Protoplasma (2017) 254:271–283
DOI 10.1007/s00709-016-0941-7

CrossMark

ORIGINAL ARTICLE

Jasmonates are induced by the PAMP flg22 but not the cell death-inducing elicitor Harpin in *Vitis rupestris*

Xiaoli Chang[1,3] **· Mitsunori Seo**[2] **· Yumiko Takebayashi**[2] **· Yuji Kamiya**[2] **·**
Michael Riemann[3] **· Peter Nick**[3]

Received: 11 October 2014 / Accepted: 2 January 2016 / Published online: 15 January 2016
© Springer-Verlag Wien 2016

Abstract Plants employ two layers of defence that differ with respect to cell death: pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). In our previous work, we have comparatively mapped the molecular events in a cell system derived from the wild American grape *Vitis rupestris*, where cell death-independent defence can be triggered by PAMP flg22, whereas the elicitor Harpin activates a cell death-related ETI-like response. Both defence responses overlapped with respect to early events, such as calcium influx, apoplastic alkalinisation, oxidative burst, mitogen-activated protein kinase (MAPK) signalling, activation of defence-related genes and accumulation of phytoalexins. However, timing and amplitude of early signals differed. In the current study, we address the role of jasmonates (JAs) as key signalling compounds in hypersensitive cell death. We find, in *V. rupestris*, that jasmonic acid and its bioactive conjugate jasmonoyl-isoleucine (JA-Ile) rapidly accumulate in response to flg22 but not in response to Harpin. However, Harpin can induce programmed cell death, whereas exogenous methyl jasmonate (MeJA) fails to do so, although both signals induce a similar response of defence genes. Also in a second cell line from *V. vinifera* cv. 'Pinot Noir', where Harpin cannot activate cell death and where flg22 fails to induce JA and JA-Ile, defence genes are activated in a similar manner. These findings indicate that the signal pathway culminating in cell death must act independently from the events culminating in the accumulation of toxic stilbenes.

Keywords Defence signalling · Effector-triggered immunity (ETI) · Jasmonic acid (JA) · PAMP-triggered immunity (PTI) · *Vitis*

Handling Editor: Reimer Stick

Electronic supplementary material The online version of this article (doi:10.1007/s00709-016-0941-7) contains supplementary material, which is available to authorized users.

✉ Xiaoli Chang
xl_changkit@126.com

[1] Department of Plant Pathology, Agricultural College, Sichuan Agricultural University, Chengdu 611130, People's Republic of China

[2] RIKEN Center for Sustainable Resource Science, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

[3] Molecular Cell Biology, Botanical Institute, Karlsruhe Institute of Technology, Kaiserstr. 2, 76131 Karlsruhe, Germany

Introduction

Plants are exposed to a wide range of abiotic and biotic stresses. Among the biotic stresses, invasion by microbial pathogens and wounding by herbivorous insects are most important. Whereas insect attack is mostly encountered by morphological barriers or the production of unpalatable or even toxic compounds in response to the wounding, the defence of pathogens has to be more sophisticated. Especially biotrophic pathogens circumvent or quell the defence system. Thus, similar to animals, plant immunity must be able to specifically discriminate non-self molecules to activate effective defence. In contrast to mammalian immunity, plants lack mobile defence cells, which means that immunity must be present in all cells and therefore has to be innate. For this reason, plant immunity is composed of two levels that have been described by the so-called zig-zag model (Jones and Dangl 2006). The first level is activated by a fairly general pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs), such as bacterial flagellin or fungal chitin that are recognised

by evolutionarily conserved surface pattern recognition receptors (PRRs), activating so-called PAMP-triggered immunity (PTI) (Nürnberger and Lipka 2005; Bittel and Robatzek 2007; Robatzek 2014). This mechanism ensures a broadband immunity to whole classes of microbial pathogens. Since the trigger is linked with essential molecules of the pathogen, pathogens cannot respond to the selective pressure by a loss of these PAMPs. Instead, more specialised (often biotrophic) pathogens have evolved so-called effector molecules that can quell PTI. To encounter the attack of such specialised pathogens, many plants have evolved a second layer of immunity, where specific plant resistance (R) proteins can recognise microbial effectors and reinstall immunity. This second layer, termed effector-triggered immunity (ETI), often culminates in a hypersensitive response (HR), a plant-specific form of programmed cell death providing an efficient strategy to block biotrophic pathogens (Boller and Felix 2009; Jones and Dangl 2006). Although programmed cell death is generally assigned to ETI and not to PTI, this classical dichotomy has come under debate (Thomma et al. 2011).

The cellular and molecular events underlying PTI share some commonalities across the eukaryotes (Nürnberger and Lipka 2005). Some of the early cellular responses have been identified including a depolarisation of the plasma membrane (Felix et al. 1999), activation of ion channels (Jeworutzki et al. 2010), activation of mitogen-activated protein kinase (MAPK) cascades, activation of WRKY transcription factors (Gómez-Gómez and Boller 2000; Nürnberger et al. 2004), generation of reactive oxygen species (ROS) and transcription of defence-related genes (Zipfel et al. 2006; Chinchilla et al. 2007). However, most of these responses have also been observed in the context of ETI but differ in kinetics and intensity (Tsuda and Katagiri 2010). The molecular mechanisms underlying this differential output are largely unclear.

Plant hormones have been discussed as modulators of plant immunity (for reviews, see Pieterse et al. 2009, 2012; Wasternack and Hause 2013), with jasmonic acid (JA) and its derivatives (for review, see Browse 2009) and salicylic acid (SA) as prime candidates (for review, see Vlot et al. 2009). Activation of plant defence by herbivores, necrotrophic pathogens as well as wounding is generally correlated by activation of the JA pathway (reviewed in Bostock 2005; Howe and Jander 2008). JA is synthesised via the oxylipin pathway and subsequently either metabolised to methyl jasmonate (MeJA) by the JA carboxyl methyl transferase (Seo et al. 2001) or conjugated to isoleucine by the JA conjugate synthase JAR1 (Staswick and Tiryaki, 2004) to yield the biological highly active jasmonoyl-isoleucine (JA-Ile) (Fonseca et al. 2009). JA-Ile is recognised by a receptor complex leading to proteasomal degradation of a transcriptional repressor releasing transcriptional activators (for review, see Pauwels et al. 2009). Under physiological conditions, JA signalling remains transient. Shut-off mechanisms include transcriptional activation of the JAZ repressors (for recent review, see Kazan 2015) but also degradation of jasmonic acid (Heitz et al. 2012) by catabolic cytochrome P_{450} proteins and cleavage of the conjugate by hydrolases (Widemann et al. 2013). Although activation of the JA pathway is often correlated with HR, the actual evidence for a causal relationship has remained scarce. JA has been shown to accumulate after infiltration with the non-host pathogen *Pseudomonas syringae* pv. *phaseolicola* prior to the development of HR-like lesions, and this effect was not observed by a bacterial mutant that failed to produce those lesions (Kenton et al. 1999). In a further example, grapevine leaves have been sprayed with exogenous MeJA and were observed to produce local necrotic spots resembling a hypersensitive-like response (Repka 2001, 2013). As antagonist of JA signalling (Pieterse et al. 2012; Spoel et al. 2003), the SA pathway has acquired considerable interest. SA biosynthesis is triggered during both PTI and ETI (Bernoux et al. 2011; Mishina and Zeier 2007) and often followed by activation of PR proteins, discussed with respect to systemic acquired resistance (SAR) (Glazebrook 2005; Grant and Lamb 2006). Several molecular mechanisms have been proposed to convey the antagonism between SA and JA signalling (reviewed in Pieterse et al. 2012). The biological function of this antagonistic interaction might be to optimise the balance between defence and growth by optimal allocation of energy resources (for review, see Jaillais and Chory 2010; Kazan and Manners 2008).

