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Bacterial Elicitor XTH and Salicylic Acid Regulate the Expression of Defence-Related Genes in Potato

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Abstract

Potato (*Solanum tuberosum*) is a major staple food crop susceptible to numerous pathogens and pests. Utilising biotic elicitors offers a promising integrative approach for crop management, potentially reducing losses and chemical treatments. One such elicitor, an inactive suspension of *Xanthomonas axonopodis* (XTH), has previously been shown to enhance potato resistance against pathogens, like the bacterium causing blackleg disease. However, the underlying mechanism of this resistance remains unclear. Thus, this study investigated the effect of XTH on the defence metabolism in potato plants and compared it to the response elicited by exogenous salicylic acid (SA), a well-known defence-signalling molecule. We analysed the expression of marker genes for defence response pathways, including JA/ET-responsive genes (*StPin2*, *StERF1*, and *StJAZ1/TIFY10A-like*) and SA-responsive genes (*StPR-1b*, *StPR-2*, and *StChtA*). Potato plants were treated with either SA and XTH, and both treated and systemic leaves were analysed. XTH upregulated all analysed genes locally and systemically within the first 24 h, except for *StChtA*. The XTH-mediated upregulation of *StPAL* and *Pin2* genes suggests this elicitor might trigger responses via the jasmonic acid pathway. Exogenous application of SA induced the systemic expression of *StPR*, *StChtA*, *StJAZ1/TIFY10A-like*, and *StERF* in potato plants. Our results indicate that XTH modulates the expression of defence-related genes in potato plants by simultaneously activating both the salicylic acid and jasmonic acid signalling pathways. This dual activation suggests that XTH could be a valuable resource for crop management in potato cultivation, potentially reducing the need for chemical pesticides.

Keywords Biotic inducer · Disease management · Phytohormones · *Solanum tuberosum* · Systemic resistance · *Xanthomonas*

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Introduction

Potatoes (*Solanum tuberosum* L.) constitute one of the most consumed and economically important staple food crops, recognised for their role in food security and poverty alleviation (FAO 2021; Zhang et al. 2024). While the nutritional content is affected by various factors like weather, potato variety, and soil conditions, potatoes are a good source of carbohydrates, potassium, phosphorus, magnesium, vitamins, proteins, and other potentially bioactive metabolites (Beals 2019; Bhutto et al. 2024). Beyond nutrition, potatoes support diverse industry sectors, including food, beverages, bioplastic, and bioenergy (Sampaio et al. 2020). Moreover, potato waste can be repurposed in the animal and human consumption, in the production of bio-packing and industrial bioprocesses, for instance, as a culture medium base for microorganisms used in biotechnological processes (Kot et al. 2020).

Annual global potato production reaches approximately 375 million tons (Devaux et al. 2020; FAOSTAT 2023). However, productivity is hampered by abiotic and biotic stressors resulting in substantial yield losses (Savary et al. 2019). Pathogens like *Alternaria* spp., *Fusarium* spp., *Rhizoctonia solani*, and *Phytophthora infestans* reduce potato yield by infecting healthy tissues and causing plants to grow smaller and produce fewer tubers. Although chemical agents have been extensively used to control plant diseases, the potential environmental and human health risks caused by pesticides are of concern (Kroschel et al. 2020). Understanding the mechanisms by which plants defend themselves against pathogens can contribute to the development of new strategies that are more sustainable, harmless, eco-friendly, and cost-effective.

Plants are well equipped with natural biochemical compounds to cope with pathogens. Upon infection by a pathogen, the plant host perceives pathogen-associated molecular patterns (PAMPs), which are conserved structures like flagellin, lipopolysaccharides, and chitin. These PAMPs are recognised by membrane receptors known as pattern recognition receptors (PRRs), which launch a signalling cascade resulting in the activation of defence responses (Chang et al. 2022). The signalling pathways often involve key hormones, such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), which modulate the plant response against pathogens. The responses mediated by SA play an important role in resistance against biotrophic pathogens, while JA and ET are often involved in defence responses against necrotrophs (Bigeard et al. 2015). The SA signalling pathway is controlled in plants by the regulatory protein NONEXPRESSOR OF *PR* GENES1 (NPR1) (Wu et al. 2012). SA triggers NPR1 translocation to the nucleus by inducing changes in the cellular redox state where NPR1 interacts with members of the TGA family of transcription factors to activate downstream responses, such as the expression of *PATHOGENESIS-RELATED* (*PR*) genes (Shigenaga and Argueso 2016). Activation of the SA signalling pathway at the infection site can trigger a similar response in distal plant parts to protect undamaged tissues,

process referred to as systemic acquired resistance (SAR) (Zehra et al. 2021). The JA signalling pathway is divided into two major branches: the MYC branch, associated with the defence against herbivores, and the ERF branch, which requires the collaboration with ET and mediates resistance against necrotrophic pathogens (Ruan et al. 2019). In *Arabidopsis thaliana*, the ERF branch is regulated by members of the APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) family of transcription factors, such as ERF1 and ORA59 (Pieterse et al. 2012). One central step in the JA signalling pathway is the COI1-mediated degradation of JAZ (JASMONATE ZIM-DOMAIN) repressor proteins, which activates JA downstream responses (Ruan et al. 2019).

