Heliyon 10 (2024) e30929

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Elicitation of salicylic acid and methyl jasmonate provides molecular and physiological evidence for potato susceptibility to infection by *Erwinia carotovora* subsp. *carotovora*

Sherien E. Sobhy [a,1], Asma A. Al-Huqail [b], Faheema Khan [b], Gehad Abd-Allah Ragab [c], Mohamed A. El-sheikh [d], Asia R. Ahmed [d], Ahmed A. Saleh [e,f,*,1,2], Elsayed E. Hafez [a,1]

[a] *Plant Protection and Bimolecular Diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, New Borg El-Arab, 21934, Egypt*
[b] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia*
[c] *Botany Department, Faculty of Science, Tanta University, Tanta, 31527, Egypt*
[d] *Plant Pathology Department, Faculty of Agriculture, Damanhour University, Egypt*
[e] *College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China*
[f] *Animal and Fish Production Department, Faculty of Agriculture (Al-Shatby), Alexandria University, Alexandria City, 11865, Egypt*

ARTICLE INFO

Keywords:
E. Carotovora
Potato cultivars
Antioxidant responses
Salicylic acid
Methyl jasmonate

ABSTRACT

Among the range of severe plant diseases, bacterial soft rot caused by *Erwinia carotovora* is a significant threat to crops. This study aimed to examine the varying response patterns of distinct potato cultivars to the influence of *E. carotovora*. Furthermore, it seeks to highlight the potential role of salicylic acid (SA) and methyl jasmonate (MeJA) in stimulating the antioxidant defence system. We collected eight bacterial isolates from diseased and rotted tubers which were morphologically and physiologically identified as *E. carotovora* subsp. *carotovora*. We conducted a greenhouse experiment to analyse the antioxidant responses of three different potato cultivars (Diamont, Kara, and Karros) at various time intervals (2, 4, 6, 8, 12, and 24 h) after bacterial infection (hpi). We assessed the extent of disease damage by applying a foliar spray of 0.9 mM salicylic acid (SA) and 70 μM methyl jasmonate (MeJA). Inoculating with *Ecc* led to an increase in total phenolic levels, as well as the activities and gene expression of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POX) as time progressed. Additionally, the application of SA and MeJA resulted in a further increase relative to the diseased treatments. The Karros cultivar, unlike the Diamont and Kara cultivars, demonstrated the highest expression levels of PAL, PPO and POX through inoculation, reflecting its higher levels of activity and resistance. Furthermore, the genetic response of potato cultivars to infection at 0 hpi varied depending on their susceptibility. The examination of the rate of PAL activity upregulation following SA or MeJA stimulation clarifies the cultivars' susceptibility over time. In conclusion, the study identified E. carotovora subsp. carotovora as the most virulent isolate causing soft rot

* Corresponding author.
E-mail addresses: sherienmorsey4@gmail.com (S.E. Sobhy), aalhuqail@ksu.edu.sa (A.A. Al-Huqail), kfaheema@ksu.edu.sa (F. Khan), Gehad.ragab@science.tanta.edu.eg (G. Abd-Allah Ragab), melsheikh67@yahoo.com (M.A. El-sheikh), Asia55@agr.dmu.edu.eg (A.R. Ahmed), Elemlak1339@Gmail.Com (A.A. Saleh), elsayed_hafez@yahoo.com (E.E. Hafez).
[1] These authors contributed equally to the current work.
[2] Current address: Yangzhou University, Yangzhou, 225009, China.

https://doi.org/10.1016/j.heliyon.2024.e30929
Received 17 November 2023; Received in revised form 29 April 2024; Accepted 8 May 2024
Available online 9 May 2024

disease in potato tubers. It further revealed that the Karros cultivar displayed superior resistance with high activities and gene expression of *PAL*, *PPO* and *POX*, while the cv. Diamont exhibited sensitivity. Additionally, foliar exposure to SA and MeJA induced antioxidant responses, enhancing the potato plants' resistance against *Ecc* pathogenesis and overall plant defence.

1. Introduction

Global agriculture, as a crucial economic asset, suffers significant losses due to pathogens that severely hinder yield productivity and quality [1]. The increased demand for vegetables, including potatoes, tomatoes, and eggplants, has engaged approximately 800 million people and contributed to more than 30 % of global agricultural production [2]. Potato (*Solanum tuberosum* L.) is a staple vegetable crop that plays a crucial role in food security due to its significant nutritional value, providing essential vitamins, proteins, minerals, and carbohydrates [3]. In addition to its significance as a crop, potatoes are also used as a food source and serve as a feedstock for various processed products. The potato crop faces constant threats from various pathogens in both the cultivated areas and during storage, leading to severe diseases that disrupt crop quality and quantity [4]. Moreover, potatoes have a wide distribution pattern, providing trait diversity and ensuring tolerance stability [5]. Therefore, the primary research goals include investigating the ability of different potato varieties to resist pathogens and enhancing plant tolerance against diseases.

Bacterial soft rot caused by *Erwinia carotovora* poses a significant threat to crops, leading to a loss in yields due to the rapid degradation of tuber tissue. *E. carotovora* is a type of facultative anaerobic, non-spore-forming, gram-negative enterobacteria that can invade a wide range of important crops [6]. The virulence of *E. carotovora* is attributed to the production of plant cell wall-hydrolysing enzymes, such as pectinases and cellulases, which are utilized by the bacterial cells [7,8]. *E. carotovora* has been widely recognized as the primary pathogen responsible for soft rot in crops like potatoes [9] and onions [10], especially under conditions of high moisture during storage.

To enhance plant stress tolerance, a comprehensive understanding of the physiological, biochemical and molecular mechanisms involved is crucial. Validation of such mechanisms and their underlying genetic basis is imperative for plants to develop adaptive responses to stress [11]. Among the different plant hormones, salicylic acid (SA) and Methyl jasmonate (MeJA) have been extensively investigated for their roles in mitigating the detrimental effects of both abiotic and biotic stressors [12–15].

SA acts as a signalling molecule that triggers a cascade of defence responses in plants. Also, it acts as a signal that moves throughout the plant, inducing the expression of defence genes and strengthening plant defences against future attacks [12–15]. One of the major roles of SA in plant defence is its involvement in the activation of systemic acquired resistance (SAR) [16,17]. SAR is a mechanism by which plants develop long-lasting resistance against a wide range of pathogens after being exposed to local infection. Additionally, SA plays a crucial role in the activation of hypersensitive response (HR), a rapid cell death at the site of infection [18,19]. HR is an effective defence mechanism employed by plants to restrict the spread of pathogens and is often accompanied by the production of toxic compounds that inhibit the growth of pathogens [20].

MeJA is a plant hormone that is involved in plant defence responses, particularly against herbivorous insects and necrotrophic pathogens [21]. MeJA is synthesized from linolenic acid and functions as a signalling molecule that regulates the expression of numerous defence genes [22]. When plants are attacked by herbivorous insects, MeJA is rapidly synthesized and promotes the production of defence-related compounds such as protease inhibitors, toxins, and volatile organic compounds (VOCs) [23]. These compounds deter further damage by inhibiting the feeding and attracting natural enemies of the herbivores. In response to necrotrophic pathogens, MeJA can also induce the production of defence compounds that inhibit the growth and spread of these pathogens. However, the role of MeJA in plant defence against pathogens is complex and can vary depending on the specific pathogen and plant species involved [24,25].

As a plant growth modulator, MeJA is known to regulate and induce induced systemic resistance (SR), thereby enhancing plant defence mechanisms. It exerts an indirect effect on promoting systemic acquired resistance (SAR) by modulating the transcription coactivator non-expressor of pathogenesis-related genes 1 (NPR1) [26]. MeJA also activates antioxidant responses in plants and helps mitigate oxidative damage by scavenging excessive reactive oxygen species (ROS) production in infected plants [27]. Moreover, the exogenous application of MeJA can rapidly induce endogenous jasmonic acid (JA) synthesis and signal transduction, thereby regulating the immune response and enhancing the plants' defensive capacity against various pathogens [28].

Collectively, SA and MeJA act as signalling molecules that initiate an array of defence mechanisms, including the activation of defence-related genes, the production of toxic compounds, and the recruitment of beneficial organisms to combat pathogens. Understanding the intricacies of these defence responses is crucial for developing effective strategies to enhance plant resistance against pathogens [15].

Additionally, plant disease resistance involves the activation of defence responses that hinder infection at different stages of the host-pathogen interaction [29]. These defences include physical and chemical barriers, as well as the activation of enzymes. The activities of certain enzymes, such as PAL, PPO and POX, are altered during the interaction between plants and pathogens [30–32]. In resistant cultivars, there are notable increases in the activities of PAL, PPO and POX enzymes, as well as phenolic compounds, following infection [33]. These changes in enzyme activity and phenolic content contribute to the plants' antioxidant response and help counteract oxidative stress. PAL is responsible for converting L-phenylalanine into *trans*-cinnamic acid, which leads to the synthesis of defensive compounds [34]. PPO catalyses the oxidation of phenols to more toxic quinones, while POX enhances plant resistance by producing lignin [35]. Phenolic compounds' have been found to mitigate the severity of pathogen-related diseases and

decrease the activity of bacterial enzymes. They also contribute to the formation of physical barriers that impede pathogen spread.

Against this backdrop, the objectives of this study were to investigate the response patterns of different potato cultivars to the influence of *E. carotovora*, also emphasizing the potential of SA and MeJA in stimulating the antioxidant defence system.

2. Materials and methods

2.1. Isolation and purification of bacteria

Diseased potato tubers exhibiting distinctive bacterial soft rot symptoms were collected from local markets, fields, and cold storehouses in El-Beheira governorate. The tubers with rotted parts were carefully cleaned with tap water and surface sterilized using 70 % ethanol. Slices of the inside tissues were then macerated in sterile water in glass tubes. A loop of the resulting suspension was streaked on the surface of nutrient agar media in 9-cm Petri dishes [36]. The plates were stored at 27 °C for 48 h to allow complete bacterial growth. Bacteria were then purified using the single colony isolation technique.

