants between treatment and control were compared using paired sample *t* tests with a 95% confidence interval of the difference. Correlation of enzyme activities with phenolic acids was conducted using bivariate correlation analysis, significant difference is at 0.05 level.

Results

Aphid performance

Compared with control seedlings, intrinsic rate of natural increase (r_m) and fecundity of the grain aphids as well as apterous rate were significantly lower in the SA-induced seedlings than that in the controls ($df = 29$, $t_{rm} = 2.408$, $P_{rm} = 0.023$; $t_{fecundity} = 3.664$, $P_{fecundity} = 0.001$; $t_{apterous\ rate} = 2.262$, $P_{apterous\ rate} = 0.031$; fig. 1a–c), whereas female reproduction time, time to onset of reproduction and lifetime of the aphids that fed on SA-treated seedlings were not significantly affected ($P > 0.05$) (fig. 1d). Aphid body mass was significantly reduced on day 7 post-feeding treated wheat seedlings with 1.0 mM SA ($t = 7.748$, $P = 0.016$), but on day 5 post-feeding, significant suppression of aphid body mass was not observed in SA-treated wheat seedlings ($t = 1.015$, $P = 0.417$) (fig. 2). Results indicate the SA-induced wheat seedlings can significantly inhibit aphid key indices that determine aphid growth and population development.

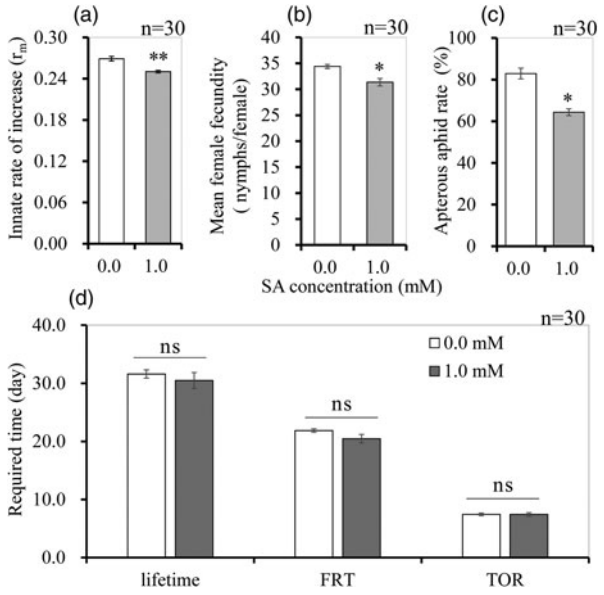

Figure 1. Effect of SA-treated wheat seedlings on key aphid performance. Data (mean ± SD) was displayed. *, ** and *** indicated significant difference at 0.05, 0.01 and 0.001 levels, respectively, ns is no significance $P > 0.05$ ($n = 30$). (a) innate rate of increase; (b) female fecundity; (c) apterous aphid rate; (d) lifetime, female reproduction time (FRT) and time to onset of female reproduction (TOR).

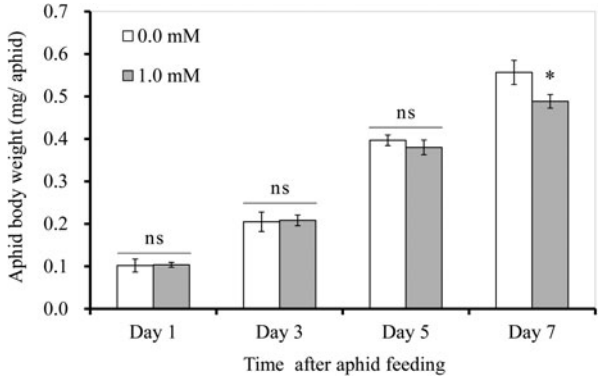

Figure 2. Effect of SA-treated wheat seedlings on aphid biomass. Data (mean ± SD) was displayed. Asterisk (*) indicated significant difference at 0.05 level, ns is no significance $P > 0.05$.

PAL and 4CL activities

In the wheat seedlings treated with SA, the PAL activity was significantly higher than that of controls at 48 and 72 h.p.t ($t_{48} = -8.100$, $P = 0.015$; $t_{72} = -11.414$, $P = 0.008$) (fig. 3a), whereas a significant suppression of 4CL activity was shown at all tested time points ($t_{24} = 17.427$, $P = 0.003$; $t_{48} = 14.571$, $P = 0.005$; $t_{72} = 13.994$, $P = 0.005$) (fig. 3b). This indicates that SA-induced PAL activity in wheat seedlings is associated with the time of duration, but the application of exogenous SA can suppress 4CL activity in wheat seedlings.