Harnessing the natural defence mechanisms in host plants is a sustainable approach to plant disease management (Devaux et al. 2020). Inducing immune-related pathways to enhance their expression and bring about a more robust defence response is a promising area of research. Plant defence is mediated by elicitors, which are components that, once recognised, initiate the plant immune responses. These elicitors can be classified as biotic (including oligosaccharides, lipids, proteins, or pathogen toxins) or abiotic (such as physical or chemical agents) (Zehra et al. 2021). Both chemicals (e.g. salicylic acid, benzothiadiazole, and benzoic acid) and elicitors from microbial origin induce defence responses, including expression of pathogenesis-related genes and activation of defence-related enzymes (Meena et al. 2022). To this end, several natural and synthetic compounds have been shown to enhance the resistance of plants to pathogens and herbivores (Ahmad and Sharma 2023). For example, reticine-A, extracted from *Citrus reticulata* fruit peel, was tested against tobacco mosaic virus (TMV) and was more efficient than the commercial elicitor benzothiadiazole (BTH). Reticine-A induced hypersensitive reaction, systemic accumulation of H_2O_2 and SA, leading to an increased expression of defence-related enzymes and up-regulation of PR proteins through the expression of *NPR1* and SA biosynthesis genes (Wang et al. 2021). Our previous research has demonstrated that challenging plants with *Xanthomonas axonopodis*, an incompatible organism, induces defence responses in potato plants via a poorly understood mechanism (Poiatti et al. 2009). More recently, the use of an inactivated cell suspension of *X. axonopodis* (called XTH, US-8932844-B2) delayed the progression of black leg disease caused by *Pectobacterium atrosepticum* in detached potato leaves via activation of secondary metabolism and oxidative stress–related enzymes (Faillace et al. 2019).

Given the potential of biotic inducers or elicitors to stimulate plant defence mechanisms, elucidating the role of the biotic inducer XTH in diverse defence-signalling pathways could advance sustainable potato production. Thus, this study aimed to determine the pathway of XTH action on modulating the defence metabolism in *S. tuberosum* plants and to compare this response with that elicited by exogenous SA, a well-known defence-signalling molecule. To this end, we analysed the relative expression of JA/ET-responsive genes (*StPin2*, *StERF1*, and *StJAZ1/TIFY10A-like*) and SA-responsive genes (*StPR-1b*, *StPR-2*, and *StChtA*) to infer the putative phytohormone involvement in XTH-induced defence responses.

Materials and Methods

Plant Material and Growing Conditions

Potato tubers (*Solanum tuberosum* cv. Agata) were obtained from a local potato distributor in Southern Brazil. The tubers were initially washed with detergent to remove the surface dirt, followed by disinfection with a 1% sodium hypochlorite solution for 20 min. Subsequently, tubers were treated with 25 mg L^{-1} GA_3 for 30 min to induce uniform sprouting. The tubers were then placed under controlled conditions (14 h photoperiod and 25 ± 2 °C) for 20 days. Once sprouted, the tubers were planted in individual 11-L pots containing non-autoclaved soil to simulate natural field conditions. Four-week-old plants grown in a greenhouse were used for the experiments.

Preparation of the Elicitors and Plant Treatment

Two different elicitors, XTH and SA, were used in this study. The autoclaved bacterial suspension, designated as XTH, was prepared as previously described (Faillace et al. 2019). Briefly, *Xanthomonas axonopodis* pv. *citri* was cultured in liquid LB medium supplemented with 10 g L^{-1} sucrose for 72 h at 25 °C. Bacterial cells were pelleted and washed twice with sterile distilled water, resuspended to an optical density (OD_{600nm}) of 1.0, autoclaved for 20 min at 121 °C and stored at − 20 °C. Salicylic acid (SA) used as an elicitor to compare the induced responses with those triggered by the XTH was prepared as an aqueous solution (50 mM, Sigma-Aldrich®).

Elicitors were applied to a single leaflet (designated as 'treated leaf') of a mature leaf. Application was carried out by gently spreading the elicitor solution on the adaxial surface of the leaflet, until dripping point. Control plants consisted of untreated plants. Independent experiments were conducted for each elicitor (XTH and SA), using 81 plants per experiment. Control data were averaged, statistically analysed, and as no differences were found between control treatments. This combined control was then compared to both XTH and SA treatments.

Analysis of the Comparative Effect of XTH and SA on Defence Responses of Potato Plants

To investigate the pathway of XTH action on modulating the potato defence metabolism and how this response compares to those triggered by SA, the relative expression of defence-related genes was conducted. The treated leaf and the upper adjacent leaf (designated 'systemic leaf') were carefully collected at 0, 6, 12, 18, and 24 h after the onset of the experiment, immediately frozen in liquid nitrogen and stored at − 80 °C. Samples were used for RNA extraction using the CTAB method described by Gambino et al. (2008). The RNA integrity and quantity were evaluated through agarose gel electrophoresis and UV light absorption (280, 260, and 230 nm), respectively. To eliminate residual genomic DNA, the total RNA was treated with

DNase (Turbo DNA-*free*™ Kit—Ambion®), and cDNA was synthesised using the high-capacity cDNA reverse transcription kit (Applied Biosystems®), following the manufacturer's instructions.