PTI and ETI lead to a radically different output, although most of the cellular events are shared. It is their time course and amplitude that differs. Where is the switch, where differences in signal quantity are transformed into a new quality of output (programmed cell death)? To approach this question, experimental systems are needed, where defence-related cell death can be studied side by side, under otherwise the *same* conditions with a defence response that is not culminating in cell death. To achieve this, we have established an experimental system based on two grapevine cell lines, where basal immunity (PTI) can be induced by the bacterial PAMP flg22, whereas the bacterial elicitor Harpin will trigger a defence response culminating in cell death (Chang and Nick 2012). Although Harpin is not a classical effector, this cell death response is dependent on the genotype of the cell line: it is absent in a cell line derived from *Vitis vinifera* cv. 'Pinot Noir' but strong in a cell line derived from the North American wild grape species *Vitis rupestris*. Due to the genotype dependence and the programmed cell death, we use for this Harpin-triggered response of *V. rupestris* the term "ETI-like response." Despite the qualitatively different response, the early molecular events such as activation of H^+ and Ca^{2+} ion channels, generation of ROS, transcription of defence-related genes as well as cytoskeletal reorganisation overlap between the two cell lines as well as between the two forms of defence (Chang and Nick 2012). However, timing and magnitude of

oxidative burst and the synthesis of toxic phytoalexins differed. In the current work, we have addressed the role of JA and SA signalling in flg22- or Harpin-triggered immunity in *V. rupestris*. We find that flg22 induces a rapid, strong and transient accumulation of jasmonic acid and its bioactive conjugate JA-Ile, whereas Harpin produces a response that is almost one order of magnitude weaker. None of the two elicitors can induce SA. Exogenous (constitutively administered) MeJA but not exogenous salicylic acid leads to the accumulation of Δ-viniferin. However, although these highly toxic stilbenes accumulate to higher levels than for induction by Harpin, MeJA (in contrast to Harpin) does not lead to cell death. These findings indicate that the signalling pathway culminating in cell death must act independently from the events culminating in the accumulation of toxic stilbenes.

Material and methods

Cell culture and treatment

Cell suspension cultures of *V. rupestris* and *V. vinifera* cv. 'Pinot Noir' were established from leaves as described previously (Seibicke 2002). Cells were maintained and subcultured referred to Chang et al. (2011) in liquid MS medium on an orbital shaker (KS250 basic, IKA Labortechnik, Germany) at 150 rpm, 25 °C, in the dark.

The bacterial peptide flg22, a 22-amino acid peptide, was purchased from a commercial producer (GenScript, Luxembourg) and diluted in sterile H_2O. A commercially available Harpin elicitor [Messenger, EDEN Bioscience Corporation, Washington, USA, active ingredient: 3 % (*w/w*) Harpin protein] was prepared into 300 mg ml^{-1} stock solution. SA was dissolved in sterile H_2O to obtain a stock solution of 2 mM, and MeJA was purchased as a liquid stock of 4.5 mM in methanol. Evans Blue (Sigma-Aldrich, Deisenhofen, Germany) was prepared as a solution of 2.5 % (*w*/*v*) in sterilised water and used for viability staining. All treatments were accompanied by appropriate solvent controls, and the maximal concentration of solvent used in the test samples did not exceed 0.1 % (*w*/*v*).

Determination of cell viability

To determine cell viability, cells from *V. rupestris* and cv. 'Pinot Noir' were sub-cultivated at stationary phase and separately triggered with 200 μM SA or 100 μM MeJA. These concentrations had been determined from a dose–response curve for packed cell volume (PCV) as readout for cell growth (Jovanović et al. 2010). The concentrations were selected such that they already produced were already producing a significant inhibition but still allowed the cells to proliferate at a rate, which was only around a quarter lower than in the control (Fig. S1 of the Supplementary material). To test whether there was a synergistic effect of Harpin with SA or MeJA on cell death, cells were induced with either 9 μg ml^{-1} Harpin, a combination of either SA or MeJA with Harpin or a corresponding volume of methanol as solvent control. Mortality was assessed at 24, 48 and 72 h after treatment by staining with Evans Blue (Gaff and Okong'O-Ogola 1971).

Cells were transferred into a custom-made staining chamber (Nick et al. 2000) to drain the medium and then incubated with 2.5 % (*w*/*v*) Evans Blue for 3 to 5 min. After washing three times with distilled water, cells were mounted on a slide and viewed under a light microscope (Zeiss-Axioskop 2 FS, DIC illumination, ×20 objective). Due to the breakdown of the plasma membrane, Evans Blue is capable of penetrating into dead cells, resulting in a blue staining of the cell interior. Frequency of cell death was calculated as ratio of the number of dead cells over the total number of scored cells. For each time point, 1500 cells were scored in three dependent experiments. Statistical significance was tested by analysis of variance (ANOVA) using SPSS software.

Expression analysis

To determine whether JA or SA signalling is downregulated by flg22 or Harpin, expression of the five selected genes including one stilbene synthase (*StSy*) referred to Kortekamp (2006), the receptor for SA (non-expressed pathogenesis-associated protein 1, *NPR1*, Le Henanff 2009) and three JA pathway-related genes (the jasmonate ZIM/tify-domain protein 1, *JAZ1*; encoding an F-box protein, *COI1*; a basic helix-loop-helix transcription factor, *MYC2*) according to Ismail et al. (2012), was analysed. Cells were treated with 1 μM flg22, or with 9 μg ml^{-1} Harpin, or with water as solvent control for 1 h (Chang and Nick 2012). Total RNA was extracted after the respective treatment, and cDNA was synthesised as previously described (Qiao et al. 2010). Transcripts were amplified by RT-PCR using the primers listed in Table S1 of the Supplementary material. Values for relative transcript abundance were calculated using elongation factor 1α as internal standard (Reid et al. 2006).

To test whether SA or MeJA elicited the defence-related gene expression (phenylalanine ammonia lyase gene, *PAL*; two pathogenesis-associated protein genes, *PR5* and *PR10*; stilbene synthase, *StSy*; *JAZ1*), and also modulate the response of gene expression to Harpin to affect gene transcripts, cells were challenged by either 9 μg ml^{-1} Harpin alone, 200 μM SA alone, 100 μM MeJA alone, SA combined with Harpin or MeJA with Harpin, for 1 h, respectively. The abundance of transcripts was evaluated by semi-quantitative RT-PCR as described above. All data represent the mean from at least three independent experimental series. Bars are standard errors. Statistical significance was tested by ANOVA.

Quantification of stilbene biosynthesis

To measure the hormonal effect on the final products of the stilbene synthase, cells were challenged with either 200 μM SA or 100 μM MeJA, and stilbene accumulation was followed over time by high performance liquid chromatography (HPLC). Cells were drained from culture medium by a vacuum of 800 Pa (Vacuubrand CVC2, Brand, Germany), shock-frozen in liquid nitrogen and then stored at −80°C until further analysis. Aliquots of 3 g fresh weight of untreated control or treated cells were homogenised with 20 ml of 80 % (*v*/*v*) methanol in water by an ultrasonic processor (UP100H, Hielscher, Germany) for 3 min. The homogenate was incubated for 2 h in the dark at room temperature in a rotatory shaker and filtered through filter paper by vacuum with 500 Pa. The filtrate was concentrated to a residual volume of 5 ml in a glass tube at 40°C (Heating Bath B490, BÜCHI, Germany) at 280 rpm (Rotavapor R-205, BÜCHI, Germany), under a vacuum of 80 Pa (Vacuubrand CVC2, Brand, Germany). Stilbenes were extracted from the aqueous phase by adding 2 ml of 5 % (*w*/*v*) $NaHCO_3$ and three aliquots of 5 ml ethyl acetate. The pooled ethyl acetate phases were completely dried and the residue suspended in 2 ml of methanol prior to injection into the HPLC.

Analysis of stilbenes was carried out on an HPLC (Agilent, 1200 series, Waldbronn, Germany) as described previously (Chang et al. 2011). *Trans*-resveratrol, *trans*-piceid and Δ-viniferin were quantified using external standards on the basis of retention time and UV–VIS spectra. The standards for *trans*-resveratrol (Sigma-Aldrich, Deisenhofen, Germany), *trans*-piceid (Phytolab, Vestenbergsgreuth, Germany) and δ-viniferin (kind gift of Dr. Kassemeyer, State Institute of Viticulture, Freiburg) were dissolved in methanol at a concentration of 100 mg l^{-1}. Calibration curves for quantification of the samples were determined using these standards and found to be linear ($r^2 > 0.99$). At least three independent experimental series were conducted.

Quantification of plant hormones

Phytohormone contents were quantified for both cell lines with three biological replicates at 0.5, 1, 3 and 6 h after addition of either 1 μM flg22, or 9 μg ml^{-1} Harpin or equal volumes of water as solvent control. All samples were collected by removing the cell medium using a Büchner funnel under vacuum. Both cell sediment and medium were shock-frozen in liquid nitrogen, freeze-dried at −50 °C for 2 days and weighed. Plant hormones were extracted as described previously (Yoshimoto et al. 2009) with some modifications: Lyophilized cell sediments or culture media were homogenised in 4 ml of 80 % acetonitrile (MeCN) containing 1 % acetic acid and extracted for 30 min with internal standards ($^{13}C_6$-JA-Ile, d_2-JA and d_6-SA). After centrifugation at 1663×*g* for 20 min, the supernatant was collected and the sediment extracted again with 4 ml of 80 % MeCN containing 1 % acetic acid. Either 1 ml (for cell sediments) or 4 ml (for culture media) of the supernatant was processed further for hormone analysis. After removal of MeCN from the supernatant, the acidic aqueous extract was loaded onto an Oasis HLB column cartridge (30 mg, 1 ml Waters, Milford, MA, USA) and washed with 1 ml of water containing 1 % acetic acid to remove highly polar impurities. Plant hormones were eluted with 2 ml of 80 % MeCN containing 1 % acetic acid. After removing MeCN in the remaining eluate, the acidic water extract was loaded onto an Oasis WAX column cartridge (30 mg, 1 ml). After washing with 1 ml of water containing 1 % acetic acid, neutral compounds were eliminated with 2 ml of 80 % MeCN, and acidic compounds were eluted with 2 ml of 80 % MeCN containing 1 % acetic acid. Hormones were quantified by liquid chromatography–electrospray ionisation–tandem mass spectrometry as described in detail in Yoshimoto et al. (2009).