PPO, POD and CAT activities

Our results revealed that exogenous SA significantly increased PPO activity in wheat seedlings at both 48 and 72 h.p.t. ($t_{48} = -8.176$, $P = 0.015$; $t_{72} = -10.708$, $P = 0.009$), but inhibited the activity at 24 h.p.t. ($t_{24} = 38.864$, $P = 0.001$) (fig. 3c). POD activity in SA-induced wheat seedlings was significantly higher than that in control wheat seedlings only at 72 h.p.t. ($t_{72} = -7.567$, $P = 0.017$) (fig. 3d). Notably, CAT activity in SA-treated seedlings was significantly reduced at all tested time points post-treatment ($t_{24} = 6.065$, $P = 0.026$; $t_{48} = 14.850$, $P = 0.005$; $t_{72} = 20.415$, $P = 0.002$) (fig. 3e). The findings show that the inducing effect of SA on PPO and POD activities is enhanced with time p.s.t., CAT activity in wheat seedlings always is inhibited by exogenous SA at all sampling time.

Phenolic compound content

When wheat seedlings were treated with exogenous SA, four phenolic acid content dynamics in the seedlings can be regulated at sampling time points. Coumaric acid content was significantly reduced at 48 h, but increased at 96 h p.s.t. ($t_{48} = 6.289$, $P = 0.024$; $t_{96} = -5.362$, $P = 0.033$) (fig. 4a). Ferulic acid content in SA-treated wheat seedlings was significantly higher than that in control ones at 24 and 72 h and lower than that in control ones at 48 h ($t_{24} = -21.671$, $P = 0.002$; $t_{48} = 73.635$, $P < 0.001$; $t_{72} = -45.813$, $P < 0.001$) (fig. 4b). A significantly low caffeic acid content was displayed in SA-treated wheat seedlings at 24 and 48 h p.s.t. ($t_{24} = 6.953$, $P = 0.020$; $t_{48} = 24.371$, $P = 0.002$) (fig. 4c). Regarding chlorogenic acid content, exogenous SA triggered a significant increase in SA-treated wheat seedlings at 24, 48 and 96 h, but significantly inhibited chlorogenic acid content at 72 h ($t_{24} = -136.858$, $P < 0.001$; $t_{48} = -21.886$, $P = 0.002$; $t_{72} = 55.932$, $P < 0.001$; $t_{96} = -82.674$, $P < 0.001$) (fig. 4d). In terms of increase or inhibition, there is a fast response (24 h or/and 48 h) to exogenous SA in ferulic acid, caffeic acid and chlorogenic acid contents,

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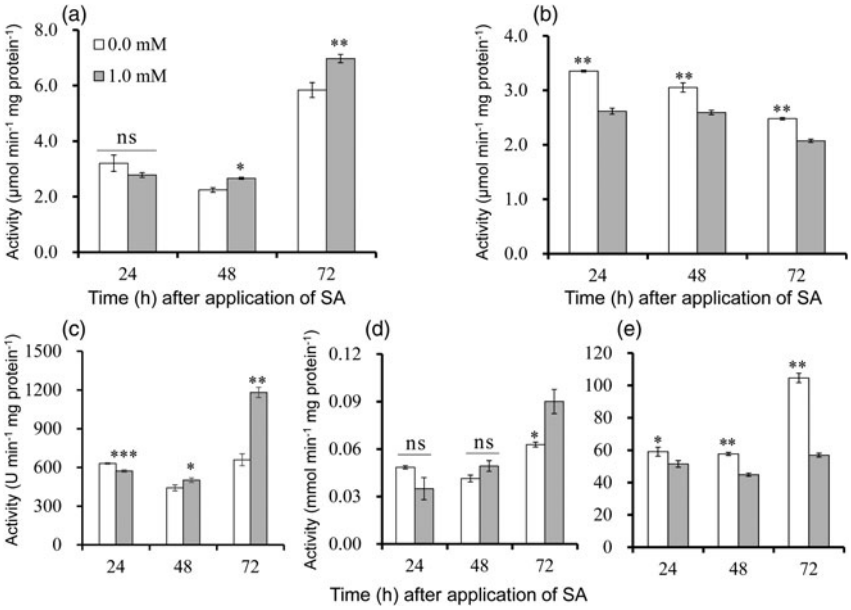

Figure 3. Effect of exogenous SA on the activity of defence-related enzymes in wheat seedlings. The enzyme activity (mean ± SD) was plotted. *, ** and *** indicated significant difference at 0.05, 0.01 and 0.001 levels, respectively, ns is no significance $P > 0.05$. (a) PAL (phenylalanine-ammonia-lyase); (b) 4CL (4-coumarate:coenzyme A ligase); (c) PPO (polyphenol oxidase); (d) POD (peroxidase); (e) CAT (catalase).