For differential expression of the genes associated to defence metabolism, quantitative RT-PCR (RT-qPCR) was performed using primers for JA/ET-responsive genes (*StPin2*, *StERF1*, and *StJAZ1/TIFY10A-like*) and SA-responsive genes (*StPR-1b*, *StPR-2*, and *StChtA*), listed in Table 1. Reactions were run on a StepOne™ Real-Time PCR System (Applied Biosystems®), using SYBR® Green I (Invitrogen™) as the fluorescent reporter signal and ROX (Invitrogen™) as the passive reference dye. Cycling conditions were: 95 °C for 5 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min, with a final step consisting of a dissociation curve ranging from 60 to 95 °C. The specificity of the PCR amplifications was confirmed by sequencing of the amplification products, and the formation of primer-dimers was monitored by the presence of a single peak in the melt curve analysis. Target genes were normalised using *elongation factor 1-alpha* (*EF-1α*) as the reference gene (Nicot et al. 2005), and the mean relative gene expression was calculated according to Pfaffl (2001). Estimation of qPCR efficiencies was assessed using the LinRegPCR software v. 2014.6.

Identification of Putative TIFY and ERF Proteins

Potato TIFY proteins were identified in Phytozome v11 database (*S. tuberosum* genome v3.4) by searching for gene models that contained a putative TIFY domain

Table 1 List and sequence of primers used in the study. Primers were designed using the Primer3Plus v2.3.6 web interface (http://primer3plus.com)

Gene	Abbreviation	Primers (5'-3')
Pathogenesis-related protein 1b	*StPR-1b*	PR1b-F: TACCAACCAATGTGCAAGCG
		PR1b-R: TTGTCCGACCCAGTTTCCAA
Endo-1,3-β-glucanase	*StPR-2*	PR2-F: ATGGAACGAACAGGAGGAGG
		PR2-R: ATAGGTCCAGGCTTTCTCGG
Acidic class II chitinase	*StChtA*	ChtA-F: AATAGAGTGCCAGGGTACGG
		ChtA-R: CACCAGTGGGAACATTCAGC
Phenylalanine ammonia lyase	*StPAL*	PAL-F: GCAGTTGGTTCTGSTATGGC
		PAL-R: ACCAGGGTGATGCTTCAACT
Ethylene response factor 1	*StERF1*	ERF1-F: GGTTTAAATGAGCCGGAGCC
		ERF1-R: CCCCGGCTCTGAACTTCTAA
JAZ1/TIFY10A-like	*StJAZ1/TIFY10A-like*	JAZ1-F: GCGAGGCGGAATTCACTTAC
		JAZ1-R: GCACCTAATCCCAACCATGC
Proteinase inhibitor II	*StPin2*	Pin2-F: GGTACTTGTAAGCGCGATGG
		Pin2-R: CTGCACAACAGTTGGTGCAT
Elongation factor 1-alpha	*StEF-1α*	EF1α-F: CTGCACTGTGATTGATGCCC
		EF1α-R: ACCAGCTTCAAAACCACCAG

(Pfam PF06200) in the predicted amino acid sequence. All amino acid sequences obtained were then blasted in NCBI against *A. thaliana* non-redundant protein database using BLASTP algorithm. To further investigate the evolutionary relationship between potato and *A. thaliana* TIFY proteins, all sequences (including all members of the *A. thaliana* TIFY superfamily) were aligned using ClustalO (http://www.ebi.ac.uk/Tools/msa/clustalo/), and the resulting alignment was submitted to a Bayesian phylogenetic analysis using MrBayes v3.2.5, setting the mixed amino acid substitution model as default. Five million generations were run, and trees were sampled every 100 generations. The first 25% trees were discarded as burn-in, and the remaining ones were summarised in a consensus tree. Branches with less than 50% credibility value were condensed.

The potato StERF1 protein (accession code NM_001288674) was aligned against all members of the *A. thaliana* ERF and DREB subfamilies of the AP2/ERF superfamily, using ClustalO (http://www.ebi.ac.uk/Tools/msa/clustalo/). Six potato ERFs and four potato DREBs proteins identified by Bouaziz et al. (2015), as well as the tomato Pti4 (NM_001347076) and Pti5 (NM_001247058) proteins were also included in the analysis. The resulting alignment was submitted to a Bayesian phylogenetic analysis using the same parameters described above for the TIFY proteins.

Statistical Analysis

Relative gene expression data were analysed using Student's *t* test ($p \leq 0.05$) at each timepoint to compare treated or systemic leaves with untreated control plants. Data were analysed using the SPSS v25.0 software and expressed as means ± standard error. Graphs were generated with GraphPad Prism v.6.0 software (GraphPad Software Inc., 2015).