Results

The JA response factor JAZ1 and the SA receptor NPR1 are specifically induced by both flg22 and Harpin

Our previous study showed that the response of the grapevine defence marker gene *StSy* was triggered by both flg22 and Harpin (Chang and Nick 2012). To understand whether this gene activation was correlated with activation of genes involved in JA/SA signalling, we investigated the expression of three genes related to JA signalling *(JAZ1*, *MYC2*, *COI1*), the gene encoding the SA receptor *NPR1* and the target gene *StSy. COI1* as F-Box protein involved in targeting the JAZ reporter for proteolytic degradation was not expected to respond on the transcriptional level and was selected as constitutive control factor of JA signalling as well as the transcriptional activator *MYC2*, which is released from repression upon JA-Ile-induced degradation of the JA response factor *JAZ1*. Consistent with our previous results (Chang and Nick 2012), both elicitors induced *StSy* transcripts to similar levels slightly reduced in cv. 'Pinot Noir' compared to *V. rupestris* (Fig. 1), whereas transcripts of *COI1* and *MYC2* were induced in none of the cell lines. Both elicitors were also able to induce the JA response factor *JAZ1* in both cell lines. Again, this induction was more pronounced in *V. rupestris* (around four- to five– fold) compared to cv. 'Pinot Noir' (around threefold). Both elicitors induced transcripts for the SA receptor *NPR1* in *V. rupestris*, whereas in cv. 'Pinot Noir', only Harpin produced a marginal induction that remained, however, at the verge of significance. Thus, both elicitors produced a strong induction of the defence marker *StSy*, accompanied by upregulation of a gene for JA

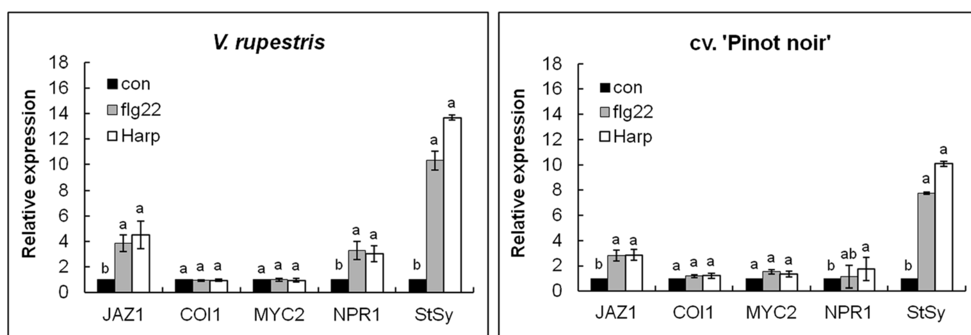

Fig. 1 Gene transcription induced by flg22 or Harpin in *V. rupestris* and cv. 'Pinot Noir'. Flg22- or Harpin-triggered gene expressions involved in SA or JA signalling pathway were examined in *V. rupestris* and cv. 'Pinot Noir'. Cells were treated for 1 h with either 1 μM flg22, or 9 μg ml^{-1} Harpin (Harp) or with water. Transcripts were measured for five selected defence genes including one grapevine defence-related genes (*StSy*, stilbene synthase), one gene related to salicylic acid (SA) signalling (*NPR1*, non-expressed pathogenesis-associated protein 1), three genes related to jasmonic acid (JA) signalling (*MYC2*, encoding a basic helix-loop-helix transcription factor; *COI1*, a JA-isoleucine receptor protein CORONATINE INSENSITIVE1; *JAZ1*, the jasmonate ZIM/tify-domain protein, a transcriptional repressor). Quantitative analysis of transcripts was followed by semi-quantitative RT-PCR using elongation factor 1α as an internal standard. The data represent averages from three independent experimental series; *error bars* represent standard errors. Expression difference of defence gene as compared to solvent control was analysed using ANOVA, and different *lowercases* show the significance at $P = 5$ %

responsiveness, and, confined to *V. rupestris*, also a gene encoding a SA receptor.

JA and JA-Ile are strongly and transiently induced by flg22 in *V. rupestris*

Since MeJA could induce the phenylpropanoid pathway in absence of elicitor, we asked further whether JA and its bioactive conjugate and JA-isoleucine conjugate (JA-Ile) accumulated in response to flg22 and Harpin, and we compared this with the response of SA as second important player in plant defence (Jaillais and Chory 2010). In fact, both JA and JA-Ile were rapidly and strongly induced by flg22 in *V. rupestris* (Fig. 2a, b, left-hand graphs), with already more than 500 μg g^{-1} dry weight (DW) of JA and more than 150 μg g^{-1} of JA-Ile. This strong accumulation was transient, however. Already 30 min later, the abundance of JA had dropped to about 50 % of the maximum at 30 min; for JA-Ile, the decrease was around 30 %. In contrast to *V. rupestris*, neither JA nor JA-Ile was induced by flg22 in *V. vinifera* cv. 'Pinot Noir' (Fig. 2a, b, left-hand graphs). Interestingly, compared to flg22, in *V. rupestris*, Harpin induced only about 15 % of JA (around 70 μg g^{-1} DW) and about 25 % of JA-Ile (around 40 μg g^{-1} DW). Although these levels of JA are still sufficient to activate JA signalling, this response was not only much weaker than that for flg22 but also followed a completely different temporal pattern: it was strongly delayed (from 1 h) and it was not transient. A similar difference between the two genotypes was observed for JA-Ile. Again, there was no significant induction of JA or JA-Ile in cv. 'Pinot Noir' (Fig. 2a, b, right-hand graphs). Unlike JA or JA-Ile, SA did not show any significant induction, independently of cell line or elicitor (Fig. 2c). In summary, although both flg22 and Harpin can induce JA and JA-Ile in *V. rupestris* (in sharp contrast to cv. 'Pinot Noir'), there is no significant induction of SA. The accumulation of jasmonates was much stronger in response to flg22 compared to Harpin (around six times for JA, around four times for JA-Ile). It was also more rapid and clearly transient for flg22, delayed and sluggish for Harpin. This is interesting in the context of the observation that induction of *JAZ*1 30 min later (Fig. 1) was comparable for both elicitors. Also, the somewhat reduced but still substantial induction of *JAZ*1 at 60 min (Fig. 1) is not preceded by any induction of JA or JA-Ile in cv. 'Pinot Noir'.

MeJA, but not SA, induces defence gene expression

Since flg22 and Harpin can induce JA/JA-Ile accumulation in *V. rupestris* (Fig. 2) and since both elicitors can induce *StSy* (Fig. 1), we addressed in the next step the responsiveness of defence genes in the two *Vitis* cell lines to MeJA or SA. We investigated the transcripts for *StSy* along with phenylalanine ammonia lyase (*PAL*) as first committed step of the phenylpropanoide pathway (Fig. 3), two pathogenesis-associated protein genes (*PR5* and *PR10*) and the JA response factor *JAZ1*, after treatment with either exogenous SA or MeJA. The concentrations for these hormones were derived from a dose–response study on cell growth and chosen such that they produced a significant effect on growth but still left proliferation to a large extent (around 70~80 %) functional (in Fig. S1 of the Supplementary material). We observed that SA did not cause any significant change, neither of *PAL* and *StSy* transcripts nor of *PR5*, *PR10* or *JAZ1*, in any of the cell lines except a slight induction of *PR5* in *V. rupestris* (that was on the verge of being significant). In contrast to SA, MeJA induced all transcripts of *PAL*, *StSy*, *PR10* and *JAZ1* but slightly inhibited *PR5* in *V. rupestris*. MeJA induced the transcripts of *JAZ1* and *StSy* as well as those of *PR10* around 2~3-fold