2.2. Tests of bacterial identification

Bacterial identification followed the method described in Bergey's Manual of Systematic Bacteriology [37,38]. Morphological and biochemical analyses were conducted on the isolated and purified bacteria [39–41].

2.3. Pathogenicity tests

A bacterial suspension was prepared from a 48-h-old culture. Uniform potato tubers weighing approximately 60 g from the highly susceptible cultivar (Diamont) were thoroughly cleaned and superficially sterilized. The tubers were then cut in half, and a 0.5 cm diameter pore was made in the centre of each half using a sterilized cork borer. A total of 250 μl of the bacterial suspension was pipetted into one half, while sterile water was used in the other half. The concentration of bacteria in a bacterial suspension for the experiment was 1×10^5 cells/ml. The infected Diamont tubers were stored in an incubator at 28 °C for two days. Then diameters of the rotten areas were measured using the method described by a reliable study [42].

2.4. Sensitivity to E. carotovora subsp. carotovora in different potato cultivars

Twelve different potato cultivars (Rossita, Mondial, Tarose, Karros, Hermis, Diamont, Kara, Belleny, Sisi, Brun, Crispy and Sponta) were assessed to evaluate their sensitivity to *E. carotovora* isolate. A highly virulent *E. carotovora* isolate (*E. carotovora* 1) was used, based on the rotting ability test. Tubers of both the control and treated groups were injected as described in the method of the pathogenicity experiment. Three replicates of each treatment were conducted. The diameters of the rotten areas were measured 48 h after incubation, serving as a measure of potato cultivar sensitivity to infection.

2.5. Greenhouse experiment

To study the potential of certain elicitors (AS and MeJA) on potato plants against *E. carotovora*, undamaged potato tubers from three cultivars (Diamont, Kara and Karros) weighing approximately 60 g were superficially sterilized by immersing them in 1 % sodium hypochlorite for 5 min, followed by washing the sterilized potatoes with sterile water twice. The sterilized potatoes were then planted in 30 cm plastic pots filled with sterile peat moss, with one potato per pot. After five weeks, the emerging shoots were sprayed with a solution of 70 μM methyl jasmonate and 0.9 mM salicylic acid [43,44]. After 7 days, small pores were made on the leaf surface using a micropipette. The leaves were then inoculated with a suspension of the highly pathogenic isolate *E. carotovora* 1 and placed in a controlled chamber at 25 ± 2 °C [43]. Ten pots were used per treatment, and each treatment was replicated three times for accuracy [44]. Leaves from the treated and control (water spray instead of elicitor) plants were collected at 2, 4, 6, 8, 12 and 24 h post inoculation (hpi) for further measurements.

2.6. Plant physiological assessments

2.6.1. Estimation of antioxidant enzymes

To extract phenylalanine ammonia lyase (PAL), 1 g of fresh potato leaves collected at 2, 4, 6, 8, 12 and 24 hpi was mixed with 3 ml of 0.1 M sodium borate buffer (pH 7.0) containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrrolidone (PVP). PAL activity was estimated according to Dickerson et al. [45]. The amount of *trans*-cinnamic acid produced was calculated using its extinction coefficient of 9.6 mM^{-1} cm^{-1} based on a standard calibration curve.

Peroxidase activity (POX) was evaluated using the method described by Hammerschmidt et al. [46]. Sodium phosphate buffer (0.1 M, pH 6.5) was used for enzyme extraction. The reaction mixture consisted of the enzyme extract and 0.05 M pyrogallol, with the addition of 1 % H_2O_2. POX activity was measured at 420 nm and expressed as a change in absorbance per min. per 1 g of fresh weight (μM/gFM min^{-1}). Polyphenol oxidase (PPO) activity was determined using a published method according to Mayer et al. [47] with minor modifications. The reaction mixture contained 0.01 M catechol was evaluated at 495 nm. PPO activity was expressed as a change in absorbance per min. per 1 g of fresh weight (μM/gFM min^{-1}).

2.6.2. Estimation of total phenolics

Total phenolic content in the treated and untreated leaves was assessed according to Zieslin et al. [48] with minor modifications. Plant material was mixed with 80 % methanol and agitated at 70 °C for 15 min. The resulting methanolic extract was then incubated for 1 h with Folin Ciocateau reagent and 20 % sodium carbonate at 25 °C and measured at 725 nm.

2.7. Molecular evaluation

2.7.1. RNA isolation and cDNA formation

Total RNA was extracted from potato leaves collected at 0, 2, 6 and 24 hpi using the BioTeke Corporation RNA Isolation kit II Guanidium isothiocyanate Method (Maxim Biotech INC, USA) following the manufacturer's procedure. The purified RNA was stored at −80 °C for further experiments.

Subsequently, reverse transcription (RT) of mRNA into complementary DNA (cDNA) was performed in the presence of dNTPs. The reaction conditions involved activating the enzyme at 42 °C for 1 h, followed by a denaturation step at 72 °C for 10 min to stop the reaction using the oligo (dT) primer (5'-TTTTTTTTTTTTTTT-3'). The resulting product was then stored at −20 °C for subsequent use.

2.7.2. Quantification of POX, PPO and PAL genes relative expression using real-time quantitative PCR

Three primers (POX, PPO and PAL) were used in this study, as listed in Table 1. The primers were obtained from Pharmacia Biotech (Amersham Pharmacia Biotech., UK Limited, HP 79NA, England). The samples were analyzed using the Fermentase kit, with a total reaction volume of 25 μL. Each reaction contained 12.5 μL of 2x Quantitech SYBR® Green RT Mix, 1 μL of 25 pmol/μL forward primer, 1 μL of 25 pmol/μL reverse primer, 1 μL of cDNA (50 ng), and topped off with RNase-free water. The reactions were carried out using the Rotor-Gene-6000 system (Qiagen, USA) with the following thermal cycling conditions: an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Data acquisition was performed during the extension stage. Quantification and calculation of fold change and relative expression of the target genes were conducted [49,50]. The result normalized to housekeeping gene 16 S rRNA.

2.8. Statistical analysis

A randomized experiment consisting of three replicates was used and the results in triplicate were analyzed using one way Analysis of Variance (ANOVA) with the assistance of "CoSTAT" software under windows. The statistical analysis was performed on a Windows platform. The mean values, representing an average of the three replicates, were computed alongside their respective standard deviations (±SD), and were subsequently displayed. To determine statistical significance, a significance level of $p \leq 0.05$ was employed.

3. Results

3.1. Characterization of causal bacterial pathogen

Eight isolates of *E. carotovora* were obtained from diseased potato tuber samples exhibiting clear symptoms of soft rot. All isolates demonstrated motility, rod shape, non-spore formation, and Gram-negative characteristics. Positive reactions were observed for catalase activity, gelatin liquefaction, and acid production from lactose, arabinose, mannose, raffinose and sorbitol. Additionally, the isolates showed growth in the presence of 6 % NaCl and at a temperature of 36 °C. However, the isolates did not exhibit starch hydrolysis, sensitivity to erythromycin, or the ability to produce acid from maltose, adonitol and dextrin, as indicated in Table 2.

3.2. Pathogenicity tests

To assess the pathogenicity of the eight identified isolates of *E. carotovora*, specific potato tubers (cv. Diamont) were subjected to infection. As shown in Table 3, the tested bacteria displayed varying levels of virulence compared to the healthy control. Isolates *Ecc*1, *Ecc*2 and *Ecc*6 were found to be the most virulent on cv. Diamont, while *Ecc*3 and *Ecc*8 were moderately virulent. Conversely, isolate *Ecc5* exhibited weak virulence compared to the untreated control ($p < 0.05$).

Table 1
Sequence of specific primers used in RT-PCR.

Genes		Primer sequence	Annealing (°C)
Phenylalanine ammonia lyaes (PAL)	F	TTCAAGGCTACTCTGGC	60
	R	CAAGCCATTGTGGAGAT	
Peroxidase (POX)	F	GCTTTGTCAGGGGTTGTGAT	
	R	TGCATCTCTAGCAACCAACG	
Polyphenol oxidase (PLX)	F	CATGCTCTTGATGAGGCGTA	
	R	CCATCTATGGAACGGGAAGA	

Table 2
Morphological, physiological, and biochemical attitude of *E. carotovora* isolates attained from infested potato tubers.

Characteristics	Bacterial isolates							
	Ecc1	Ecc2	Ecc3	Ecc4	Ecc5	Ecc6	Ecc7	Ecc8
Cell shape (Rods, single)	+	+	+	+	+	+	+	+
Sporulation	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+
Gram reaction	-	-	-	-	-	-	-	-
Catalase activity	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+
Hydrolysis of starch	-	-	-	-	-	-	-	-
Sensitivity to erythromycin	-	-	-	-	-	-	-	-
Soft rot symptoms	+	+	+	+	+	+	+	+
Growth on NaCl 6 %	+	+	+	+	+	+	+	+
Growth at 36 °C	+	+	+	+	+	+	+	+
Production of acid from								
Arabinose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Manose	+	+	+	+	+	+	+	+
Maltose	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-
Raffinose	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Dextrin	-	-	-	-	-	-	-	-

+; positive reaction, -; negative reaction.

Table 3
Pathogenicity tests of eight isolates of *E. carotovora* on Diamont potato tubers.

Isolate	Diameter of the rotted area (cm)[a]
Control	1.00 ± 0.05^{e}
Ecc1	3.58 ± 0.09^{a}
Ecc2	3.23 ± 0.15^{ab}
Ecc3	2.80 ± 0.13^{bcd}
Ecc4	2.42 ± 0.19^{d}
Ecc5	1.47 ± 0.06^{e}
Ecc6	3.12 ± 0.19^{abc}
Ecc7	2.43 ± 0.23^{cd}
Ecc8	2.87 ± 0.19^{bcd}

[a] Data are an average of three replicates ± SD. Means followed by the same letter(s) are not significantly different at ($p \leq 0.05$).