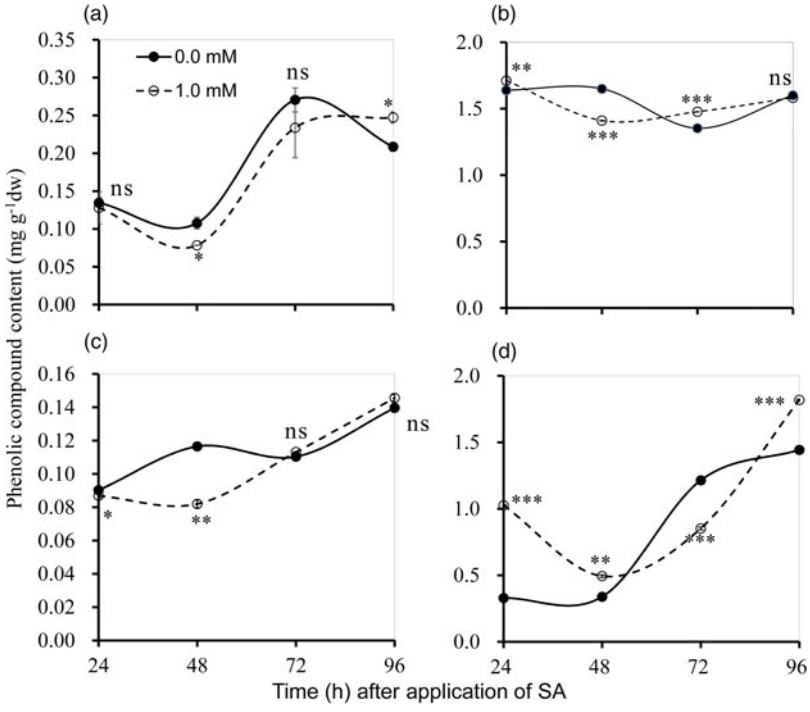

Figure 4. Content dynamics of coumaric acid (a), ferulic acid (b), caffeic acid (c) and chlorogenic acid (d) in SA-treated and control wheat seedlings. Second- or third-leaf wheat seedlings were treated with a SA spray for 24 h. Then, the leaves were harvested at 24, 48, 72 and 96 h.p.t. for determining the contents of phenolic compounds in leaf tissues through HPLC. Data are represented as the mean ± SD from three replicates. *, ** and *** indicated significant difference at 0.05, 0.01 and 0.001 levels, respectively, ns is no significance $P > 0.05$.

whereas in SA-treated wheat seedlings, the coumaric acid content presents a delayed response to exogenous SA (at 96 h).

Correlation of defence-related enzymes with phenolic compounds

To understand the association of defence-related enzyme activity with the synthesis of phenolic acid, in SA-treated wheat seedlings or control ones, the correlation between each enzyme activity and compound content was analysed with sampling time. In wheat seedlings of control, CAT activity was significantly negatively and positively correlated with ferulic acid content ($P = 0.005$) and chlorogenic acid content ($P = 0.022$), respectively, but not in SA-treated wheat ones ($P > 0.05$). On the contrary, induced PPO activity in wheat seedlings by exogenous SA was significantly associated with caffeic acid content ($P = 0.031$). No significant correlation was observed between other enzyme activities and phenolic acid contents in SA-treated and control wheat seedlings ($P > 0.05$) (table 1).

Discussion

Chemical defence against herbivores and pathogens is prevalent in plants. The synthesis of defensive compounds in plants is always related to some secondary metabolic pathways, among

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Table 1. The correlation of defence-related enzyme activity with phenolic compound content in SA-induced wheat seedlings.