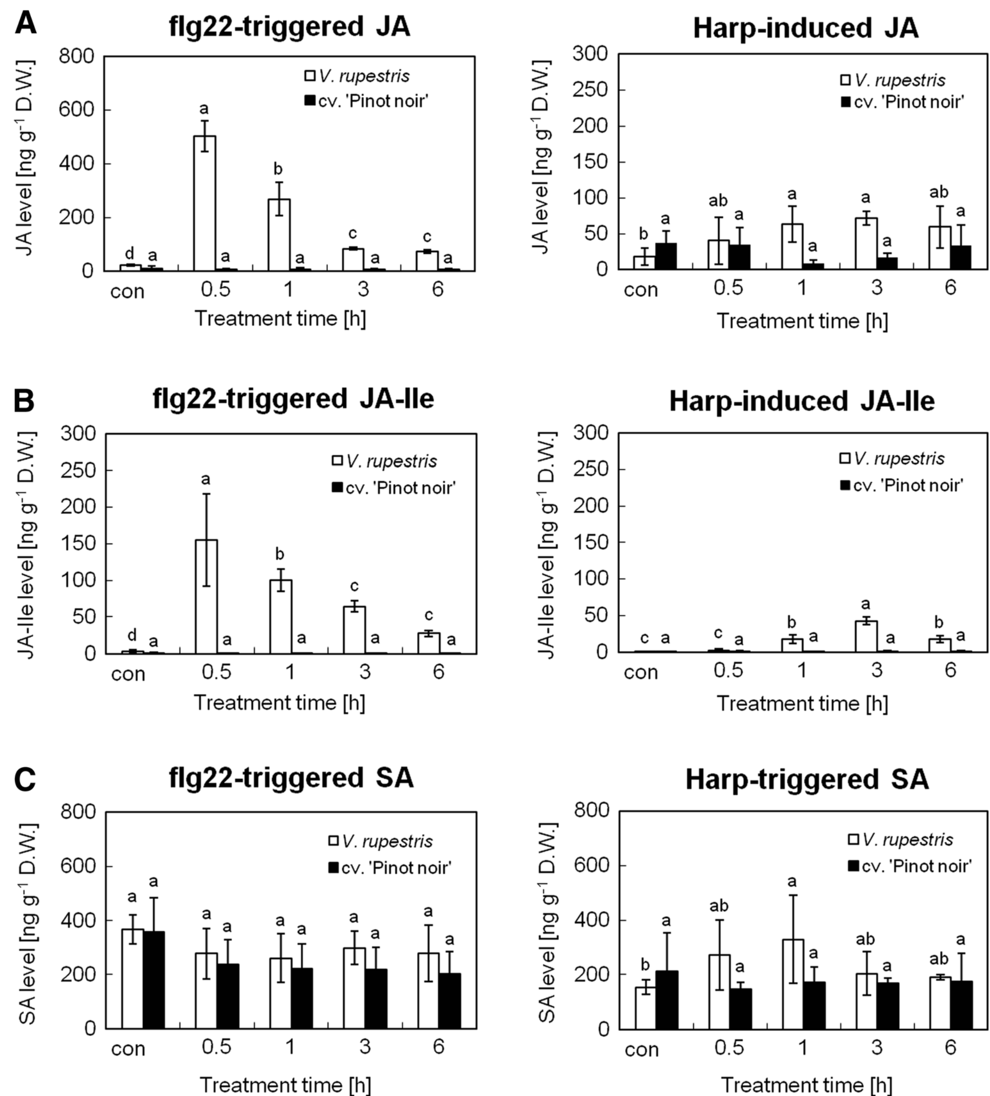

Fig. 2 Accumulation of JA, JA-Ile and SA induced by flg22 and Harpin in the two cell lines. Cells of *V. rupestris* and cv. 'Pinot Noir' were challenged with 1 μM flg22, 9 μg ml^{-1} Harpin (Harp) or the equal water as control for 0.5, 1, 3 or 6 h. Contents of JA (**a**), JA-Ile (**b**) and SA (**c**) from dried cell sediment were extracted and quantified by liquid chromatography–electrospray ionisation–tandem mass spectrometry using $^{13}C_6$-JA-Ile, d_2-JA and d_6-SA as internal standards. Data was obtained from at least three independent biological repeats. *Error bars* represent standard errors. Significance levels of differences were analysed as compared to water control in each cell line using ANOVA, and different *lowercase letters* indicate the significance at $P = 5$ %

stronger in *V. rupestris* compared to cv. 'Pinot Noir', whereas the induction of *PAL* was equal between the cell lines. In summary, exogenous MeJA can induce the expression of the phytoalexin synthesis genes *PAL* and *StSy*, as well as the defence gene *PR10*, whereas exogenous SA cannot. MeJA (but not SA) also upregulates the JA response factor *JAZ1*.

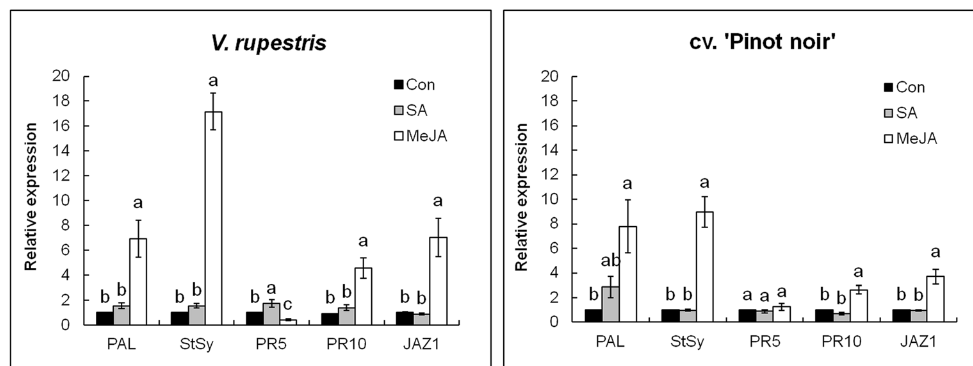

Fig. 3 Defence-related gene expression induced by SA or MeJA in *V. rupestris* and cv. 'Pinot Noir'. Cells were challenged by 100 μM MeJA or 200 μM SA as compared to water control for 1 h. The quantification was calculated relative to elongation factor 1α from four independent experimental series. *Error bars* represent standard errors. Different *lowercase letters* indicate significance levels of differences as compared to the solvent control using ANOVA with the significance at $P = 5$ %

MeJA and SA induce different accumulation of toxic stilbenes in *Vitis*

In grapevine, the biological function of stilbene synthase is to produce the cytotoxic stilbene *trans*-resveratrol, which can then be further oxidised oligomers such as Δ-viniferin, which is accompanied by programmed cell death (Chang et al. 2011). Alternatively, in some grapevine genotypes, such as cv. 'Pinot Noir', resveratrol is conjugated to yield *trans*-piceid that is sequestered into the vacuole. To interpret the functional relevance of the differential response of JA and JA-Ile accumulation triggered by flg22 or Harpin, we investigated the accumulation of these three final metabolites (*trans*-piceid, Δ-viniferin, *trans*-resveratrol) in response to MeJA and SA by HPLC (Fig. 4). Only residual amounts of *trans*-resveratrol (around 2.5 μg g^{-1} fresh weight (FW)) accumulated in *V. rupestris* at 10 h after addition of either MeJA or SA; for cv. 'Pinot Noir', the accumulation was even fivefold lower. This contrasts with the around 20 μg g^{-1} FW found in *V. rupestris* at 10 h in response to Harpin treatment (Chang and Nick 2012). However, SA induced a strong accumulation of *trans*-piceid (approximately 23.1 μg g^{-1} FW at 10 h) only in cv. 'Pinot Noir', whereas in *V. rupestris*, modest levels of the toxic Δ-viniferin (84.4 μg g^{-1} FW) were produced. In contrast to SA, MeJA induced Δ-viniferin very strongly in *V. rupestris* (about 731.9 μg g^{-1} FW at 10 h, i.e., almost ninefold higher levels than SA). These Δ-viniferin contents were also around tenfold higher than those found after treatment with Harpin (Chang and Nick 2012). In contrast, MeJA failed to induce any Δ-viniferin in cv. 'Pinot Noir'. Conversely, cv. 'Pinot Noir' accumulated *trans*-piceid in response to MeJA to a level that was around 15 % of that seen after treatment with SA. Thus, while MeJA efficiently induced *StSy* transcripts in both genotypes, the corresponding metabolite pattern strongly differed: whereas cv. 'Pinot Noir' accumulated modest levels of the inactive *trans*-piceid, *V. rupestris* accumulated low levels of resveratrol and extremely high levels of the oxidative resveratrol dimer Δ-viniferin. In contrast, the response to SA