3.3. Sensitivity of different potato cultivars to Ecc

Based on the results presented in Table 3, the highly virulent isolate *Ecc1* was selected to assess the sensitivity of 12 potato cultivars (Rossita, Mondial, Tarouse, Karros, Hermis, Diamont, Kara, Belleny, Sisi, Brun, Crispy and Sponta). As shown in Fig. 1 and Table 4, different degrees of susceptibility to the *Ecc1* isolate were observed among the potato cultivars. The Karros and Belleny cultivars exhibited the highest resistance, while Tarouse, Diamont, Rossita and Mondial cultivars were found to be the most susceptibility ($p < 0.05$). Additionally, Kara, Sisi and Crispy cultivars displayed moderate susceptibility to *Ecc1* infection.

3.4. Physiological response of potato plants against E. carotovora and MeJA and SA as certain elicitors

3.4.1. Phenylalanine ammonia lyase activity

Fig. 2 illustrates the significant activation of PAL activity in the leaves of infested Kara and Karros cultivars compared to the non-diseased control. The cv. Karros exhibited a notable increase in PAL activity at 12 h post-invasion (hpi), surpassing the control. However, there was no significant increment in PAL activity observed in the cv. Diamont tissues after *Ecc*1 invasion compared to the control during most time intervals.

Moreover, the treatment with SA and MeJA sprays resulted in a significant enhancement of PAL activity in all cultivars, surpassing both the control and diseased samples. Among the elicitors, SA exhibited the most effective impact. In the cv. Diamont, SA treatment led to a gradual increase in PAL activity at 2, 4 and 6 hpi, followed by a decrease. On the other hand, MeJA triggered a short-lived response, with the highest PAL activity observed at 0 hpi, followed by a gradual decrease over time.

In the case of cv. Kara, SA treatment showed a progressive increase in PAL activity over time, with a more continual impact up to 12 hpi. On the contrary, MeJA caused a gradual up-regulation of PAL activity, reaching the highest increase of 41 % at 6 hpi compared to

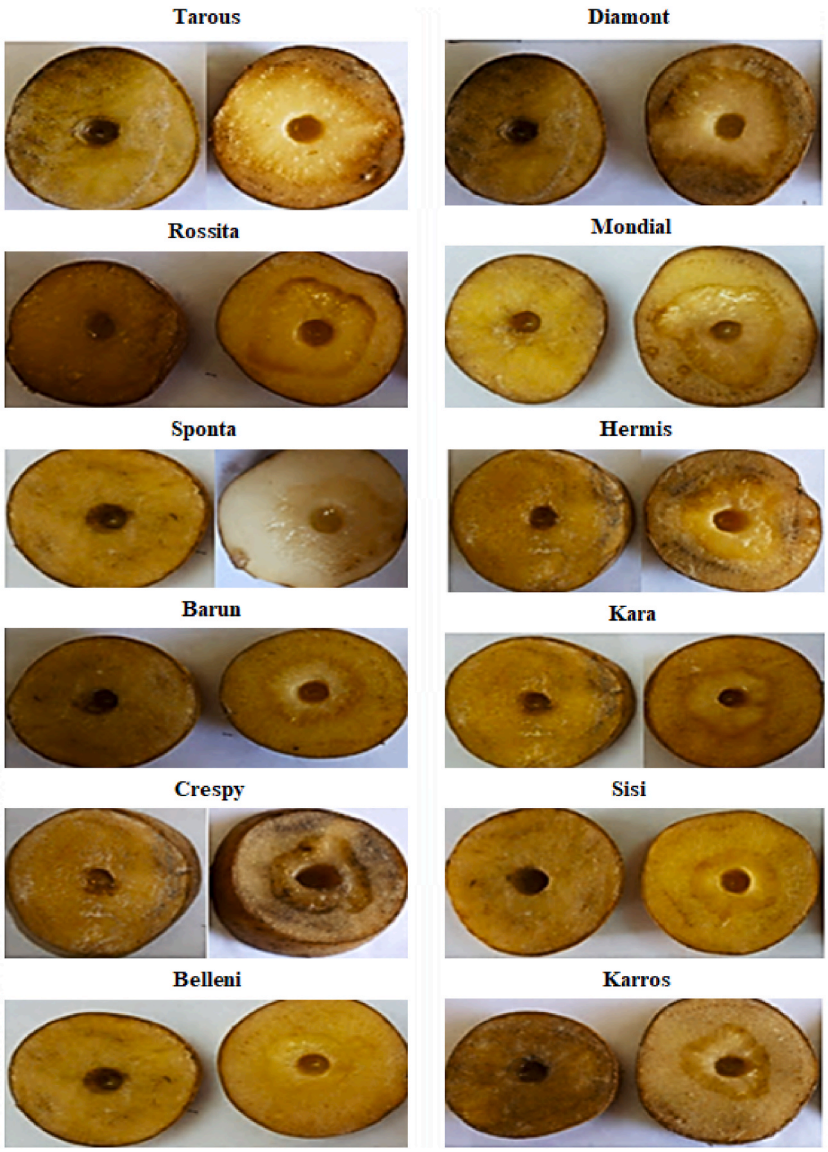

Fig. 1. Artificially inoculated tubers of 12 potato cultivars with *Ecc*1 isolate.

Table 4
Sensitivity of different twelve potato cultivars injected with *Ecc1* the highly virulent isolate.

Cultivars	Diameter of rotted area (cm)*	cultivars	Diameter of rotted area (cm)*
Karros	2.53 ± 0.19^{g}	Herims	3.12 ± 0.17^{bcde}
Rossita	3.38 ± 0.13^{ab}	Diamont	3.42 ± 0.24^{ab}
Kara	2.83 ± 0.08^{defg}	Belleny	2.53 ± 0.37^{g}
Mondial	3.27 ± 0.27^{abc}	Sponta	3.15 ± 0.27^{bcd}
Barun	2.93 ± 0.33^{cdef}	Crispy	2.76 ± 0.56^{efg}
Sisi	2.60 ± 0.10^{fg}	Tarous	3.63 ± 0.18^{a}

Data are a mean average of three replicates ± SD. Means followed by the same letter (s) are not significantly varied at ($P \leq 0.05$).

the stress treatment.

Notably, cv. Karros exhibited a more positive response to SA and MeJA treatments, showing a steady impact over time compared to other cultivars. PAL activity gradually increased and reached its maximum at 12 hpi in both SA and MeJA treatments, with increases of 40 % and 44 %, respectively, relative to the stress treatment.

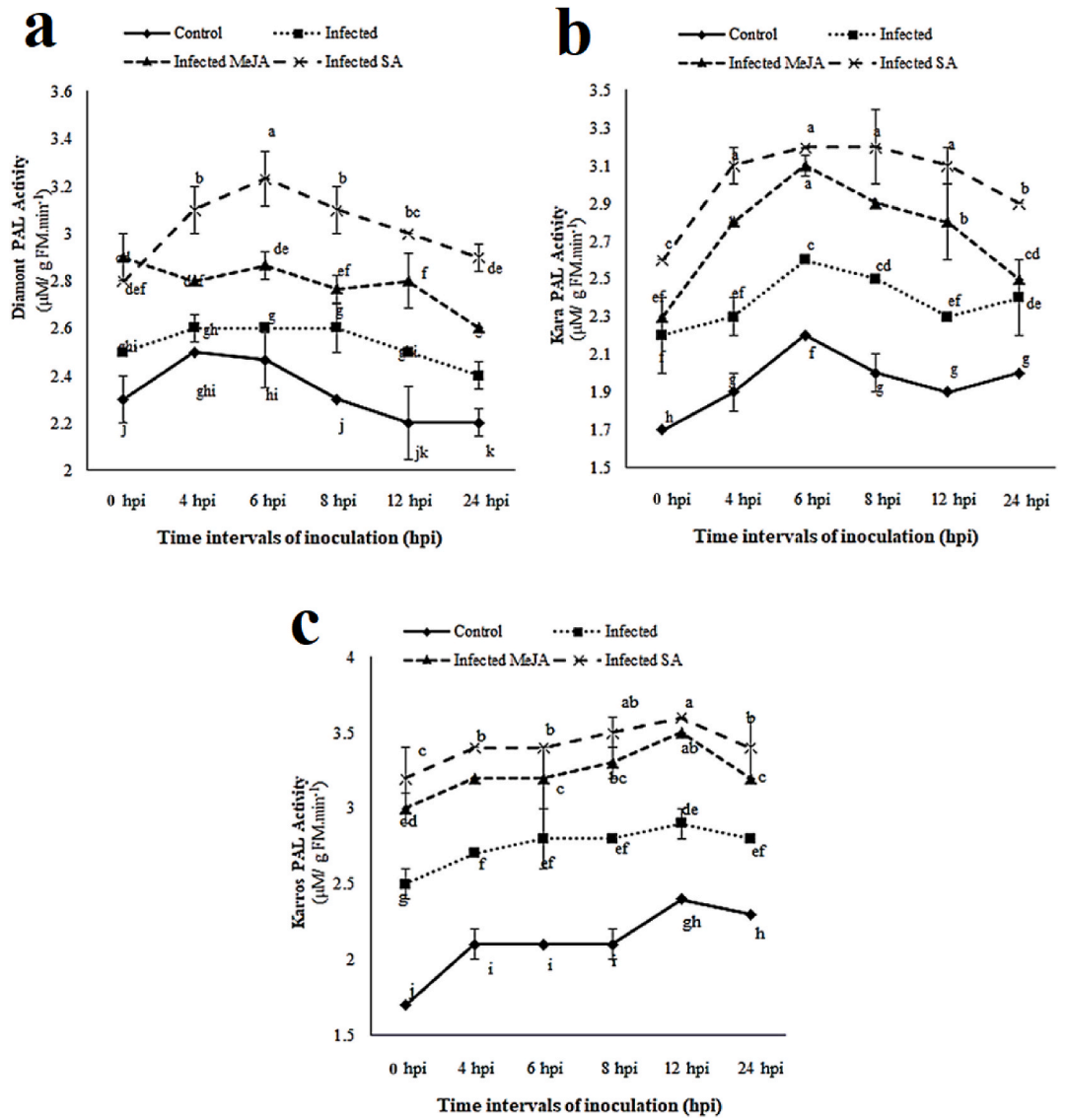

Fig. 2. Effect of *Ecc* infection on PAL activity (µM/g FM.min^{-1}) of cvs; **a)** Diamont, **b)** Kara and **c)** Karros at different time intervals of inoculation (2, 4, 6, 8, 12 and 24 hpi). Data are mean of triplicates ±SD and dissimilar letters designate statistically significant variations at $p \leq 0.05$.