		Control		Treatment	
Enzyme	Compound	*r*	*P*	*r*	*P*
PAL	CoA	0.994	0.068	0.957	0.187
	FA	−0.974	0.145	−0.284	0.816
	CA	0.035	0.978	0.993	0.077
	ChA	0.964	0.172	0.220	0.859
4CL	CoA	−0.877	0.319	−0.937	0.226
	FA	0.929	0.242	0.343	0.777
	CA	−0.5996	0.591	−0.984	0.116
	ChA	−0.944	0.215	−0.159	0.898
PPO	CoA	0.718	0.490	0.975	0.142
	FA	−0.629	0.567	−0.215	0.862
	CA	−0.588	0.600	0.999	0.031*
	ChA	0.595	0.594	0.289	0.813
POD	CoA	0.985	0.112	0.842	0.363
	FA	−0.956	0.189	−0.535	0.641
	CA	−0.034	0.978	0.922	0.252
	ChA	0.943	0.216	−0.055	0.965
CAT	CoA	0.992	0.082	0.966	0.166
	FA	−1.000	0.005**	0.264	0.830
	CA	0.267	0.828	0.907	0.277
	ChA	0.999	0.022*	0.701	0.506

PAL, phenylalanine-ammonia-lyase; 4CL, 4-coumarate:coenzyme A ligase; PPO, polyphenol oxidase; POD, peroxidase; CAT, catalase; CoA, coumaric acid; FA, ferulic acid; CA, caffeic acid; ChA, chlorogenic acid.
Asterisk (* or **) following *P* value indicated a significant correlation at 0.05 or 0.01 level.

which the phenylpropanoid pathway has been regarded to be associated with plant resistance to herbivorous insects (Alvarez, 2014). Plant hormones such as JA and SA are increasingly recognized as crucial mediators in the interactions of plants with the associated organisms (Lazebnik *et al.*, 2014), generally through the activation of defence-related enzymes and accumulation of some secondary compounds in the phenylpropanoid pathway to potentiate plant defence against pests (Chu *et al.*, 2011). Considerable evidence has shown that these hormones can trigger the activities of defensive enzymes or the accumulation of plant secondary metabolites involved in the resistance to herbivores or/and pathogens (Robert-Seilaniantz *et al.*, 2011); however, few studies explored specific hormone-mediated association among defence-related enzyme activity, compound content and plant defence against pests. Our findings in the current study revealed that exogenous SA application triggered the activities of some defence-related enzymes and increased specific phenolic content in aphid-susceptible wheat plants at a specific period, and reduced biological indices of *S. avenae*.

Phytohormones, particularly JA and SA as well as their derivatives, have been considered to regulate plant defences against either pathogens or herbivores. The activation of the defence system by phytohormones is favourable for plant resistance to herbivores (Thaler *et al.*, 2010). Application of exogenous SA reduces *Lolium multiflorum* resistance to *Rhopalosiphum padi* by reducing the concentration of loline alkaloids (Bastias *et al.*, 2018). Previous evidence also indicated no significant effect of SA application on the biological index of *S. avenae* in wheat seedlings (Cao *et al.*, 2014). However, contradictory to this notion, our results showed exogenous SA significantly repressed the intrinsic rate of natural increase of aphid (r_m), female fecundity and apterous rate, and aphid body weight at day 7 post-feeding wheat seedlings as well (figs 1a–c and 2). This indicated that the application of exogenous SA improved wheat resistance to the aphid. The difference may result from the following reasons, first, the wheat variety adopted in our study is susceptible to *S. avenae*, their seedlings may be more sensitive to exogenous stimuli such as insect feeding, pathogen infection (Han *et al.*, 2009; Thaler *et al.*, 2010) and

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phytohormones. Second, to enhance SA activation, we used previous SA-treated methods (Ananieva *et al.*, 2004) that SA-sprayed wheat seedlings were covered with a sealed glass box for 24 h. Therefore, the inducible effect of exogenous phytohormone on plant defence system might be closely associated with a variety of characteristics (Han *et al.*, 2009) and SA treatment methods.

SA and its derivatives mediate plant systemic acquired resistance to pathogens (Park *et al.*, 2007), some evidence suggests that SA could fine-tune plant defence responses to specific pests by eliciting JA signalling pathway (Smith *et al.*, 2009) in some dicotyledonous plants, and resulting in the accumulation of some products downstream in the pathway via crosstalk (Engelberth *et al.*, 2011; Thaler *et al.*, 2012). In our study, exogenous SA also activate PAL activity in wheat plants, a monocotyledon (fig. 3a), but inhibited 4CL activity with sampling time points in both SA-treated and control wheat plants (fig. 3b). Until recently, although no evidence indicates how phytohormones affect 4CL activity in plants, some 4CL isoforms were thought to participate in the biosynthetic pathway of flavonoids and lignin as well as in the production of additional phenolic compounds (Ehlting *et al.*, 1999). Further studies will facilitate understanding the effect of phytohormones on 4CL activity and its biological function.