Results and Discussion

To understand the defence mechanisms induced by XTH, we first conducted experiments to assess the defence responses activated by salicylic acid (SA) in potato plants. SA is recognised as an elicitor of SAR, a response that is often characterised by accumulation of SA and the activation of large set of defence-related (*PR*) genes, via *NPR1* pathway (Pieterse et al. 2014).

SA Induces Local, but not Systemic Expression of PAL

Exogenous application of SA was able to induce the expression of several SA-responsive *PR* genes, such as *PATHOGENESIS-RELATED PROTEIN 1B* (*StPR-1b*), *ENDO-β-1,3-GLUCANASE* (*StPR-2*), and the acidic *CLASS II CHITINASE A* (*StChtA*), in treated and systemic potato leaves (Fig. 1a–c). Gene expression in systemic leaves was induced at 6 h and returned to basal levels at 12 h. PR proteins belong to 17 different families, including β-1,3-glucanases, chitinases, peroxidases, and thionines, which explains their diverse functions in plant defence (Jain

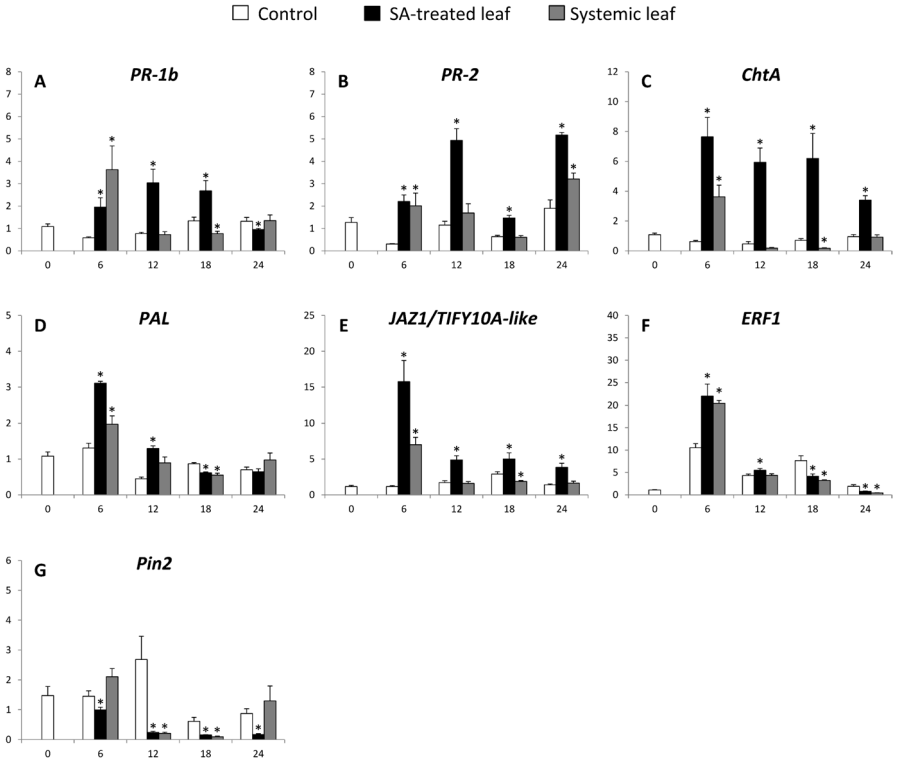

Fig. 1 Relative expression of defence-related genes in potato plants elicited with salicylic acid (SA) over time (in h). SA-treated and systemic leaves were compared to the leaves from untreated plants. Asterisks represent statistical differences from control according to Student's *t* test ($p \leq 0.05$). Error bars represent the standard error of the mean

and Khurana 2018). It is widely accepted that acidic PR proteins accumulate in plant tissues and organs in response to SA, playing an important role in defences against biotrophic pathogens, characteristic of hypersensitive responses and SAR (Jain and Khurana 2018). In potato, treatment with the elicitor SA was effective against the oomycete *Phytophthora infestans*, likely through the induction of PR proteins. However, the systemic plant protection may have also resulted from the synergism between multiple factors (Astha and Sangha 2019). Interestingly, SA treatment induced the expression of *PHENYLALANINE AMMONIA LYASE* (*StPAL*) in treated leaves, but not in systemic leaves (Fig. 1d). During defence responses, *PAL* expression in plants has been previously associated with the SA-signalling pathway (Derksen et al. 2013). However, there are a few contradictory reports regarding *StPAL* responsiveness to immune hormones in potato. Derksen et al. (2013) affirmed that both *StPAL-1* and *StPAL-2* are SA-responsive genes, but Arseneault et al. (2014) stated that *StPAL-2* is a JA-responsive gene. In the present study, the total *StPAL* transcript levels (*StPAL-1* + *StPAL-2*) were analysed, and we observed a longer local (6–12 h response of *StPAL* to SA compared to systemic leaves (Fig. 1d). PAL is

a key enzyme involved in the biosynthesis of SA in potato plants. While SA levels were not evaluated in this study, our findings suggest that the increment in phenylpropanoid pathway was not intense in systemic leaves during SA-mediated responses. Previous studies reported that arachidonic acid and infection of potato leaves with *P. infestans*–induced SAR and local accumulation of SA but failed to induce SA systemic accumulation (Coquoz et al. 1995). Given that the phenylpropanoid pathway is the primary source of SA synthesis in potato (Coquoz et al. 1998), the transient *StPAL* expression in systemic leaves may indicate a reduced SA level, even during SA-mediated defence response (Fig. 1d).