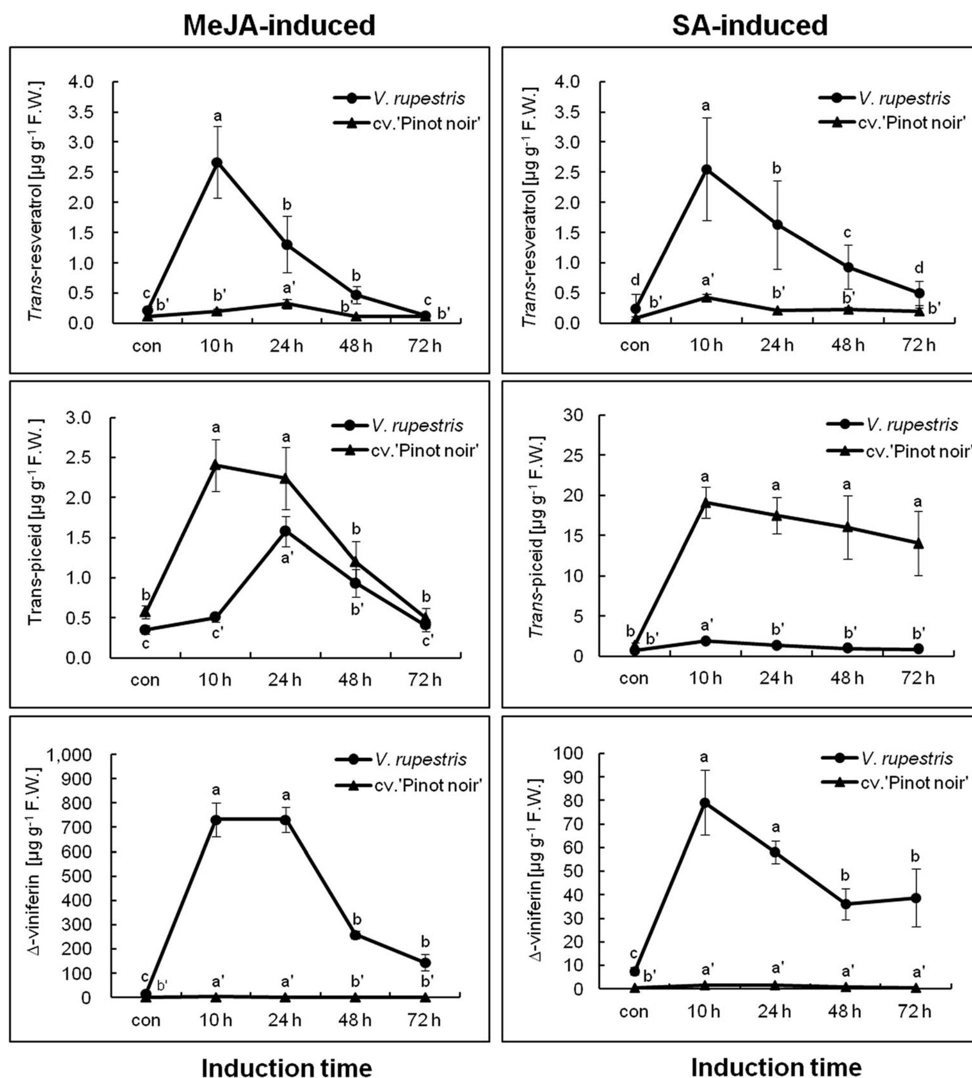

Fig. 4 Accumulation of stilbenes in response to SA and MeJA in *V. rupestris* and cv. 'Pinot Noir'. Time courses for the accumulation of *trans*-resveratrol (**a**), *trans*-piceid (**b**) and Δ-viniferin (**c**) after treatment with 200 μM SA or 100 μM MeJA in *V. rupestris* (**a**) and cv. 'Pinot Noir' (**b**) are plotted as mean values and standard errors from at least five independent experimental series. *Different lowercase letters* indicate difference significance at $P = 5$ % as compared to water control. *Arrows* at the bottom show that the stilbene accumulation of these samples was hardly detected

was very weak in *V. rupestris* with only about 10 % of Δ-viniferin formed at comparable (low) levels of *trans*-resveratrol. On the other hand, SA was effective in cv. 'Pinot Noir' inducing a significant accumulation of the inactive resveratrol glycosid *trans*-piceid.

MeJA and Harpin can synergistically induce cell death in cv. 'Pinot Noir'

Hypersensitive cell death is a characteristic feature of the advanced level of immunity such as ETI. Our previous studies have shown that Harpin induced cell death in *V. rupestris* but not in cv. 'Pinot Noir', whereas flg22, a 22-amino acid peptide, usually used as a PAMP, did not induce any significant cell death independently of the cell line (Chang and Nick 2012), although both elicitors induced qualitatively similar patterns of gene activation in both lines that were just moderately different in amplitude. Since we had found that Harpin induced the bioactive jasmonate conjugate JA-Ile exclusively in *V. rupestris*, but not at all in cv. 'Pinot Noir' (Fig. 2), we wondered whether a combination of Harpin with MeJA would be able to elicit cell death in a cell line (cv. 'Pinot Noir') that does not show any significant cell death in response to this elicitor. In preparation of this experiment, we examined the cell death in response to exogenous MeJA or SA alone (Fig. 5). Although both hormones could induce a small but significant response (around 5~8 % increased mortality compared to the untreated control) in *V. rupestris* (Fig. 5, left), there was almost no cell death in cv. 'Pinot Noir' (Fig. 5, right).

In the next step, we investigated combinations between Harpin and exogenous SA or MeJA with respect to cell death. For *V. rupestris*, MeJA increased cell death over Harpin alone (Fig. 6a, left). This increase was in the range of the mortality induced by MeJA alone (Fig. 5, left). In other words, Harpin and MeJA acted additively with respect to cell death at later time (48 and 72 h). The situation was qualitatively different for cv. 'Pinot Noir' (Fig. 6a, right). Here, the combination of Harpin and MeJA increased mortality by more than twofold, which contrasts with the absence of any mortality in response to MeJA alone at late time point (Fig. 5, right). In other words, Harpin and MeJA acted synergistically with respect to cell death as late as 48 h. Unlike MeJA, simultaneous treatment with SA did not modulate the frequency of cell death induced by Harpin, not even in an additive manner in neither of the two cell lines.

To see whether the modulation of cell death would correlate with a modulation of gene expression, we investigated the transcripts of *StSy* and *PAL*, along with *PR5* and *PR10*, and *JAZ1* (Fig. 6b) under the same conditions. We observed that although SA alone had failed to cause any change in these transcripts (Fig. 3), it significantly reduced the induction of *StSy* transcripts by Harpin (Fig. 6b). The same pattern was observed, however, at reduced amplitude, for *PAL*. In contrast to SA, MeJA did not alter the induction of these transcripts by Harpin. The pattern for *JAZ*1, although at a 2~3-fold lower level of modulation, was very similar: SA inhibited Harpin induction, whereas combination of MeJA with Harpin cannot be added in Harpin induction. In contrast, there was no significant change of *PR*5 triggered by Harpin when neither SA nor MeJA did exhibit any responsiveness to Harpin effect on transcripts of *PR*5 and *PR*10. In summary, although SA has little influence on Harpin-triggered cell death, it can downregulate Harpin-triggered expression of defence genes. In contrast, MeJA clearly modulates Harpin-inducible cell death (Fig. 6a), it does not alter Harpin-triggered expression of defence genes (Fig. 6b).

Discussion

Plants respond to different stress factors by different, often very specific responses. However, this specificity is brought about by only a limited number of molecular players that even

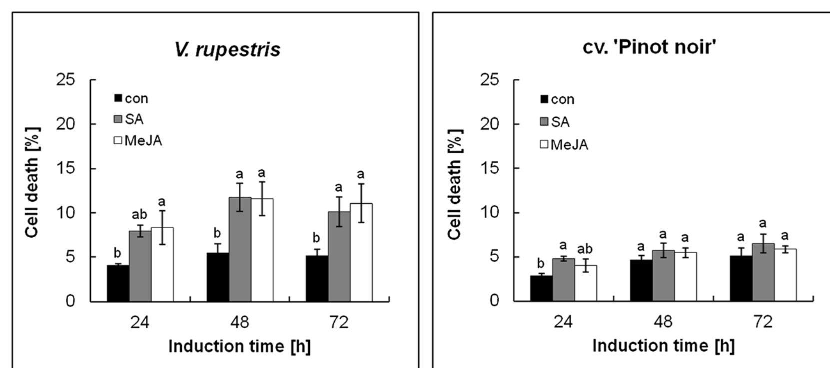

Fig. 5 Influence of SA or MeJA on cell viability in two cell lines. The mortality after treatment with SA (200 μM) and MeJA (100 μM) as compared to the solvent control for 24, 48 and 72 h in *V. rupestris* and cv. 'Pinot Noir' was followed over time scoring samples of 1500 cells for each data point. Mean values and standard errors from three independent experimental series are shown. Difference significance was depicted by different *lowercase letters* at $P = 5$ %