3.4.2. Polyphenol oxidase (PPO) activity

The infection by *Ecc* significantly triggered PPO activity in the cvs. Diamont, Kara, and Karros cultivars compared to the control (Fig. 3). Particularly, cv. Karros showed the highest increase in PPO activity after pathogen infection at 6 hpi, by 44 %, followed by a gradual decrease compared to the non-diseased control.

The foliar application of SA and MeJA resulted in a further increase in PPO activity in the Diamont, Kara, and Karros cultivars, with SA demonstrating the most favourable improvement. In the cv. Diamont, PPO activity gradually increased with SA treatment, reaching its maximum at 12 hpi, with a 30 % increase compared to the control. MeJA-induced response was nonsignificant at 0 and 4 hpi, but a significant enhancement of PPO activity was observed after 4 hpi, reaching its highest value at 24 hpi compared to the biotic stress treatment.

With time, the resistance of Kara and Karros cultivars improved due to the application of SA and MeJA, resulting in the gradual increase of PPO activity. The highest significant activity was observed at 6 hpi in both cultivars.

3.4.3. Peroxidase (POX) activity

The data in Fig. 4 demonstrated that the diseased Diamont, Kara and Karros plants exhibited a significant induction in POX activity compared to the control at all the time intervals due to *Ecc* invasion. cv. Karros showed the most notable increase in POX activity at 12 hpi, with an increase of 40 % relative to the control, followed by cv. Kara (32 % at 12 hpi) and cv. Diamont (16 % at 8 hpi).

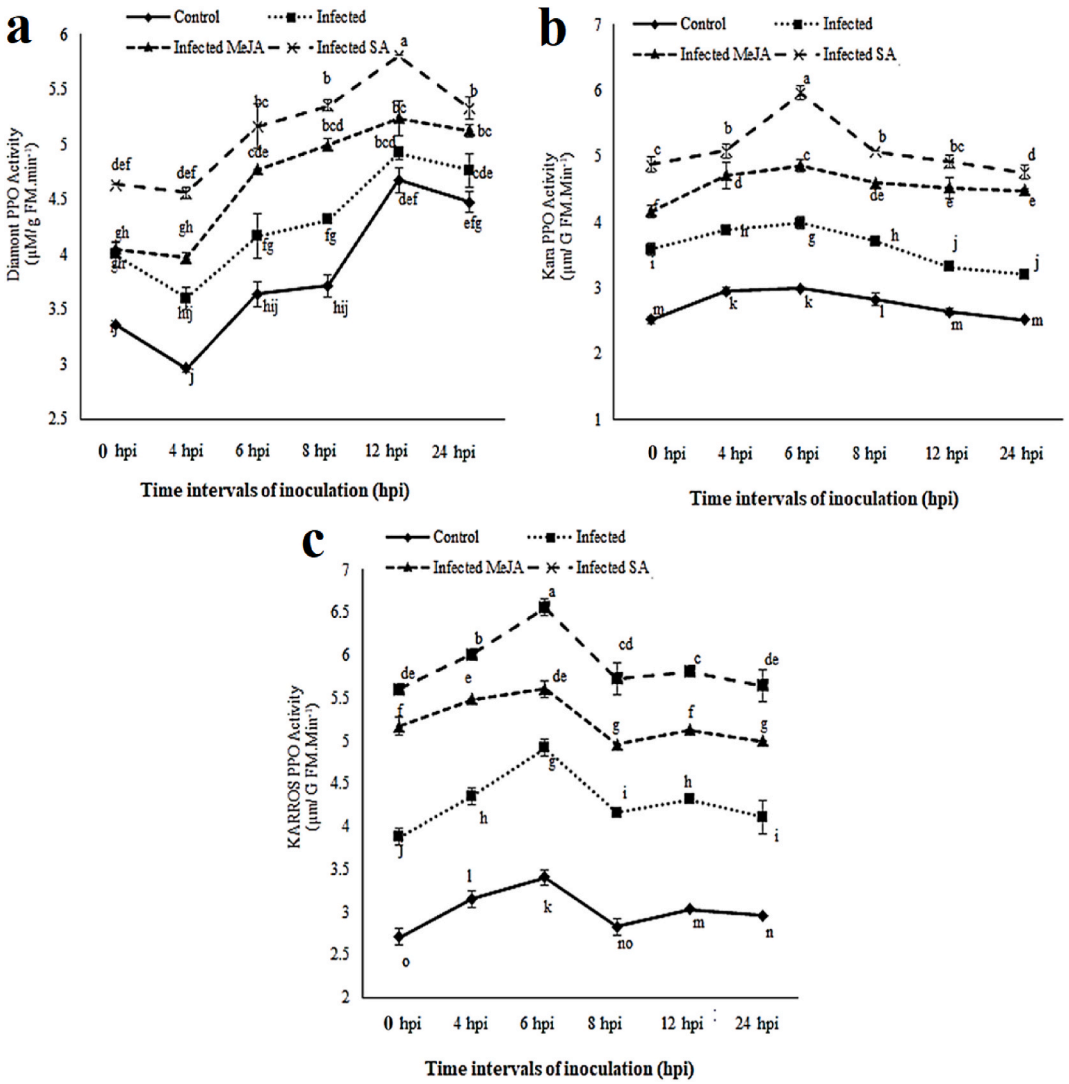

Fig. 3. Effect of Ecc infection on PPO activity (µM/g FM.min^{-1}) of cvs; **a)** Diamont, **b)** Kara and **c)** Karros at different time intervals of inoculation (2, 4, 6, 8, 12 and 24 hpi). Data are mean of triplicates ±SD and dissimilar letters designate statistically significant variations at $p \leq 0.05$.

SA and MeJA foliar exposure proved to be effective in increasing *POX* activity in the cvs. Diamont, Kara, and Karros compared to the infected treatments. In cv. Kara, SA amendment was responsible for the highest POX activity, while MeJA displayed a progressive increase over all time intervals. On the other hand, the greatest rise in POX activity was achieved by MeJA in cv. Diamont, surpassing the impact of SA. In cv. Karros, SA caused the most significant elevation in POX activity at 2, 4, 6 and 8 hpi, with the maximum increase observed at 4 hpi (32 %) relative to the stress treatment. Across all cultivars and treatments, the infected Karros plants sprayed with SA induced the most considerable increase in *POX* activity.

3.4.4. Phenolic content in potato leaves

Fig. 5 demonstrates that all potato cvs. (Diamont, Kara and Karros) exhibited an over-production of phenolic contents when infected with *Ecc*1, compared to the water-treated control. The accumulation of phenolics increased significantly over time in all cultivars following infection. Notably, the infected cv. Karros displayed the highest significant increase in phenolic content, ranging from 100 to 150 % across all time intervals, surpassing the other cultivars.

Additionally, the data presented in Fig. 5 revealed that the application of SA and MeJA significantly enhanced the total phenolic content in the infected leaves of cvs. Diamont, Kara and Karros, compared to the *Ecc*1 infected samples. In the case of cvs. Diamont and Kara, spraying with SA resulted in the highest recorded promotion of phenolics compared to their stressed counterparts. Furthermore, both SA and MeJA induced the highest phenolic content in the cv. Karros at 12 h post-infection, representing a 33.5 % increase relative to the infected treatment. Overall, the infected leaves of the cv. Karros exhibited the highest increase in total phenolic content after SA and MeJA application, when compared to the other cultivars.