SA Treatment Induced the Expression of StJAZ1/TIFY10A-like and StERF1, but Repressed StPin2

SA induced local and systemic expression of the JA/ET marker genes *JASMONATE ZIM-DOMAIN PROTEIN 1/TIFY10A-LIKE* (*StJAZ1/TIFY10A-like*) and *ETHYLENE RESPONSE FACTOR 1* (*StERF1*) in potato plants (Fig. 1e–f). The predicted *S. tuberosum* JAZ1/TIFY10A-like protein was selected amongst several candidates as a putative marker for JA signalling based on its homology with the *A. thaliana* JAZ1/TIFY10A protein (Fig. 2). SA-treated leaves expressed *StJAZ1/TIFY10A-like* during all analysed time points (Fig. 1e), while systemic leaves accumulated *StJAZ1/TIFY10A-like* mRNA levels only transiently at 6 h (Fig. 1e). *StERF1* expression was also transiently induced by SA treatment, both locally and systemically at 6 h (Fig. 1f). Antagonism between the SA and JA pathways has been documented showing how the exogenous application of SA usually impairs JA responsiveness in plants (Li et al. 2019b). However, in tissues distant from the infection site this antagonism may be negligible and does not appear to be a determining factor in resistance against pathogens of different lifestyles (Shigenaga and Argueso 2016).

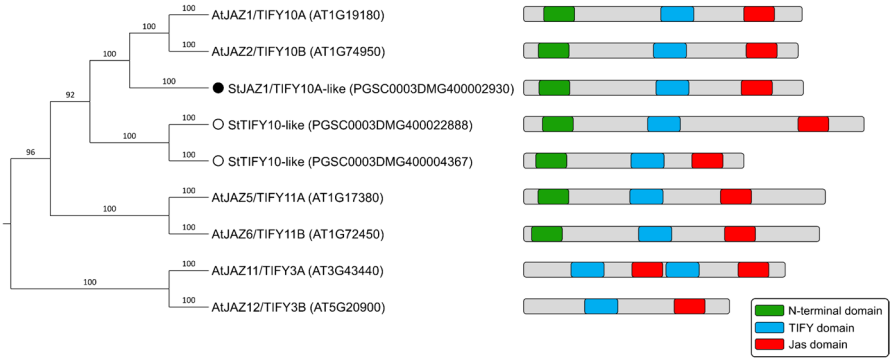

Fig. 2 Phylogenetic analysis of the JAZ subfamily groups I and II from *A. thaliana* and potato. Conserved domains were determined using MEME Suite v4.12 (http://meme-suite.org/tools/meme) and are represented with different colours in the right. The potato StJAZ1/TIFY10A-like protein described in this study is indicated with a black circle. Other potato proteins similar to *A. thaliana* TIFY10A and TIFY10B are indicated with empty circles. Support values are indicated on each branch of the phylogenetic tree. At = *A. thaliana*; St = *S. tuberosum*

Indeed, it has been suggested that SA and JA may occur at lower concentrations and act synergically, promoting broad-spectrum defence responses (Li et al. 2019b). Salzman et al. (2005) reported that sorghum plants show a transient accumulation of JA approximately 3 h after treatment with SA. From an evolutionary perspective, this mechanism may prevent the plant from remaining susceptible to the attack of necrotrophs when producing a defence response against a biotrophic pathogen, for example (Fu and Dong 2013).

In *A. thaliana*, the expression of *JAZ* genes is activated by JA (Pieterse et al. 2012). JAZ proteins, in turn, provide negative feedback regulation of the JA-signalling pathway by interacting with positive transcriptional regulators, such as MYC and EIN transcription factors (Kazan and Manners 2012). Van der Does et al. (2013) and Zander et al. (2014) reported that the mechanism by which SA suppresses the JA/ET-signalling pathway in *A. thaliana* relies on the transcriptional downregulation of the ERF transcription factor *OCTADECANOID-RESPONSIVE ARABIDOPSIS 59* (*ORA59*). Although *ORA59* appears to be a crucial node of convergence for SA-induced suppression of the JA/ET pathway, other molecular players, including NPR1, MPKs, and WRKYs, are equally important during SA-JA antagonistic interactions (Li et al. 2019a, b). By studying splicing variants of the *JAZ10* gene, Van der Does et al. (2013) concluded that *JAZ10* is not involved in the SA-mediated suppression of the JA-signalling pathway in *A. thaliana*. However, their finding does not necessarily exclude the involvement of other JAZ proteins in the SA-JA antagonism. In this context, it remains elusive whether *StJAZ1/TIFY10A-like* is involved in SA-JA antagonism in potato plants.