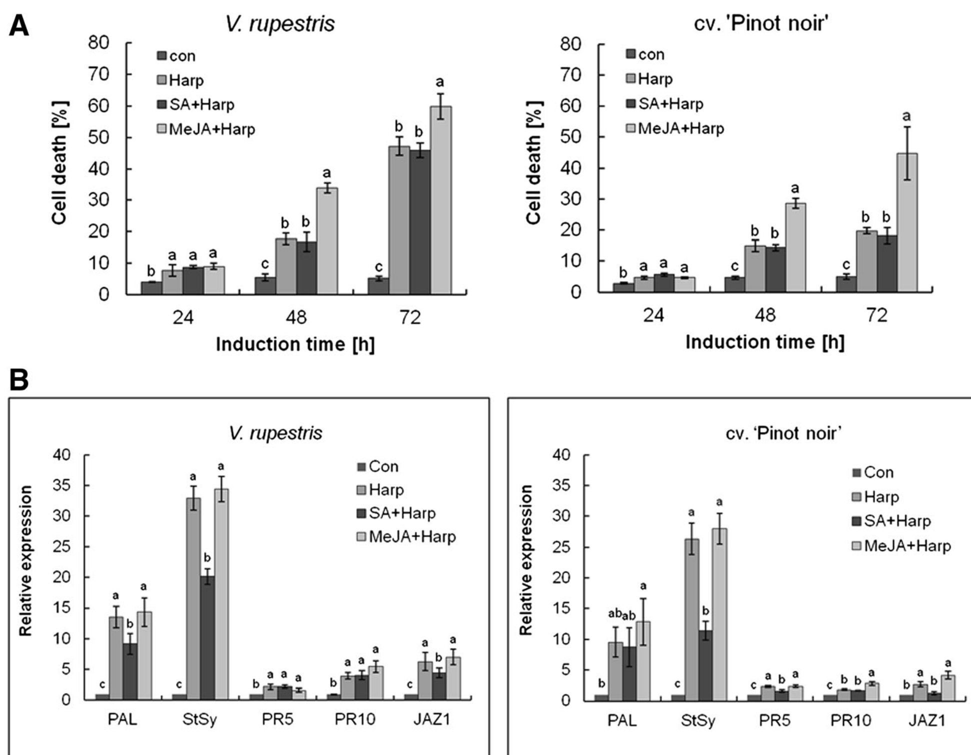

Fig. 6 Influence of SA or MeJA on Harpin-induced response in *Vitis.* **a** The mortality was tested after treatment with Harpin (Harp, 9 μg ml^{-1}), or SA (200 μM) in combination with Harpin or MeJA (100 μM) in combination with Harpin as compared to the solvent control for 24, 48 and 72 h in *V. rupestris* and cv. 'Pinot Noir' was followed over time scoring samples of 1500 cells for each data point. **b** Gene expressions were examined upon challenging by 9 μg ml^{-1} Harpin (Harp), 100 μM MeJA or 200 μM SA with Harpin protein as compared to water control for 1 h. The quantification was calculated relative to elongation factor 1α from four independent experimental series. Data show mean and standard errors from three independent experiments. Different *lowercase letters* indicate significance levels of differences as compared to the solvent control using ANOVA with the significance at $P = 5$ %

overlap in their activity, such as Ca^{2+}, ROS and jasmonates. To gain specificity with a small number of components requires that the spatiotemporal patterns of these players (their "signatures") have to be integrated and transduced into a differential output. Plant immunity can result in a qualitatively different output (basal defence versus cell death-related defence), although the molecular nature of the early events overlaps. This provides a typical example for this signature model. We have addressed this in an experimental model, where defence can be evoked either with (Harpin, *V. rupestris*) or without (flg22, cv. 'Pinot Noir') cell death. In the current work, we address the role of JA and SA as central hormones modulating defence. Our central finding is that the PAMP flg22 strongly induces jasmonic acid and its bioactive conjugate JA-Ile in *V. rupestris*. In the same cell line, exogenous MeJA can induce very high levels of toxic viniferins that are normally only seen in response to the elicitor Harpin. Nevertheless, MeJA cannot replace Harpin with respect to the induction of cell death, although it can replace Harpin with respect to the induction of defence-related genes. However, MeJA can activate cell death synergistically with Harpin in the cell line cv. 'Pinot Noir', where this elicitor alone fails to efficiently activate cell death. In contrast to MeJA, SA, although able to induce stilbene accumulation in form of inactive glycosides and to quell Harpin-induced gene expression, does not modulate Harpin-dependent cell death.

JA and cell death Jasmonic acid is well-known to regulate not only the plant response to wounding or herbivore attack but also to play a role in plant responses to pathogens. Defence-related cell death has been associated with accumulation of JA in tobacco (Kenton et al. 1999) and in *Arabidopsis* protoplasts (Zhang and Xing 2008). In grapevine, a hypersensitive-like response induced by exogenously added MeJA was observed in leaves (Repka 2001) and also in suspension cultured cells (Repka et al. 2004, 2013). In our cell system, we observed that flg22, as a PAMP which did not induce cell death (Chang and Nick 2012), induced a rapid and transient accumulation of JA and its bioactive conjugate JA-Ile in *V. rupestris*, whereas Harpin significantly induced cell death but only evoked a substantially weaker accumulation JA (reduced fourfold) and JA-Ile (reduced sixfold), respectively (Fig. 2). Although the levels accumulated in *V. rupestris* should still be sufficient to elicit jasmonate responses, it is questionable to what extent they contribute to signalling, because they were also strongly delayed in time

(with a maximum reached only 3 h after elicitation, compared to 30 min after eliCitation) in case of cv. 'Pinot Noir'. Interestingly, the strong accumulation of JA and JA-Ile in cv. 'Pinot Noir' remains transient, a phenomenon observed also for other stress responses of grapevine cells (salinity stress: Ismail et al. 2014a; mannitol stress: Ismail et al. 2015). Although this issue is still to be elucidated, there exist two molecular mechanisms that might account for the transient accumulation of JA and JA-Ile: JA activates the expression of *JAZ1*, a negative regulator of JA-dependent gene activation (Chini et al. 2009; Pauwels et al. 2009; reviewed in Kazan 2015). Since several of the JA synthesis genes are targets of JAZ1, this will not quell jasmonate signalling but also shut down the synthesis of additional jasmonate itself. This would explain why accumulation will not rise further. However, it would not explain why the abundance of JA and JA-Ile should decrease. This might be the point where a second mechanism comes in, namely, the degradation of jasmonic acid (Heitz et al. 2012) by catabolic cytochrome P450 proteins as well as the cleavage of the conjugate by hydrolases (Widemann et al. 2013).

Although the application of MeJA alone induces only a low level of cell death in both genotypes (Fig. 5), it can synergistically enhance the otherwise weak cell death response triggered by Harpin in cv. 'Pinot Noir' (Fig. 6a). This demonstrates that the accumulation of JA/JA-Ile induced by flg22 does not lead to cell death, whereas the accumulation of both compounds in the context of Harpin-induced signalling culminates in cell death. So, it is not JA/JA-Ile per se acting as signal for cell death but its interaction with different partners activated by different signalling chains. Although the molecular nature of these partners is not known, they seem to be evolutionarily conserved, since JA can induce programmed cell death also in mammalian cancer cells (reviewed in Frescher 2007) associated with signal events such as oxidative burst and MAPK induction. Both events are also observed in the cell response to flg22 and Harpin but with different temporal patterns (Chang and Nick 2012). How these differences in temporal signature are converted into the observed differences with respect to cell death still need to be elucidated.

SA and cell death SA-mediated immune responses are important components of both PTI and ETI (Tsuda et al. 2008, 2009) and can work in parallel with several other regulators of cell death (An and Mou 2011). In *Arabidopsis*, SA signalling was reported to be activated by both flg22 (Tsuda et al. 2008; Yi et al. 2014) and Harpin (Dong 2004). Interestingly, in our system, SA synthesis was not induced by either flg22 or Harpin in none of the cell lines (Fig. 2). This does not mean that these cells are not competent for SA signalling, because exogenous addition of SA inhibited Harpin-induced expression of *StSy* (Fig. 6b) and induced strong accumulation of the stilbene-glucoside piceid in cv. 'Pinot Noir' (Fig. 4). However, similar to MeJA, SA failed to induce significant cell death if administered alone (Fig. 5) and it also did not modulate Harpin-induced cell death (Fig. 6a). Thus, in contrast to JA, SA is not playing a role for Harpin-triggered cell death.