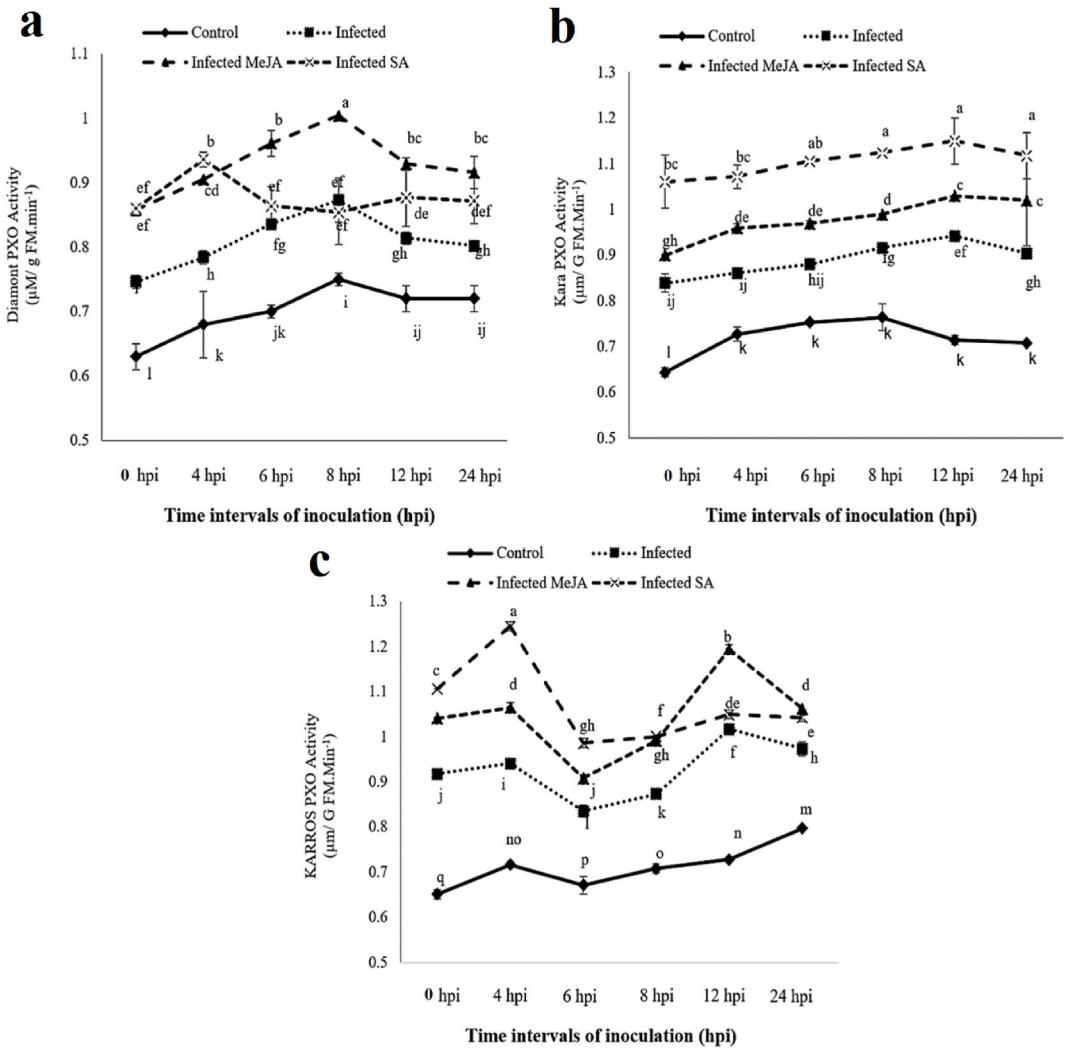

Fig. 4. Effect of Ecc infection on POX activity (µM/g FM.min^{-1}) of cvs; **a)** Diamont, **b)** Kara and **c)** Karros at different time intervals of inoculation (2, 4, 6, 8, 12 and 24 hpi). Data are mean of triplicates ±SD and dissimilar letters designate statistically significant variations at $p \leq 0.05$.

3.5. Quantification of PAL, PPO and POX gene expression

Figs. 6–8 depict the significant up-regulation of *PAL*, *PPO* and *POX* genes in the cvs. Diamont, Kara, and Karros in response to bacterial infection, gradually increasing over time. The highest improvement in gene expression levels was observed in the cv. Karros, particularly at 2- and 4-hrs post-infection (hpi), compared to the cvs. Diamont and Kara. At 0 hpi of *Ecc* invasion, the three cultivars showed variable genetic reactions based on their sensitivity. Notably, cvs. Diamont and Kara exhibited a rapid response with significant enhancement in PAL, PPO and POX expression starting at 0 h. Overall, the cv. Karros displayed the greatest expression levels of PAL, PPO and POX at 6, 12 and 24 hpi, respectively, compared to their control levels, with an increase percentage of 150, 94 and 130 %, respectively.

Moreover, the application of SA and MeJA significantly triggered the expression levels of *PAL*, *PPO* and *POX* genes in comparison to control and infested treatments. Among the treatments, SA exhibited the most effective impact, causing distinct improvement in *PAL* and *PPO* genes' expression over time in all cultivars. In comparison to the control, PAL reached its maximum expression level at 0 hpi (a 111 % increase), while *PPO* gene recorded a 57 % increase at 24 hpi in the cv. Diamont. Furthermore, PAL expression in the cvs. Kara and Karros significantly increased at 6 hpi, by 124 % and 113 % respectively, compared to the control. The potato leaves of the cv. Karros, sprayed with SA, displayed the highest increase in *PAL* and *PPO* genes' expression among the three cultivars.

With regards to *POX* gene expression, the application of SA and MeJA led to a progressive elevation over time in the cvs. Diamont and Kara, peaking at 24 hpi. Notably, SA effectively increased *POX* gene expression by 47 % in the Kara cultivar, while MeJA had a favourable impact of 25 % in the cv. Diamont at 24 hpi, relative to the diseased treatment. Conversely, the cv. Karros exhibited maximum *POX* gene expression with MeJA treatment at 24 hpi, while SA resulted in marked over-expression of the *POX* gene at 0 hpi,

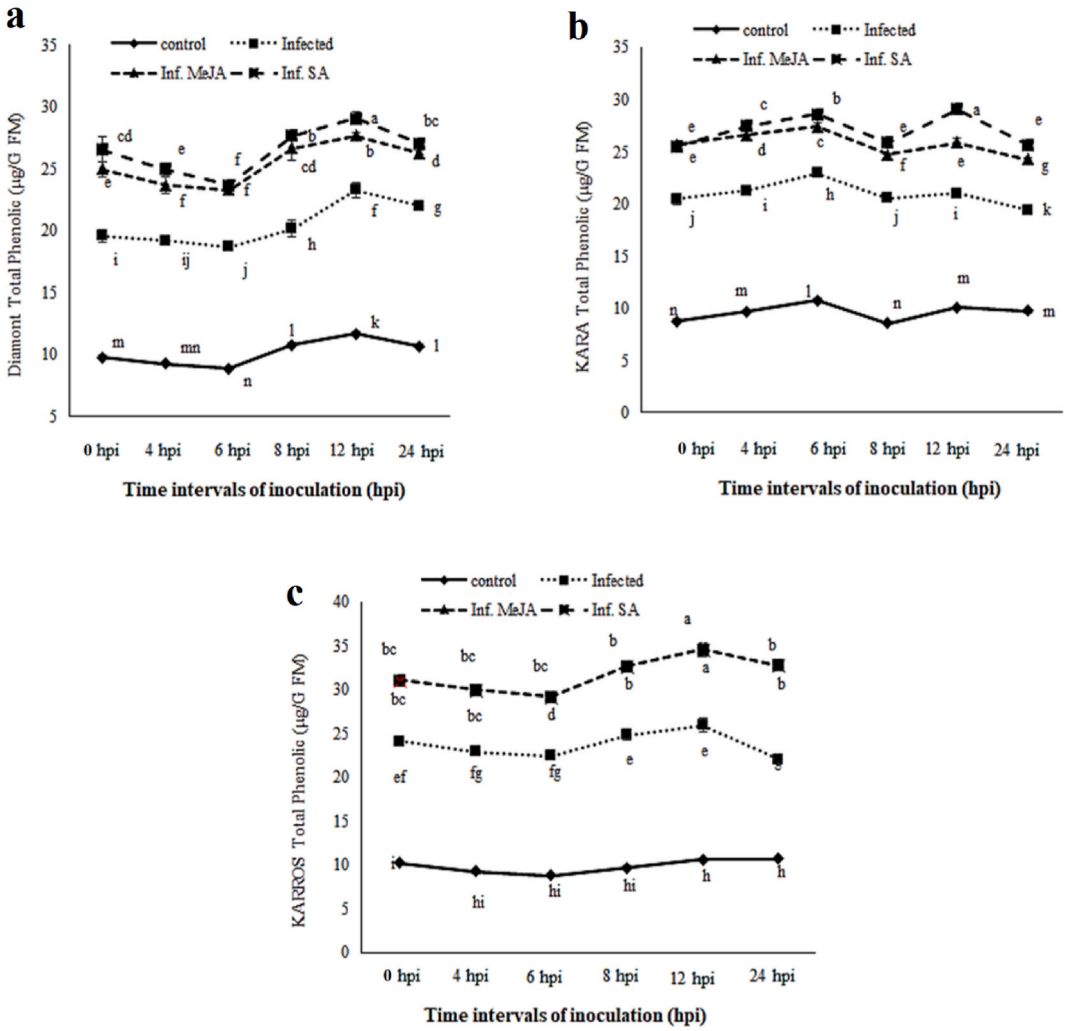

Fig. 5. Effect of *Ecc* infection on total phenolic content of cvs; **a)** Diamont, **b)** Kara and **c)** Karros at different time intervals of inoculation (2, 4, 6, 8, 12 and 24 hpi). Data are mean of triplicates ±SD and dissimilar letters designate statistically significant variations at $p \leq 0.05$.

surpassing the levels observed in the cvs. Diamont and Kara.

4. Discussion

Potatoes are a vital economic crop in Egypt and many other countries. However, their quality and production are often hindered by various factors, including the prevalence of soft rot disease, which is primarily caused by *E. carotovora*. This phytopathogen enters the plant through different natural openings, such as stomata, lenticels and wounds [51].

In the current study, in order to assess susceptibility to bacterial soft rot, it was crucial to examine the defence response mechanisms in various potato cultivars. We observed varying degrees of the rotten area, indicating differences in the virulence of *E. carotovora* subsp. *carotovora* isolates on different potato cultivars. This finding aligns with a previous study where potato parts inoculated with different bacterial isolates exhibited rapid rot and discoloration after 24 h [52]. A previous research has also confirmed the variation in sensitivity to bacterial soft rot among potato cultivars [33]. These differences in susceptibility may be attributed to factors such as increased dry constituent content, calcium content and pectin methylation [53]. Additionally, the activity of cell wall hydrolysing enzymes is influenced by the natural cell wall configuration, which can be altered by high cell wall content and normal constituents in the middle lamella [54]. On the other hand, several reliable studies confirmed that the genetic factors and the function of the plant's defence system have a significant effect [55–57].