Antioxidases such as PPO, POD and CAT play a crucial role in the phenylpropanoid pathway, some of their products are often vital defence compounds against herbivores and pathogens (Dong *et al.*, 2010; Jonathan Gogbeu *et al.*, 2015). SA can enhance the activities of these three oxidases in many plants (Valverde *et al.*, 2015), but the activities of POD and CAT are suppressed by SA in other plants (Mohase and van der Westhuizen, 2002). Our findings showed that potentiated activities of PPO and POD at 48 and 72 h were observed in the SA-treated wheat seedlings and dependent on sampling time (fig. 3c, d). These results further confirmed the conclusions made by others (Ananieva *et al.*, 2004; Tasgin *et al.*, 2006). Interestingly, SA reduced CAT activity in the present study, this result was consistent with previous reports in wheat plants (Mohase and van der Westhuizen, 2002) and the apoplast of wheat leaves (Tasgin *et al.*, 2006), but are different from the results observed in barley seedlings (Ananieva *et al.*, 2004) and cherry fruits (Valverde *et al.*, 2015). Therefore, CAT activity induced by SA appears to be dependent on plant species.

Plant phenolics have been considered to be vital defence components against biotic and abiotic stresses, plant hormones are indispensable regulators in the synthesis of these defence compounds (Dong *et al.*, 2010; Lajara *et al.*, 2015). Exogenous application of plant hormones is an effective way to induce the defence reactions in plants and results in stronger plant defence against particular herbivores and pathogens because of the increased contents of some defence compounds (Mohase and van der Westhuizen, 2002; Park *et al.*, 2007). SA-inducible phenolic metabolism has been reported in SA-treated plants (Kováčik *et al.*, 2009). The levels of coumaric acid at 96 h.p.t., ferulic acid at 24 and 72 h.p.t. and chlorogenic acid at 24, 48 and 96 h.p.t. were significantly increased in SA-treated wheat seedlings, but caffeic acid contents at 24 and 48 h.p.t. were significantly reduced in SA-treated wheat seedlings (fig. 4c). The results indicate that exogenous SA affects the dynamics of phenolic metabolism in wheat plants, suggesting that the SA-induced role may be different in various phenolic acids.

The biosynthesis pathways of many aromatic compounds have the first three enzymatic steps in common with the phenylpropanoid pathway in plants. That is, PAL, cinnamate 4-hydroxylase and 4CL in the upstream portion of the pathway successively catalyse phenylalanine deamination, hydroxylation and synthesis of *p*-coumaroyl CoA ester (Li *et al.*, 2015). Growing evidence showed that both endogenous and exogenous SA stimulated the accumulation of phenylpropanoids through the activation of PAL in plants (Kováčik *et al.*, 2009; Dong *et al.*, 2010); however, little is known about the relationship between SA, PAL and the accumulation of specific phenolics in plants. Our results indicate that the accumulation of all tested phenolic acids was not statistically significantly correlated with PAL and 4CL activities in SA-treated wheat seedlings (table 1). This implies that the sensitivity of the metabolism of these compounds to PAL and 4CL could be associated with the location of their metabolic branches, in which they are synthesized (Li *et al.*, 2015). Although the first and last shared step is catalysed by PAL and 4CL (Li *et al.*, 2015), respectively, and PAL activity at 48 and 72 h.p.t. was induced by exogenous SA, 4CL activity was significantly reduced in SA-treated wheat seedlings (fig. 3b). This suggests that PAL and 4CL may not be directly involved in the metabolism of terminal compounds that could belong to metabolic branches in wheat tissue.

Plants can defend themselves against pathogens and herbivores by using phenolics in two ways: first, large quantities of phenolic compounds are accumulated in plant tissues to prevent infestation, and second, some compounds with immense antibiotic capability are synthesized by oxidizing phenols of low toxicity in plant tissues (Chrzanowski *et al.*, 2003). As mentioned, the exogenous application of SA has been reported to trigger the increases in PPO, POD and CAT activity and the content of total phenolics and individual phenolic acids in a variety of plant species (Ananieva *et al.*, 2004; Dong *et al.*, 2010). PPO can oxidize mono- or dihydroxy phenols to the corresponding quinones, which provide resistance to pathogens and herbivores (Wang and Constabel, 2004). POD and CAT oxidize phenolic compounds in the presence of H_2O_2 or reactive oxygen species in plants (Fry, 2004). These enzymatic activities were found to be inhibited by some substances and oxidative products (Le Bourvellec *et al.*, 2004). Our results showed that a significant positive correlation existed between PPO activity and caffeic acid content in SA-treated wheat seedlings but not in control seedlings (table 1). CAT activity in wheat seedlings of control was negatively and positively correlated with ferulic and chlorogenic acids, respectively, whereas the correlation was not observed in SA-treated wheat seedlings (table 1). The findings suggest that exogenous SA could change the constitutive content of phenolic acids in wheat plants by interfering with the activities of some defence-related enzymes in the phenylpropanoid pathway.