Phytoalexins and cell death There is an accumulating body of evidence demonstrating that resveratrol and its oxidised oligomers, the viniferins, are closely correlated with toxicity to pathogens and contribute to the necrosis-like HR at infection sites in *Vitis* cultivars (Jeandet et al. 2002; Pezet et al. 2004a, b; Alonso-Villaverde et al. 2011), whereas the glucoside piceid does not show any toxicity against *Plasmopara viticola* (Pezet et al. 2003). Our previous studies showed that Harpin induced a strong accumulation of resveratrol and its highly toxic oxidative dimer, δ-viniferin, whereas flg22 only elicited residual amounts of both products (Chang and Nick 2012). Furthermore, exogenous resveratrol induced unrestrained oxidative burst, actin bundling and programmed cell death in grapevine cells (Chang et al. 2011). Accumulation of stilbenes by MeJA has also been reported for several other grapevine cell lines (Belhadj et al. 2008; Krisa et al. 1999; Tassoni et al. 2005). In the current study, exogenous MeJA induced only low levels of resveratrol but high amounts of the toxic Δ-viniferin in *V. rupestris*. In contrast, SA, although inducing similar (low) amounts of resveratrol *V. rupestris*, will produce only tenfold lower levels of Δ-viniferin in *V. rupestris.* In contrast, it will accumulate high levels of the inactive piceid in cv. 'Pinot Noir' (Fig. 4). This means that the first product of the pathway, resveratrol, is converted to different derivates, depending on genotype and triggering hormone. It should be noted that the readout for resveratrol represents only the steady-state levels of this stilbene, dependent on the rate of synthesis and the rate of conversion into piceid or viniferins. In contrast, the values for piceid and viniferins as the final products will read out the integral over time. It is therefore easily understood why resveratrol can be relatively low, although piceid or viniferins are observed at high abundance. Interestingly, the strong accumulation of Δ-viniferin by MeJA in *V. rupestris* is not linked with cell death (Fig. 5). Thus, although Harpin is inducing strong levels of Δ-viniferin (Chang and Nick 2012) and although Harpin is also strongly inducing cell death (Qiao et al. 2010), strong induction of Δ-viniferin (MeJA, *V. rupestris*) is not sufficient to induce cell death. In other words, MeJA cannot mimic Harpin in this respect.

The responsiveness of stilbene synthesis to JA and SA differs qualitatively between the two lines, although the pattern of defence-gene expression is comparable. This discrepancy indicates that the regulation of stilbene metabolism must involve a strong post-translational component, and that this post-translational component is dependent of genetic factors. This conclusion is confirming the results from a comparative study of stilbene accumulation in leaves of an extensive

population of *V. sylvestris* (Duan et al. 2015). Thus, although there is a correlation between accumulation of stilbenes in non-glycosylated form and the ability to undergo defence-related cell death, the non-glycosylated stilbenes are not the cause of defence-related cell death. However, both events seem to be genetically coupled: cv. 'Pinot Noir' will always produce glycosylated piceid and be reluctant to die (but can be assisted to do so, if JA signalling is activated), and *V. rupestris* prefers accumulating non-glycosylated stilbenes, i.e., viniferins, and more easily activates cell death. The correlation of type of accumulated stilbene and the pathogen response of the respective donor plants is also seen in other genotypes: a cell culture derived from the pathogen-susceptible *V. vinifera* cultivar Gamay Fréaux produced large quantities of piceid but only traces of resveratrol upon elicitation with MeJA (Aumont et al. 2004). In contrast, a cell culture derived from the North American species *Vitis berlandieri* that is highly pathogen resistant and exhibits HR upon challenge by *P. viticola* produces large quantities of resveratrol and viniferins instead (Donnez et al. 2011). Our finding that the stilbene accumulation of two cell lines differs in their response to JA (accumulation of viniferin in *V. rupestris*) versus SA (accumulation of piceid in cv. 'Pinot Noir') implies that the genotypic differences within *Vitis* with respect to accumulation of piceid versus viniferins (Duan et al. 2015) might be linked with differences in the responsiveness to JA versus SA signalling.

Conclusion and outlook The role of JA signalling in defence-related cell death depends on the context (which elicitor? which genotype?) supporting the "signature model". In the context of PTI, JA is modulating defence-gene expression, stilbene accumulation, but does not trigger cell death. In the context of "ETI-like" defence as triggered by Harpin, JA is not modulating defence gene expression but can synergistically activate cell death. SA cannot activate defence gene expression, but it can quell Harpin-triggered gene expression (interestingly, the effect on the final stilbene products is different in cv. 'Pinot Noir', which again indicates post-translational control). SA is not linked with cell death in this context. This means that the role of JA for phytoalexin synthesis and the role of JA for cell death are different, which implicates a split of signalling. We find further that phytoalexin synthesis can be uncoupled from cell death, supporting its role for basal immunity. Consistent with the strong overlap between basal and cell death-related immunity, the accumulation of stilbenes is also activated in the context of Harpin-triggered defence.

Working hypothesis The accumulation of JA and JA-Ile induced during basal immunity might play a dual role—it activates defence gene expression, but it also interferes negatively with cell death. In case of Harpin (which also activates the basal immunity, but with some delay, see for instance the calcium influx in Chang and Nick 2012), the cells are already irreversibly committed for cell death when JA synthesis initiates (see Fig. 2). Thus, JA and JA-Ile, although still able to activate the gene expression, will not be able to suppress cell death. The PAMP flg22 activates calcium influx much more swiftly than Harpin (Chang and Nick 2012), such that JA and JA-Ile accumulate timely enough to quell the signalling events responsible for cell death. The bifurcation between basal immunity versus cell death-related immunity is thus correlated with differences in the temporal signature of jasmonate accumulation. A similar correlation between jasmonate signatures and adaptive versus cell death-related responses has also been found for the response of grapevine cells to salt stress (reviewed in Ismail et al. 2014b). Future work will be dedicated to identify molecular events participating in the signal chain responsible for cell death and to see their interaction with JA.

Acknowledgments We gratefully acknowledge Dr. Yusuke Jikumaru for his support of hormone analysis in this study. This work was supported by a fellowship of the Chinese Scholarship Council to Xiaoli Chang and by funds from the BACCHUS Interreg project.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests. All forms of financial support are acknowledged in the contribution. No agreement is signed with any sponsor of the research reported that forbids our publishing of this research without the prior approval of the sponsor.

References

An C, Mou Z (2011) Salicylic acid and its function in plant immunity. J Integr Plant Biol 53(6):412–428

Alonso-Villaverde V, Voinesco F, Viret O, Spring JL, Gindro K (2011) The effectiveness of stilbenes in resistant Vitaceae: ultrastructural and biochemical events during *Plasmopara viticola* infection process. Plant Physiol Biochem 49:265–274

Aumont V, Larronde F, Richard T, Budzinski H, Decendit A, Deffieux G, Krisa S, Mérillon JM (2004) Production of highly ^{13}C-labeled polyphenols in *Vitis vinifera* cell bioreactor cultures. J Biotechnol 109: 287–294

Belhadj A, Telef N, Saigne C, Cluzet S, Barrieu F, Hamdi S, Mérillon JM (2008) Effect of methyl jasmonate in combination with carbohydrates on gene expression of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures. Plant Physiol Biochem 46: 493–499

Bernoux M, Ellis JG, Dodds PN (2011) New insights in plant immunity signaling activation. Curr Opin Plant Biol 14:512–518

Bittel P, Robatzek S (2007) Microbe-associated molecular patterns (MAMPs) probe plant immunity. Curr Opin Plant Biol 10:335–341

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

Bostock RM (2005) Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu Rev Phytopathol 43:545–580

Browse J (2009) Jasmonate passes muster: a receptor and targets for the defense hormone. Annu Rev Plant Biol 60:183–205

Chang X, Heene E, Qiao F, Nick P (2011) The phytoalexin resveratrol regulates the initiation of hypersensitive cell death in *Vitis* cell. PLoS ONE 6:e26405

Chang X, Nick P (2012) Defence signalling triggered by Flg22 and Harpin is integrated into a different stilbene output in *Vitis* cells. PLoS ONE 7, e40446

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448:497–500

Chini A, Boter M, Solano R (2009) Plant oxylipins: COI1/JAZs/MYC2 as the core jasmonic acid-signalling module. FEBS J 276:4682–4692

Dong X (2004) NPR1, all things considered. Curr Opin Plant Biol 7:547–552

Donnez D, Kim KH, Antoine S, Conreux A, De Luca V, Jeandet P, Clément C, Courot E (2011) Bioproduction of resveratrol and viniferins by an elicited grapevine cell culture in a 2L stirred bioreactor. Process Biochem 46:1056–1062

Duan D, Halter D, Baltenweck R, Tisch C, Tröster V, Kortekamp A, Hugueney P, Nick P (2015) Genetic diversity of stilbene metabolism in Vitis sylvestris. J Exp Bot 66(11):3243–3257