Among the potato cultivars examined, cv. Karros demonstrated the most notable increases in PAL, PPO and POX activities over time in response to infection. This was followed by cv. Kara and then cv. Diamont. Additionally, infected cv. Karros exhibited the highest phenolic content throughout all time intervals, suggesting a higher level of resistance compared to cvs. Kara and Diamont. However, the susceptibility of cv. Diamont to bacterial infection could be attributed to the relatively insignificant increase in PAL activity

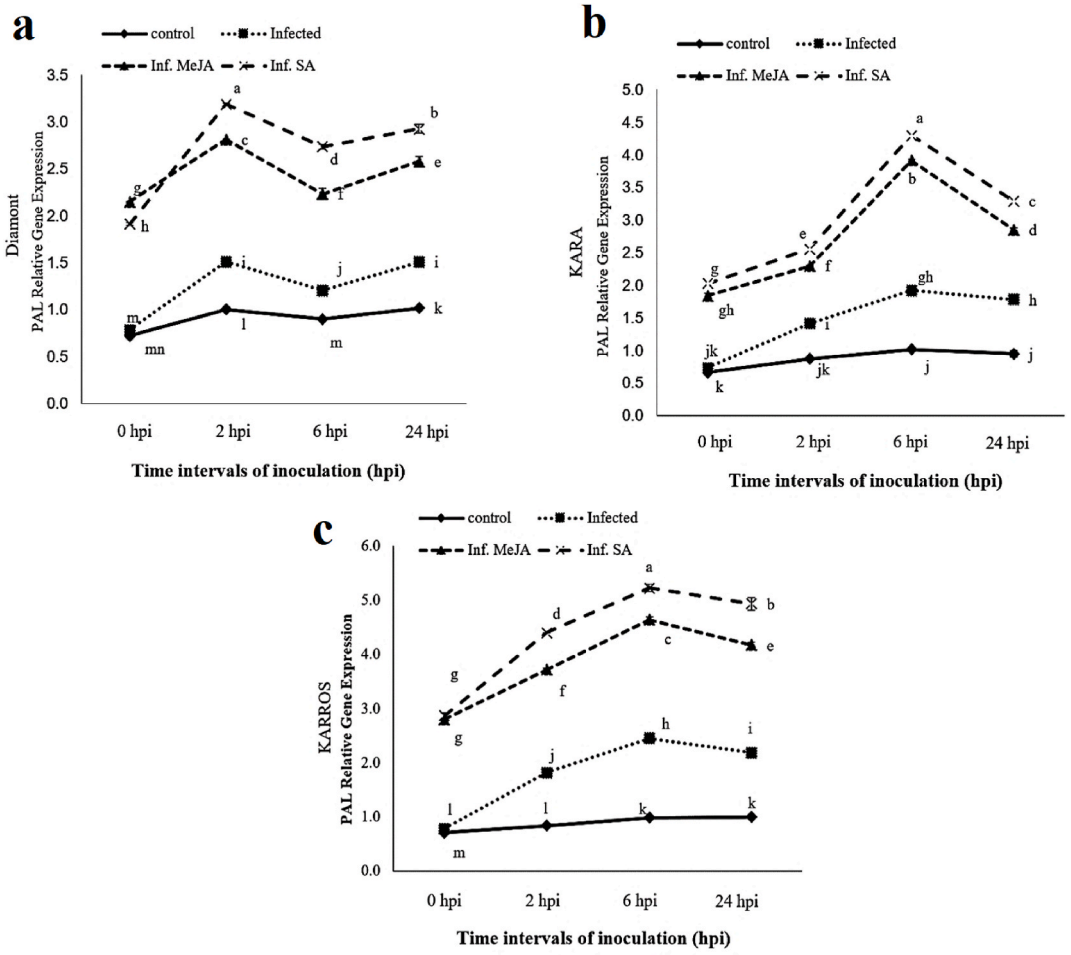

Fig. 6. Effect of Ecc infection on *PAL* relative gene expression of cvs; **a)** Diamont, **b)** Kara and **c)** Karros at different time intervals of inoculation (0, 2, 6 and 24 hpi). Data are mean of triplicates ±SD and dissimilar letters designate statistically significant variations at $p \leq 0.05$.

compared to other cultivars during most time intervals [58].

To investigate the interactions between plant tissues and bacterial infection, as well as observe their genetic responses, the expression levels of PAL, PPO and POX were monitored over time in the studied cultivars. Results showed a gradual upregulation of *PAL*, *PPO* and *POX* genes in cvs. Diamont, Kara and Karros following bacterial infection, indicating their activation [59].

The genetic response of potato cultivars upon *Ecc* invasion was found to differ at 0 hpi, depending on their susceptibility. Significant increases in *PAL, PPO* and *POX* expression levels were observed in the cvs. Diamont and Kara at 0 hpi, indicating a rapid response and sensitivity compared to the control. This divergence in resistance between the three cultivars became evident immediately after infection due to variations in the expression of antioxidant genes. Alternatively, when comparing their reactions, the cv. Karros exhibited the highest expression levels at 2 and 4 hpi, which correlated with the induction of PAL, PPO and POX activity, highlighting its elevated and rapid resistance [60].

In order to combat biotic stress factors in plant crops, SA and MeJA have been proposed as viable solutions for controlling soft rot. In the present study, direct application of SA and MeJA to potato tubers was not performed; instead, the tubers were inoculated with these substances, resulting in varying levels of inhibition of soft rot disease. SA, whether induced endogenously or applied exogenously, acts as a signal that stimulates specific plant defence responses, including the expression of pathogenesis-related genes, activation of antioxidant enzymes, and resistance to pathogens [35,61]. The results of this study demonstrated that spraying with either SA or MeJA significantly enhanced the antioxidant machinery and total phenolic content in the leaves of cvs. Diamont, Kara and Karros after infection with *Ecc*1, compared to stressed samples and control. These findings aligned with a previous study of Creelman et al. [62].

Over time, SA exhibited the most notable enhancement of antioxidant response and improved resistance compared to MeJA. Consequently, the foliar spray of SA induced the most significant increase in PAL, PPO and POX activity, as well as phenolic production, followed by the MeJA treatments in all cultivars. Consistent with previous reports, SA application in potato tubers increased resistance to bacterial soft rot infection initiated by *Ecc* [27]. The effect of SA was not directly on the growth of the pathogen, but rather

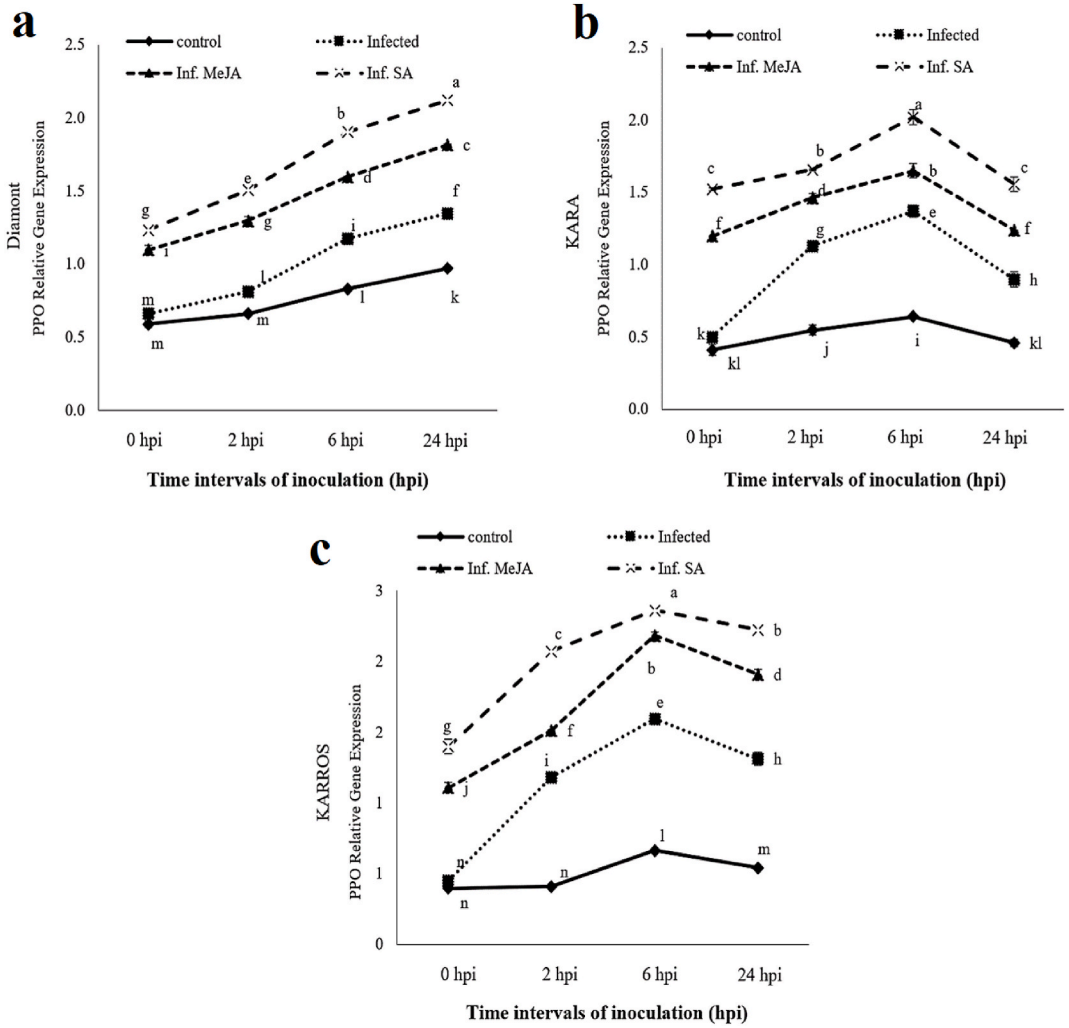

Fig. 7. Effect of *Ecc* infection on *PPO* relative gene expression of cvs; **a)** Diamont, **b)** Kara and **c)** Karros at different time intervals of inoculation (0, 2, 6 and 24 hpi). Data are mean of triplicates ±SD and dissimilar letters designate statistically significant variations at $p \leq 0.05$.

an induction of plant defence responses [63,64], consequently enhancing stress tolerance by reducing oxidative stress. Similarly, it has been observed that the severity of bacterial soft rot was completely reduced with the application of SA at a concentration of 0.9 mM before or after inoculation with the pathogen [65]. Additionally, spraying SA significantly decreased the severity of bacterial leaf spot in tomato plants infected with a virulent *Xanthomonas vesicatoria* strain by activating the activities of POX, CAT and PPO [29].