In summary, our findings demonstrated that the application of SA in aphid-susceptible wheat seedlings significantly reduced r_m, female fecundity, apterous rate and aphid biomass of *S. avenae*, these reduced indices reflect the inadaptation of aphids to host plants. It is worth noting that high-quality wheat varieties such as strong-gluten wheat often are susceptible to aphids in China, and can provide sufficient nutrition for aphid growth and development (Wang *et al.*, 2006). Therefore, the application of SA in wheat production may provide an environmental-friendly way of controlling the aphid population to reduce wheat loss.

On the other hand, exogenous SA also potentiated the activities of PAL, PPO and POD with time, but reduced 4CL and CAT activities, and the constitutive contents of some phenolic acids were also modified in SA-treated wheat seedlings.

https://doi.org/10.1017/S0007485321000237 Published online by Cambridge University Press

Nevertheless, a correlation between defence-related enzyme activity with phenolic acid content was not observed by a simple correlation analysis, suggesting metabolic complexity of phenolic acids in plants. Therefore, we yet have no enough information about the phytochemical metabolism regulated by SA. Further studies are needed to determine the precise mechanism of SA-mediated relationship between a defence-related enzyme and phytochemical biosynthesis.

Acknowledgements. This research was funded by the Nation Key Projects on Science and Technology Innovation of High Yield and Efficiency of Grain, grant numbers 2016YFD0300203.

Conflict of interest. None.

References

Aksoy M (2020) A new insight into purification of polyphenol oxidase and inhibition effect of curcumin and quercetin on potato polyphenol oxidase. *Protein Expression and Purification* **171**, 105612.

Alba JM, Schimmel BC, Glas JJ, Ataide LM, Pappas ML, Villarroel CA, Schuurink RC, Sabelis MW and Kant MR (2015) Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytologist* **205**, 828–840.

Alvarez MA (2014) Plant secondary metabolism. In Alvarez MA (ed), *Plant Biotechnology for Health: From Secondary Metabolites to Molecular Farming*. Switzerland: Springer International Publishing, pp. 15–31.

Ananieva EA, Christov KN and Popova LP (2004) Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. *Journal of Plant Physiology* **161**, 319–328.

Aziz MA, Iftkhar A and Hanif M (2013) Life table studies on *Trilocha virescence* (Bombycidae: Lepidoptera) on *Ficus nitida*. *Asian Journal of Agriculture and Biology* **1**, 2–7.

Ballaré CL (2011) Jasmonate-induced defenses: a tale of intelligence, collaborators and rascals. *Trends in Plant Science* **16**, 249–257.

Bastias DA, Martinez-Ghersa MA, Newman JA, Card SD, Mace WJ and Gundel PE (2018) The plant hormone salicylic acid interacts with the mechanism of anti-herbivory conferred by fungal endophytes in grasses. *Plant Cell and Environment* **41**, 395–405.

Bhattarai KK, Xie Q-G, Pourshalimi D, Younglove T and Kaloshian I (2007) *Coi*1-dependent signaling pathway is not required for *Mi*-1-mediated potato aphid resistance. *Molecular Plant-Microbe Interactions* **20**, 276–282.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

Cai QN, Zhang QW and Cheo M (2004) Contribution of indole alkaloids to *Sitobion avenae* resistance in wheat. *Journal of European Entomology* **128**, 517–521.

Campos ML, Kang J-H and Howe GA (2014) Jasmonate-triggered plant immunity. *Journal of Chemical Ecology* **40**, 657–675.

Cao HH, Wang SH and Liu TX (2014) Jasmonate- and salicylate-induced defenses in wheat affect host preference and probing behavior but not performance of the grain aphid, *Sitobion avenae*. *Insect Science* **21**, 47–55.

Chrzanowski G, Ciepiela AP, Sprawka I, Sempruch C, Sytykiewicz H and Czerniewicz P (2003) Activity of polyphenoloxidase in the ears of spring wheat and triticale infested by grain aphid (*Sitobion avenae* (F.)). *Electronic Journal of Polish Agricultural Universities, Biology* **6**, art-04.

Chu HY, Wegel E and Osbourn A (2011) From hormones to secondary metabolism: the emergence of metabolic gene clusters in plants. *The Plant Journal* **66**, 66–79.

Dong J, Wan G and Liang Z (2010) Accumulation of salicylic acid-induced phenolic compounds and raised activities of secondary metabolic and antioxidative enzymes in *Salvia miltiorrhiza* cell culture. *Journal of Biotechnology* **148**, 99–104.