Nevertheless, whereas SA treatment induced the expression of *StJAZ1/TIFY10A-like* and *StERF1* in potato plants (Fig. 1e–f), the transcriptional level of *PROTEINASE INHIBITOR II* (*StPin2*) was locally (6, 12, and 24 h) and systemically (12 and 18 h) repressed by SA (Fig. 1g). *Pin2* is a well-established JA-responsive gene in both potato and *A. thaliana* plants (Turrà and Lorito 2011), and its repression by SA suggests that antagonism between the SA- and JA-signalling pathways occurred under our experimental conditions.

SA May Act Synergistically with ET in Potato

Although ET is widely recognised for its role in JA-induced resistance against necrotrophs, synergism between SA and ET has been reported (Pieterse et al. 2012). Therefore, the upregulation of *StERF1* expression in potato plants treated with SA (Fig. 1f) suggests a positive crosstalk between ET and SA in potato. In tobacco, ET perception is essential for SA accumulation and SAR development (Verberne et al. 2003). It was also shown that ET enhances the response of *A. thaliana* plants to SA via the ETHYLENE INSENSITIVE 2 (EIN2) transcription factor, resulting in increased expression of the SA marker gene *PR-1* (Pieterse et al. 2012). Thus, the expression of *StERF1* and other ET-related transcription factors during a SA-induced defence response in potato plants could contribute to enhanced expression of SA-responsive genes. Zander et al. (2014) reported that the expression of the ERF transcription factors *ORA59* and *ERF96* is greatly repressed in *A. thaliana* in the

presence of SA. However, the same was not observed for other *ERF*s (Van der Does et al. 2013; Zander et al. 2014) as the expression of several *ERF* genes remained elevated when *A. thaliana* plants were simultaneously sprayed with SA and the ET precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (Zander et al. 2014). Moreover, the tomato *Pti4* gene, a homologue of the potato *StERF1* gene (Fig. 3), was shown to be induced either by ET or SA, and it has been reported that SA can induce the expression of at least six *ERF* genes in potato (Bouaziz et al. 2015). Therefore, these findings suggest that some specific branches of the ET pathway could remain active in potato during SA-induced defence response.

The repression of *StPin2* transcription in potato plants following a treatment with SA corroborates our overall findings, since the upregulation of *JAZ* genes, as observed in *A. thaliana*, would result in stronger repression of JA-related genes, such as *Pin2*. Considering that the expression of *ERF*s is regulated by EIN and

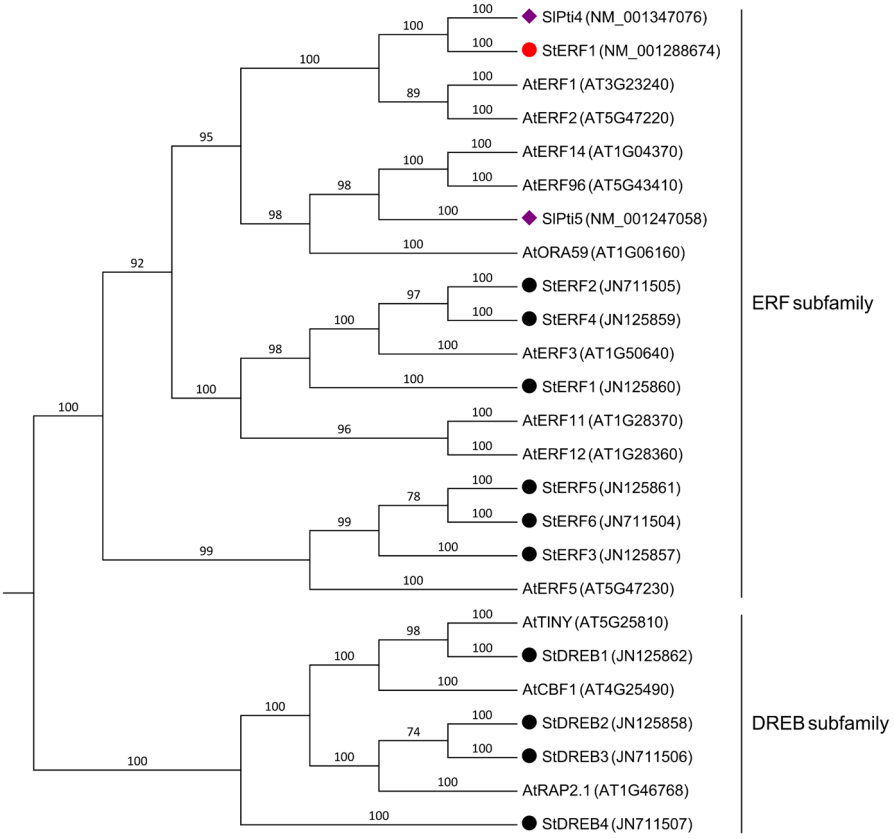

Fig. 3 Phylogenetic analysis of ERF and DREB proteins from *S. tuberosum* and *A. thaliana*. The potato ERF1 protein described in this study is indicated with a red circle on the phylogenetic tree. Potato ERF and DREB proteins described by Bouaziz et al. (2015) are indicated with black circles. The tomato Pti4 and Pti5 proteins are indicated with purple rhombuses. Branch support values are indicated respectively on the phylogenetic tree. At = *A. thaliana*; St = *S. tuberosum*; Sl = *S. lycopersicum*