It has been established that jasmonic acid can induce acquired resistance by eliciting pathogenesis-related proteins in infected plants, including proteinase inhibitors and peroxidases [66]. Protein inhibitors can impact bacterial nutrition and trigger plant resistance [67]. MeJA exposure has commonly been used to induce herbivore resistance in various plant species and has been shown to enhance the accumulation of pathogenesis-related proteins and defences, thus reducing the incidence of many crop diseases [30,68].

The sensitivity of cultivars was observed by measuring PAL activity after treatment with SA or MeJA. In the Diamont cultivar, PAL activity showed a gradual increase at 2 and 6 hpi for MeJA and SA, respectively. However, PAL activity gradually decreased over time, indicating a short-term response. In contrast, the Kara cultivar exhibited a more sustained response, with PAL activity gradually increasing until 6 and 12 hpi for MeJA and SA, respectively. The Karros cultivar demonstrated the most consistent positive response over time, with PAL activity steadily increasing until a maximum at 12 h post-treatment for both SA and MeJA.

In terms of PPO activity, the Diamont cultivar exhibited a gradual increase until reaching its peak at 12 hpi with SA. On the other hand, the Kara and Karros cultivars showed a faster response, with maximum PPO activity observed at 6 h post-treatment. The POX activity in response to SA and MeJA elicitation showed variation among the cultivars over different hpi. Across all cultivars, the highest POX activity was observed in the Karros cultivar when treated with SA at 4 hpi, indicating its crucial role in pathogen resistance. Additionally, the infected leaves of the cv. Karros exhibited the greatest increase in total phenolic content after application of both SA and MeJA. These findings provide evidence for the distinct resistance response and elicitation ability of SA [69] and MeJA [70] among the three potato cultivars studied.

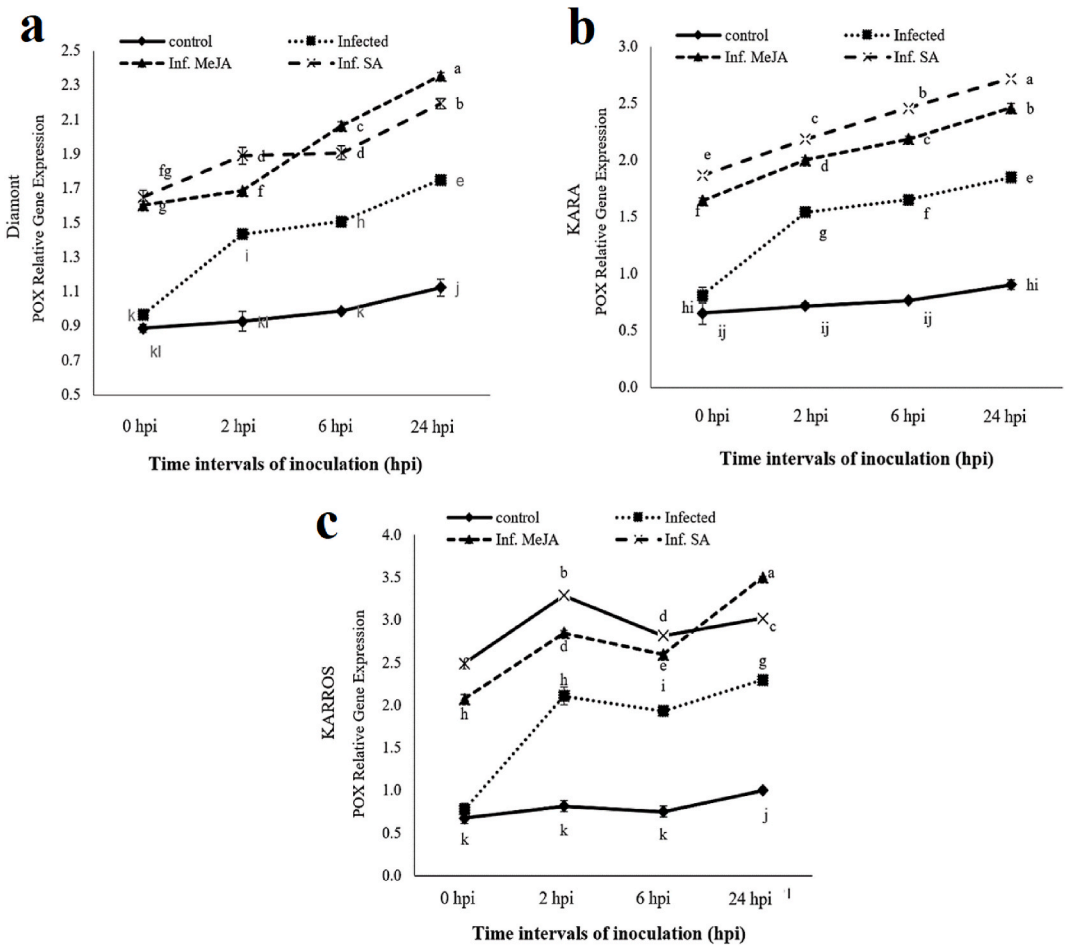

Fig. 8. Effect of Ecc infection on *POX* relative gene expression of cvs; **a)** Diamont, **b)** Kara and **c)** Karros at different time intervals of inoculation (0, 2, 6 and 24 hpi). Data are mean of triplicates ±SD and dissimilar letters designate statistically significant variations at $p \leq 0.05$.

The current findings demonstrate an increase in the expression levels of *PAL*, *PPO* and *POX* genes following the application of SA and MeJA in the three potato cultivars under study, compared to the control and infested treatments. The up-regulation of these antioxidant genes can be attributed to the important role played by SA and MeJA in inducing resistance [35]. This highlights the potential role of SA and MeJA in stimulating the antioxidant defence system. In particular, the examination of *PAL*, *POX*, and *PPO* expression levels at time 0 revealed that they were higher in SA and MeJA treatments compared to controls or infected leaves. This finding indicates an induced resistance due to the application of SA and MeJA treatments.

Consistent with the results obtained, the genetic response and resistance of potato cultivars to bacterial infection, as well as the elicitation ability of both SA and MeJA, varied over time. SA treatment notably enhanced the expression of *PAL* and *PPO* genes in all three cultivars as time progressed. In contrast, MeJA treatment resulted in the highest expression of the *POX* gene at 0 hpi in the cv. Karros, while the maximum expression in the other cultivars was observed at 24 h post-infection. These findings shed light on the variability in susceptibility among potato cultivars against *Ecc* invasion.

5. Conclusion

This study investigated different potato cultivars' responses to *E. carotovora* and the potential benefits of SA and MeJA in enhancing the antioxidant defense system. The experiment focused on Diamont, Kara, and Karros cultivars at multiple time intervals after bacterial infection. Results showed increased total phenolic levels, as well as PAL, PPO and POX activities and gene expression over time following *E. carotovora* inoculation. SA and MeJA application amplified these responses, particularly in the Karros cultivar, which exhibited superior resistance. Conversely, Diamont and Kara cultivars showed lower expression levels, indicating susceptibility. The findings highlight *E. carotovora* as a virulent pathogen causing soft rot in potato tubers and underscore Karros' robust resistance. Additionally, foliar exposure to SA and MeJA enhanced antioxidant responses, offering potential strategies for combating bacterial soft rot in potato crops.

Funding

This research was funded by Researchers Supporting Project number (RSP2024R186), King Saud University, Riyadh, Saudi Arabia.

Informed consent statement

Not applicable.

Data availability statement

All data generated or analyzed during this study are included in this manuscript and its information files.

CRediT authorship contribution statement

Sherien E. Sobhy: Writing – review & editing, Investigation, Formal analysis, Data curation. **Asma A. Al-Huqail:** Writing – review & editing, Visualization, Investigation. **Faheema Khan:** Writing – review & editing, Visualization, Investigation. **Gehad Abd-Allah Ragab:** Resources, Methodology, Data curation. **Mohamed A. El-sheikh:** Resources, Methodology, Data curation, Conceptualization. **Asia R. Ahmed:** Resources, Methodology, Data curation, Conceptualization. **Ahmed A. Saleh:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elsayed E. Hafez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors would like to extend their sincere appreciation to the Researcher Supporting Project number (RSP2024R186), King Saud University, Riyadh, Saudi Arabia.