Ehlting J, Buttner D, Wang Q, Douglas CJ, Somssich IE and Kombrink E (1999) Three 4-coumarate:coenzyme A ligases in *Arabidopsis thaliana* represent two evolutionarily divergent classes in angiosperms. *The Plant Journal* **19**, 9–20.

Engelberth J, Viswanathan S and Engelberth MJ (2011) Low concentrations of salicylic acid stimulate insect elicitor responses in *Zea mays* seedlings. *Journal of Chemical Ecology* **37**, 263–266.

Fry SC (2004) Primary cell wall metabolism: tracking the careers of wall polymers in living plant cells. *New Phytologist* **161**, 641–675.

Glinwood R, Liu X-F, Hu X-S, Keller MA, Zhao H-Y, Wu Y-F and Liu T-X (2014) Tripartite interactions of barley yellow dwarf virus, *Sitobion avenae* and wheat varieties. *PLoS ONE* **9**, e106639.

Han Y, Wang Y, Bi J-L, Yang X-Q, Huang Y, Zhao X, Hu Y and Cai Q-N (2009) Constitutive and induced activities of defense-related enzymes in aphid-resistant and aphid-susceptible cultivars of wheat. *Journal of Chemical Ecology* **35**, 176–182.

Hatcher PE, Moore J, Taylor JE, Tinney GW and Paul ND (2004) Phytohormones and plant-herbivore-pathogen interactions: integrating the molecular with the ecological. *Ecology* **85**, 59–69.

Horváth E, Janda T, Szalai G and Páldi E (2002) In vitro salicylic acid inhibition of catalase activity in maize: differences between the isozymes and a possible role in the induction of chilling tolerance. *Plant Science* **163**, 1129–1135.

Hu Y, Han Y, Zhao X, Yang X, Huang Y, Lou P and Cai Q (2008) Dynamics and effect evaluation of three phenolic compound contents in wheat varieties with different resistances to *Sitobion avenae*. *Chinese Journal of Applied & Environmental Biology* **14**, 753–756.

Jonathan Gogbeu S, Mathurin Okoma K, Beranger N'Goran KS and Odette Dogbo D (2015) Salicylic acid, phosphorous acid and fungicide Sumi 8 effects on polyphenol oxidases activities and *Cassava* resistance to anthracnose. *American Journal of Agriculture and Forestry* **3**, 109.

Kim JH and Jander G (2007) *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. *The Plant Journal* **49**, 1008–1019.

Knobloch K-H and Hahlbrock K (1977) 4-Coumarate:CoA ligase from cell suspension cultures of *Petroselinum hortense* Hoffm: partial perification, substrate specificity, and further properties. *Archives of Biochemistry and Biophysics* **184**, 237–248.

Kováčik J and Klejdus B (2012) Tissue and method specificities of phenylalanine ammonia-lyase assay. *Journal of Plant Physiology* **169**, 1317–1320.

Kováčik J, Grúz J, Bačkor M, Strnad M and Repčák M (2009) Salicylic acid-induced changes to growth and phenolic metabolism in *Matricaria chamomilla* plants. *Plant Cell Reports* **28**, 135–143.

Lajara MM, López-Orenes A, Ferrer MA and Calderón AA (2015) Long-term exposure treatments revert the initial SA-induced alterations of phenolic metabolism in grapevine cell cultures. *Plant Cell, Tissue and Organ Culture (PCTOC* **122**, 665–673.

Lazebnik J, Frago E, Dicke M and van Loon JJA (2014) Phytohormone mediation of interactions between herbivores and plant pathogens. *Journal of Chemical Ecology* **40**, 730–741.

Le Bourvellec C, Le Quéré J-M, Sanoner P, Drilleau J-F and Guyot S (2004) Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products. *Journal of Agricultural and Food Chemistry* **52**, 122–130.

Li Y, Kim JI, Pysh L and Chapple C (2015) Four isoforms of *Arabidopsis thaliana* 4-coumarate:CoA ligase (4CL) have overlapping yet distinct roles in phenylpropanoid metabolism. *Plant Physiology* **169**, 2409–2421.

Mohase L and van der Westhuizen AJ (2002) Salicylic acid is involved in resistance responses in the Russian wheat aphid-wheat interaction. *Journal of Plant Physiology* **159**, 585–590.

Park SW, Kaimoyo E, Kumar D, Mosher S and Klessig DF (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science (New York, N.Y.)* **318**, 113–116.

Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A and Van Wees SCM (2012) Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* **28**, 489–521.

Pyati P, Bandani AR, Fitches E and Gatehouse JA (2011) Protein digestion in cereal aphids (*Sitobion avenae*) as a target for plant defence by endogenous proteinase inhibitors. *Journal of Insect Physiology* **57**, 881–891.

Radwan DEM (2012) Salicylic acid induced alleviation of oxidative stress caused by clethodim in maize (*Zea mays* L.) leaves. *Pesticide Biochemistry and Physiology* **102**, 182–188.

Razmjou J, Ramazani S, Naseri B, Ganbalani GN and Dastjerdi HR (2011) Resistance and susceptibility of various wheat varieties to *Sitobion avenae* (Hemiptera: Aphididae) in Iran. *Applied Entomology and Zoology* **46**, 455–461.

Reymond P and Farmer EE (1998) Jasmonate and salicylate as global signals for defense gene expression. *Current Opinion in Plant Biology* **1**, 404–411.

Robert-Seilaniantz A, Grant M and Jones JD (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology* **49**, 317–343.

Ruelas C, Tiznado-Hernandez ME, Sanchez-Estrada A, Robles-Burgueno MR and Troncoso-Rojas R (2006) Changes in phenolic acid content during *Alternaria alternata* infection in tomato fruit. *Journal of Phytopathology* **154**, 236–244.

Saballos A, Sattler SE, Sanchez E, Foster TP, Xin Z, Kang C, Pedersen JF and Vermerris W (2012) Brown midrib2 (Bmr2) encodes the major 4-coumarate: coenzyme A ligase involved in lignin biosynthesis in sorghum (*Sorghum bicolor* (L.) Moench). *The Plant Journal* **70**, 818–830.

Smith CM and Boyko EV (2007) The molecular bases of plant resistance and defense responses to aphid feeding: current status. *Entomologia Experimentalis et Applicata* **122**, 1–16.

Smith JL, De Moraes CM and Mescher MC (2009) Jasmonate- and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. *Pest Management Science* **65**, 497–503.

Tasgin E, Atici O, Nalbantoglu B and Popova L (2006) Effects of salicylic acid and cold treatments on protein levels and on the activities of antioxidant enzymes in the apoplast of winter wheat leaves. *Phytochemistry* **67**, 710–715.

Thaler JS and Bostock RM (2004) Interactions between abscisic-acid-mediated responses and plant resistance to pathogens and insects. *Ecology* **85**, 48–58.

Thaler JS, Agrawal AA and Halitschke R (2010) Salicylate-mediated interactions between pathogens and herbivores. *Ecology* **91**, 1075–1082.

Thaler JS, Humphrey PT and Whiteman NK (2012) Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science* **17**, 260–270.

Urbanek Krajnc A and Kristl J and Ivancic A (2011) Application of salicylic acid induces antioxidant defense responses in the phloem of *Picea abies* and inhibits colonization by *Ips typographus*. *Forest Ecology and Management* **261**, 416–426.

Valiñas MA, Lanteri ML, ten Have A and Andreu AB (2017) Chlorogenic acid, anthocyanin and flavan-3-ol biosynthesis in flesh and skin of Andean potato tubers (*Solanum tuberosum* subsp. *andigena*). *Food Chemistry* **229**, 837–846.

Valverde JM, Giménez MJ, Guillén F, Valero D, Martínez-Romero D and Serrano M (2015) Methyl salicylate treatments of sweet cherry trees increase antioxidant systems in fruit at harvest and during storage. *Postharvest Biology and Technology* **109**, 106–113.

Vogt T (2010) Phenylpropanoid biosynthesis. *Molecular Plant* **3**, 2–20.

Wang J and Constabel CP (2004) Polyphenol oxidase overexpression in transgenic Populus enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). *Planta* **220**, 87–96.

Wang M, Yuan G, Chen J, Lei Z and Wu Z (2006) Occurrence and damage characteristics of wheat aphids and identification of wheat resistance to aphids. *Journal of Henan Agricultural Sciences* **7**, 58–60, (In Chinese).

Wen P-F, Chen J-Y, Kong W-F, Pan Q-H, Wan S-B and Huang W-D (2005) Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. *Plant Science* **169**, 928–934.

Zhang Z, Meng Z, Yao Y and Zhou L (2010) Change of ferulic acid content in wheat seedling. *Chinese Food Science* **31**, 271–274.

Zhang P-J, Li W-D, Huang F, Zhang J-M, Xu F-C and Lu Y-B (2013) Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid signaling. *Journal of Chemical Ecology* **39**, 612–619.