ETHYLENE INSENSITIVE-LIKE (EIL) transcription factors, which in turn are negatively regulated by JAZ proteins, it could be assumed that SA-induced expression of *StJAZ1/TIFY10A-like* should also indirectly lead to repression of *ERF1* transcription. Nevertheless, 12 JAZ repressor proteins have been described in *A. thaliana*, and different JAZ proteins bind and inactivate different classes of transcription factors (Kazan and Manners 2012). Therefore, our results suggest that StJAZ1/TIFY10A-like protein does not appear to inactivate transcription factors involved in the expression of the *StERF1* gene (Fig. 1e–f). However, this remains to be demonstrated. In addition, it is still necessary to determine whether StERF1 protein is involved in the regulation of ET-responsive genes or enhanced expression of SA-related genes (or both) during a SA-mediated defence response.

XTH-Induced Defence Response Is Partly Mediated by JA

The XTH elicitor induced the expression of defence-related genes in potato plants. Nearly all genes analysed in this study were upregulated locally and/or systemically

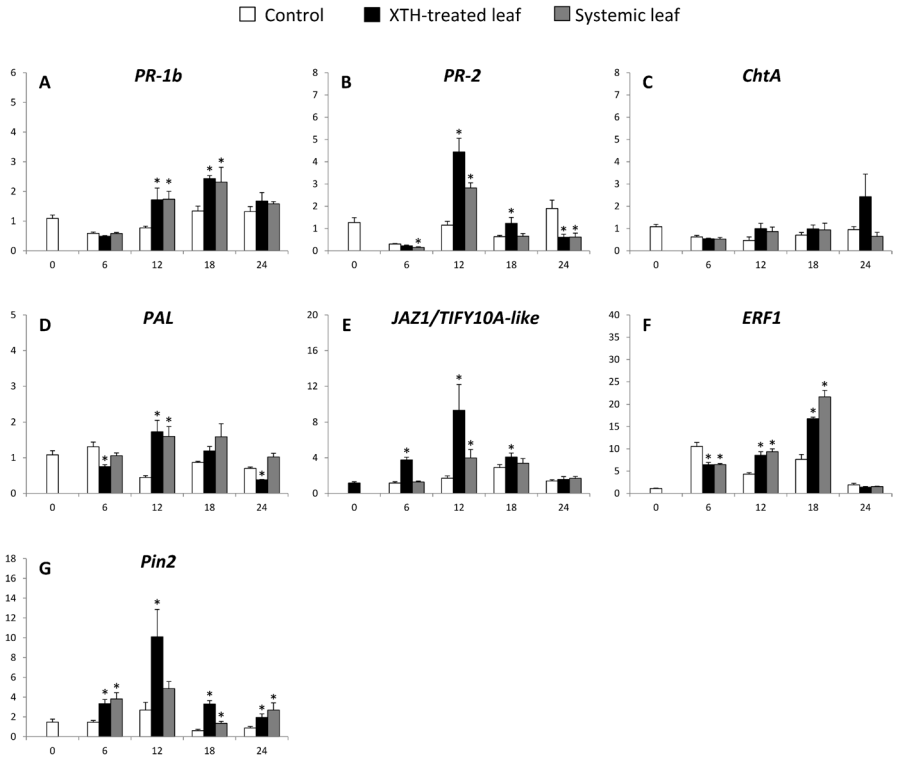

Fig. 4 Relative expression of defence-related genes in potato plants elicited with XTH over time (in h). XTH-treated and XTH-systemic leaves were compared to the leaves from untreated plants. Asterisks represent statistical differences from control according to Student's *t* test ($p \leq 0.05$). Error bars represent the standard error of the mean

by XTH during the first 24 h, except *StChtA* (Fig. 4a–g). Notably, as observed during SA application (Fig. 1a–b), XTH also induced transient expression of *StPR1-b* and *StPR-2* in treated and systemic leaves (Fig. 4a–b). In general, there was a delay in the responses triggered by XTH when compared to SA treatment (Figs. 1a–g and 4a–g). While SA was capable of activating local and systemic gene expression of SA-responsive genes as early as 6 h after the onset of the experiment (Fig. 1a–g), XTH-induced responses were generally observed at 12 h (Fig. 4a–g). This delay could be attributed to the nature of SA as a hormone, which can readily initiate transcriptional reprograming in plant cells. In contrast, XTH needs to be first recognised by membrane receptors, which then activates signalling cascades that ultimately result in the expression of defence-related genes.

Contrary to the response obtained with the SA elicitor, transient expression of *StPAL* was induced in systemic leaves by XTH at 12 h (Fig. 4d). This suggests that XTH-induced systemic *StPAL* expression is not mediated by SA but via an alternative pathway. Moreover, we observed that the expression of *StPin2*, a JA marker gene, was upregulated by XTH treatment (Fig. 4g), in contrast to what was observed with SA (Fig. 1g). Since *StPin2* is considered a robust marker for the JA-signalling pathway, this suggests that XTH-triggered defence responses are, at least partly, mediated by JA. Our results corroborate data from Derksen et al. (2013) and Arseneault et al. (2014), which suggest that *StPAL* gene is responsive to both SA and JA. In addition, the JA/ET-responsive genes, *StJAZ1/TIFY10A-like* and *StERF1*, were also upregulated by XTH treatment in potato leaves (Fig. 4e–f).