Felix G, Duran JD, Volko S, Boller T (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J 18:265–276

Frescher E (2007) Jasmonates in cancer therapy. Cancer Lett 245:1–10

Fonseca S, Chini A, Hamberg M, Adie B, Porzel A, Kramell R, Miersch O, Wasternack C, Solano R (2009) (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nat Chem Biol 5:344–350

Gaff DF, Okong'O-Ogola O (1971) The use of non-permeating pigments for testing the survival of cells. J Exp Bot 22:756–758

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43: 205–227

Gómez-Gómez L, Boller T (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. Mol Cell 5:1003–1011

Grant M, Lamb C (2006) Systemic immunity. Curr Opin Plant Biol 9: 414–420

Heitz T, Widemann E, Lugan R, Miesch L, Ullmann P, Désaubry L, Holder E, Grausem B, Kandel S, Miesch M, Werck-Reichhart D, Pinot F (2012) Cytochromes P45 CYP94C1 and CYP94B3 catalyze two successive oxidation steps of plant hormone Jasmonoyl-isoleucine for catabolic turnover. J Biol Chem 287:6296–6306

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59:41–66

Ismail A, Riemann M, Nick Peter (2012) The jasmonate pathway mediates salt tolerance in grapevines. J Exp Bot 63(5):2127–2139

Ismail A, Seo M, Takebayashi Y, Kamiya Y, Eiche E, Nick P (2014a) Salt adaptation requires efficient fine-tuning of Jasmonate signalling. Protoplasma 251:881–898

Ismail A, Takeda S, Nick P (2014b) Life and death under salt stress: sam players, different timing? J Exp Bot 65(12):2963–2979

Ismail A, Seo M, Takebayashi Y, Kamiya Y, Nick P (2015) A Balanced JA/ABA status might correlate with adaptation to osmotic stress in *Vitis* cells. J Plant Physiol 185:57–64

Jaillais Y, Chory J (2010) Unraveling the paradoxes of plant hormone signaling integration. Nat Struct Mol Biol 17:642–645

Jeandet P, Douillet-Breuil AC, Bessis R, Debord S, Sbaghi M, Adrian M (2002) Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. J Agric Food Chem 50:2731–2741

Jeworutzki E, Roeflsema MR, Anschütz U, Krol E, Elzenga JT, Felix G, Boller T, Hedrich R, Becker D (2010) Early signaling through the *Arabidopsis* pattern recognition receptor FLS2 and EFR involves Ca^{2+}-associated opening of plasma membrane anion channels. Plant J 62:367–378

Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329

Jovanović AM, Durst S, Nick P (2010) Plant cell division is specifically affected by nitrotyrosine. J Exp Bot 61:901–909

Kazan K (2015) Diverse roles of jasmonates and ethylene in abiotic stress tolerance. Trends Plant Sci 20:219–229

Kazan K, Manners JM (2008) Jasmonate signaling: toward an integrated view. Plant Physiol 146:1459–1468

Kenton P, Mur LA, Atzorn R, Wasternack C, Draper J (1999) (−)-Jasmonic acid accumulation in tobacco hypersensitive response lesions. Mol Plant Microbe Interact 12:74–78

Kortekamp A (2006) Expression analysis of defence-related genes in grapevine leaves after inoculation with a host, a nonhost pathogen. Plant Physiol Biochem 44:58–67

Krisa S, Larronde F, Budzinski H, Decendit A, Deffieux G, Mérillon JM (1999) Stilbene production by *Vitis vinifera* cell suspension cultures: methyl jasmonate induction and 13^c biolabeling. J Nat Prod 62: 1688–1690

Le Henanff G, Heitz T, Mestre P, Mutterer J, Walter B, Chong J (2009) Characterization of Vitis vinifera NPR1 homologs involved in the regulation of Pathogenesis-Related gene expression. BMC Plant Biol 9:54

Mishina TE, Zeier J (2007) Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in *Arabidopsis*. Plant J 50:500–513

Nick P, Heuing A, Ehmann B (2000) Plant chaperonins: a role in microtubule-dependent wall-formation? Protoplasma 211:234–244

Nürnberger T, Brunner F, Kemmerling B, Piater L (2004) Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev 198:249–266

Nürnberger T, Lipka V (2005) Nonhost resistance in plants: new insights into an old phenomenon. Mol Plant Pathol 6:335–345

Pauwels L, Inzé D, Goossens A (2009) Jasmonate-inducible gene: what does it mean? Trends Plant Sci 14:87–91

Pezet R, Perret C, Jean-Denis JB, Tabacchi R, Gindro K, Viret O (2003) δ-viniferin, a resveratrol dehydrodimer: one of the major stilbenes synthesized by stressed grapevine leaves. J Agric Food Chem 51: 5488–5492

Pezet R, Gindro K, Viret O, Richter H (2004a) Effects of resveratrol, viniferins, pterostilbene on Plasmopara viticola zoospore mobility, disease development. Vitis 43:145–148

Pezet R, Gindro K, Viret O, Spring JL (2004b) Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. Physiol Mol Plant P 65:297–303

Pieterse CM, Leon-Reyes A, Van der Ent S, Van Wees SC (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5:308–316

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521

Qiao F, Chang X, Nick P (2010) The cytoskeleton enhances gene expression in the response to the Harpin elicitor in grapevine. J Exp Bot 61: 4021–4031

Reid KE, Olsson N, Schlosser J, Peng F, Lund ST (2006) An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biol 6:27

Repka V (2001) Methyl jasmonate induces a hypersensitive-like response of grapevine in the absence of avirulent pathogens. Vitis 40:5–10

Repka V, Fischerová I, Šilhárová K (2004) Methyl jasmonate is a potent elicitor of multiple defense responses in grapevine leaves and cell suspension cultures. Biol Plant 48:273–283

Repka V, Čarná M, Pavlovkin J (2013) Methyl jasmonate-induced cell death in grapevine requires both lipoxygenase activity and functional octadecanoid biosynthetic pathway. Biologia 68:896–903

Robatzek S (2014) Endocytosis: at the crossroads of pattern recognition immune receptors and pathogen effectors. Plant Cell Monogr 22: 273–297

Seibicke T (2002) Untersuchungen zur induzierten Resistenz a *Vitis* spec. PhD thesis. University of Freiburg

Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Lee JS, Choi YD (2001) Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. PNAS 98:4788–4793

Spoel SH, Koornneef A, Claessens SM, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Métraux JP, Brown R, Kazan K, Van Loon LC, Dong X, Pieterse CM (2003) NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15:760–770

Staswick PE, Tiryaki I (2004) The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. Plant Cell 16:2117–2127

Tassoni A, Fornalè S, Franceschetti M, Musiani F, Michael AJ, Perry B, Bagni N (2005) Jasmonates and Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv. Barbera cell cultures. New Phytol 166:895–905

Thomma BP, Nürnberger T, Joosten MH (2011) Of PAMPs and effectors: the blurred PTI-ETI dichotomy. Plant Cell 23:4–15

Tsuda K, Sato M, Glazebrook J, Cohen JD, Katagiri F (2008) Interplay between MAMP-triggered and SA-mediated defense responses. Plant J 53(5):763–775

Tsuda K, Katagiri F (2010) Comparing signalling mechanisms engaged in pattern-triggered and effector-triggered immunity. Curr Opin Plant Biol 13:459–465

Tsuda K, Sato M, Stoddard T, Glazebrook J, Katagiri F (2009) Network properties of robust immunity in plants. PLoS Genet 5, e1000772

Vlot AC, Dempsey DA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol 47:177–206

Wasternack C, Hause B (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann Bot Lond 111:1021–1058

Widemann E, Miesch L, Lugan R, Holder E, Heinrich C, Aubert Y, Miesch M, Pinot F, Heitz T (2013) The amidohydrolases IAR3 and ILL6 contribute to Jasmonoyl-isoleucine hormone turnover and generate 12-hydroxyjasmonic acid upon wounding in Arabidopsis leaves. J Biol Chem 288:31701–31714

Yoshimoto K, Jikumaru Y, Kamiya Y, Kusano M, Consonni C, Panstruga R, Ohsumi Y, Shirasu K (2009) Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signalling during senescence and the innate immune response in *Arabidopsis*. Plant Cell 21:2914–2927

Yi SY, Shirasu K, Moon JS, Lee S, Kwon S (2014) The activated SA and JA signaling pathways have an influence on flg22-triggered oxidative burst and callose deposition. PLoS ONE 9(2), e88951

Zhang L, Xing D (2008) Methyl jasmonate induces production of reactive oxygen species and alterations in mitochondrial dynamics that precede photosynthetic dysfunction and subsequent cell death. Plant Cell Physiol 49:1092–1111

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. Cell 125:749–760