XTH-Induced Expression of SA-Responsive Genes

Contrary to findings suggesting that JA mediates XTH-triggered defence responses, we observed that two SA-responsive genes (*StPR1-b* and *StPR-2*) were also induced by XTH (Fig. 4a–b). These results indicate that XTH is involved in the simultaneous activation of JA/ET and SA-signalling pathways. Although SA and JA are traditionally reported as antagonistic hormones, synergistic effects have been reported (Li et al. 2019b). For instance, Xu et al. (2011) demonstrated that cotton defence responses to *Verticillium dahliae* involved a complex hormonal network that includes the SA, JA, and ET pathways. Davidsson et al. (2013) also discussed that SA and JA/ET play a central role in resistance against pectolytic bacteria. In *A. thaliana* plants, the defence response against the pathogen *Xanthomonas campestris* pv. *armoraciae* is primarily involves a combination of JA-, ET-, and SA-dependent pathways (Ton et al. 2002). Furthermore, Pieterse et al. (2012) reported that rhizobacteria-induced systemic resistance in *A. thaliana* also required JA, ET, and SA signalling via NPR1.

Xanthomonas species produce several macromolecules implicated in pathogenesis, such as extracellular enzymes (proteases, pectinases, and endoglucanases) and extracellular polysaccharides (xanthan gum). Other pathogenicity factors include the production of type III secretion system proteins encoded by *hrp* genes (Timilsina et al. 2020). As Gram-negative bacteria, *Xanthomonas* spp. also produce conserved structures such as lipopolysaccharides, flagellins, and glycoproteins (Timilsina et al.

2020). These molecules are potential PAMPs that can be recognised by PRRs and act as elicitors of plant immunity. Halim et al. (2009) has shown that PAMP-induced defence responses in potato plants inoculated with a PAMP from *P. infestans* require both SA and JA signalling.

In the present study, an autoclaved bacterial suspension (XTH) was spread on the surface of potato leaves. Epidermal leaf cells are expected to perceive this contact via PAMP recognition by PRRs. The upregulation of both SA- and JA-responsive genes in XTH-treated plants (Fig. 4a–g) suggests that XTH-induced defence responses, presumably occurring via PAMP trigger immunity (PTI), are mediated by both JA and SA, corroborating previous reports (Halim et al. 2009) indicating that PTI in potato may require both hormones. In addition, the activation of SA- and JA-mediated defences by the XTH elicitor could help explain the observed resistance against necrotrophic pectobacteria when potato plants were treated with XTH (Faillace et al. 2019).

Other studies demonstrate that elicitors can enhance plant resistance to pathogens by modulating signalling pathways. For instance, the protein elicitor PeFOC1, derived from *Fusarium oxysporum* f.sp. *cubense*, induced a defence response in tobacco plants against TMV and *Pseudomonas syringae* pv. *tabaci* by upregulating defence-related genes such as *Nt*PR1a, *Nt*NPR1, *Nt*PAL, *Nt*EDS1, and *Nt*LOX (Li et al. 2019a). Conversely, in potatoes, the use of the elicitor JA increased plant resistance to *P. infestans*. However, the effectiveness was influenced by the application timing and could also be affected by the cultivar used (Arévalo-Marín et al. 2021).

Conclusions

Our results suggest that the autoclaved *X. axonopodis* suspension (XTH elicitor) modulates the expression of defence-related genes in *Solanum tuberosum* plants by simultaneously activating both JA- and the SA-dependent pathways. Furthermore, our data indicate that the expression of the putative jasmonate repressor *StJAZ1/TIFY10A-like* may be regulated by SA in a JA-independent manner. The mechanism by which SA controls *StJAZ1/TIFY10A-like* expression in potato remains unknown, highlighting the need for further investigation using other molecular approaches. For instance, transgenic potato lines carrying silencing constructs for genes involved in the JA biosynthetic and/or signalling pathways could provide valuable insights. Additionally, potential targets of StJAZ1/TIFY10A-like and StERF1 should be investigated.

Since XTH may promote the concomitant activation of salicylic acid, ethylene, and jasmonate-related pathways, this elicitor might induce resistance against both biotrophic and necrotrophic microorganisms. Finally, the ability of the XTH elicitor to promote different defence-related pathways in potato plants offers a promising use for XTH as a valuable natural product for crop management, potentially reducing the use of chemical pesticides. Field trials are still necessary to test the interval of XTH application and its effectiveness against a broad range of pathogens.

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Author Contribution LVA and TS conceived and designed the experiments. TS performed the experiments. LVA, ERS, TS, VSF, and LFR analysed and interpreted the data. LVA, ERS, NRS, and TS wrote the paper, with contributions of LFR. All authors read and approved the final manuscript.

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Declarations

Competing Interests The authors declare no competing interests.

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