References

[1] A. Devaux, J.P. Goffart, P. Kromann, J. Andrade-Piedra, V. Polar, G. Hareau, The potato of the future: Opportunities and challenges in sustainable Agri-food systems, Potato Res. 64 (4) (2021) 681–720.
[2] W.V. Davis, C. Weber, S. Wechsler, G. Lucier, H. Wakefield, K. Vaiknoras, Vegetables and Pulses Outlook, U.S. Department of Agriculture, Economic Research Service, December 2023. Report No. VGS-371).
[3] S.S. Jayanty, K. Diganta, B. Raven, Effects of cooking methods on nutritional content in potato tubers, Am. J. Potato Res. 96 (2019) 183–194.
[4] M. Koch, M. Naumann, E. Pawelzik, A. Gransee, H. Thiel, The importance of nutrient management for potato production Part I: plant nutrition and yield, Potato Res. 63 (2020) 97–119.
[5] R. Chacón-Cerdas, L. Barboza-Barquero, F.J. Albertazzi, W. Rivera-Méndez, Transcription factors controlling biotic stress response in potato plants, Physiol. Mol. Plant Pathol. 112 (2020) 101527.
[6] J.R. Lamichhane, G.M. Balestra, L. Varvaro, Occurrence of potato soft rot caused by Erwinia carotovora (synonym Pectobacterium carotovorum) in Nepal: a first report, Plant Dis. 94 (3) (2010) 382.
[7] M. Pérombelon, Potato diseases caused by soft rot erwinias: an overview of pathogenesis, Plant Pathol. 51 (1) (2002) 1–12.
[8] C.B. Wegener, Induction of defence responses against Erwinia soft rot by an endogenous pectate lyase in potatoes, Physiol. Mol. Plant Pathol. 60 (2) (2002) 91–100.
[9] M.A. El-Sheikh, S.A. El-Kazzaz, E. El-Argawy, A.R. Ahmed, Characteristics and control of Erwinia carotovora subsp. carotovora affecting potato in behera governorate, Egypt, Egypt. J. Phytopathol. 39 (2) (2011) 73–89.
[10] P.S. Shelake, D. Mohapatra, M.K. Tripathi, S.K. Giri, A. Kate, M. Kumar, Inactivation of Aspergillus Niger and Erwinia carotovora in onion (*Allium cepa L.*) bulbs subjected to pulsed ozone treatment, Postharvest Biol. Technol. 192 (2022) 111998.
[11] G. DalCorso, S. Farinati, A. Furini, Regulatory networks of cadmium stress in plants, Plant Signal. Behav. 5 (6) (2010) 663–667.
[12] R. Sultana, Z. Imam, R.R. Kumar, V.S. Banu, S. Nahakpam, R. Bharti, C. Bharadwaj, A.K. Singh, R.K. Pasala, D.R. Singh, Signaling and defence mechanism of jasmonic and salicylic acid response in pulse crops: role of WRKY transcription factors in stress response, J. Plant Growth Regul. (2024) 1–17.
[13] Z. Zou, Q. Fan, X. Zhou, X. Fu, Y. Jia, H. Li, Y. Liao, Biochemical pathways of salicylic acid derived from l-phenylalanine in plants with different basal SA levels, J. Agric. Food Chem. 72 (6) (2024) 2898–2910.
[14] L. Li, H.-N. Sun, M. Zhang, T.-H. Mu, Exploring the potential in postharvest preservation of sweet potato: 2-methylbutanoic acid, isobutyric acid, perillaldehyde and salicylaldehyde, to control Rhizopus stolonifer, Postharvest Biol. Technol. 210 (2024) 112785.
[15] A. Ezzat, S. Szabó, Z. Szabó, A. Hegedűs, D. Berényi, I.J. Holb, Temporal patterns and inter-correlations among physical and antioxidant attributes and enzyme activities of apricot fruit inoculated with monilinia laxa under salicylic acid and methyl jasmonate treatments under shelf-life conditions, J Fungi 7 (5) (2021).
[16] M.I.R. Khan, M. Fatma, T.S. Per, N.A. Anjum, N.A. Khan, Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants, Front. Plant Sci. 6 (2015) 462.
[17] Z. Liu, Y. Ding, F. Wang, Y. Ye, C. Zhu, Role of salicylic acid in resistance to cadmium stress in plants, Plant Cell Rep. 35 (2016) 719–731.
[18] I. Hadizadeh, Response of Potato Tubers to Biocontrol Agent and Soft Rot Pathogen Dickeya Solani: Transcriptional Regulation and Hormone Interactions, 2024.
[19] D.P. Jayaweera, C. Dambire, D. Angelopoulou, S. Munné-Bosch, R. Swarup, R.V. Ray, Physiological, molecular, and genetic mechanism of action of the biostimulant Quantis™ for increased thermotolerance of potato (*Solanum tuberosum L.*), Chem Biol Technol Agric 11 (1) (2024) 1–19.

[20] L. Augustine, L. Varghese, S. Kappachery, V.M. Ramaswami, S.P. Surendrababu, M. Sakuntala, G. Thomas, Comparative analyses reveal a phenylalanine ammonia lyase dependent and salicylic acid mediated host resistance in Zingiber zerumbet against the necrotrophic soft rot pathogen Pythium myriotylum, Plant Sci. (2024) 111972.
[21] P. Demiwal, S.U. Nabi, J.I. Mir, M.K. Verma, S.R. Yadav, P. Roy, D. Sircar, Methyl jasmonate improves resistance in scab-susceptible Red Delicious apple by altering ROS homeostasis and enhancing phenylpropanoid biosynthesis, Plant Physiol Biochem (2024) 108371.
[22] J.-J. Cheong, Y.D. Choi, Methyl jasmonate as a vital substance in plants, Trends Genet. 19 (7) (2003) 409–413.
[23] Y. Zhang, J. Zhong, A. Munawar, Y. Cai, W. He, Y. Zhang, H. Guo, Y. Gao, Z. Zhu, W. Zhou, Knocking down a DNA demethylase gene affects potato plant defense against a specialist insect herbivore, J. Exp. Bot. 75 (1) (2024) 483–499.
[24] X. Zhang, Y. Yu, J. Zhang, X. Qian, X. Li, X. Sun, Recent progress regarding jasmonates in tea plants: biosynthesis, signaling, and function in stress responses, Int. J. Mol. Sci. 25 (2) (2024) 1079.
[25] J. Qian, C. Zhu, G. Jian, L. Zeng, Y. Yang, Release patterns and potential utility of herbivore-induced plant volatiles in crops: a review, Environ. Exp. Bot. (2024) 105659.
[26] A. Beneduzi, A. Ambrosini, L.M. Passaglia, Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents, Genet. Mol. Biol. 35 (2012) 1044–1051.
[27] A. López-Villamor, M. Nunes da Silva, M.W. Vasconcelos, Evaluation of plant elicitation with methyl-jasmonate, salicylic acid and benzo(1,2,3)-thiadiazole-7-carbothioic acid-S-methyl ester for the sustainable management of the pine wilt disease, Tree Physiol. 42 (12) (2022) 2596–2613.
[28] Y. Yang, X. Yang, X. Guo, X. Hu, D. Dong, G. Li, X. Xiong, Exogenously applied methyl Jasmonate induces early defense related genes in response to Phytophthora infestans infection in potato plants, Hortic Plant J 8 (4) (2022) 511–526.
[29] Y.E. Ibrahim, Activities of antioxidant enzymes in salicylic acid treated tomato against Xanthomonas vesicatoria, Afr. J. Microbiol. Res. 6 (27) (2012) 5678–5682.
[30] S. Sobhy, E.E.M. Abo-Kassem, K.M. Saad-Allah, E.E. Hafez, Physiological and molecular effects of calcium and salicylic acid on Fusarium graminearum-infected wheat seedlings, J. Plant Growth Regul. (2023) 1–20.
[31] K.M. Saad-Allah, G.A. Ragab, Sulfur nanoparticles mediated improvement of salt tolerance in wheat relates to decreasing oxidative stress and regulating metabolic activity, Physiol. Mol. Biol. Plants 26 (2020) 2209–2223.
[32] N. Sewelam, E.A. Abo-Kassem, S. Sobhy, S. Dowidar, Roles of calcium and ABA in alleviating drought stress effects on Triticum aestivum L. Plants, Egypt J Exp Biol (Zool) 13 (2) (2017).
[33] H.S. Mustafa, A.M. Alawami, Susceptibility of newly introduced potato cultivars to Libya to infection with bacterial soft rot and the associated physiological changes, J. Agric. Sci. Technol. 2 (8A) (2012) 976.
[34] J. Huang, M. Gu, Z. Lai, B. Fan, K. Shi, Y.-H. Zhou, J.-Q. Yu, Z. Chen, Functional analysis of the Arabidopsis PAL gene family in plant growth, development, and response to environmental stress, Plant Physiol 153 (4) (2010) 1526–1538.
[35] S. Sobhy, D. G Aseel, E.-E.M. Abo-Kassem, N. A Sewelam, K. M Saad-Allah, M. A Samy, E. E Hafez, Priming of wheat plant with weed extracts, calcium and salicylic acid for contribution to alleviating the oxidative stress imposed by Fusarium graminearum and lead toxicity, Novel Res Microbiol J 7 (2) (2023) 1932–1965.
[36] R.M. Atlas, Handbook of Media for Environmental Microbiology, CRC Press, 2005.
[37] S.T. Williams, Bergey's Manual of Systematic Bacteriology, Williams & Wilkins, 1989.
[38] D.J. Brenner, N.R. Krieg, J.T. Staley, G. Garrity, Bergey's Manual® of Systematic Bacteriology: Volume Two: the Proteobacteria (Part C), Springer, 2005.
[39] S.T. Cowan, Cowan and Steel's Manual for the Identification of Medical Bacteria, Cambridge University Press, 1993.
[40] R. Dickey, Erwinia 'Carotovora' or Soft Rot Group. Laboratory Guide for Identification of Plant Pathogenic Bacteria, 1988, pp. 44–58.
[41] D. Gross, A. Vidaver, Z. Klement, K. Rudolph, D. Sands, Methods in Phytobacteriology, 1990.
[42] J. Hollis, R. Goss, Factors influencing invasion of Potato by Erwinia carotovora, Phytopathology 40 (9) (1950).
[43] A. Zehra, M. Meena, M.K. Dubey, M. Aamir, R. Upadhyay, Synergistic effects of plant defense elicitors and Trichoderma harzianum on enhanced induction of antioxidant defense system in tomato against Fusarium wilt disease, Bot. Stud. 58 (1) (2017) 1–14.
[44] M. Montesano, G. Brader, I. Ponce De León, E.T. Palva, Multiple defence signals induced by Erwinia carotovora ssp. carotovora elicitors in potato, Mol. Plant Pathol. 6 (5) (2005) 541–549.
[45] D. Dickerson, S. Pascholati, A.E. Hagerman, L. Butler, R. Nicholson, Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with Helminthosporium maydis or Helminthosporium carbonum, Physiol. Plant Pathol. 25 (2) (1984) 111–123.
[46] R. Hammerschmidt, J. Kuć, Lignification as a mechanism for induced systemic resistance in cucumber, Physiol. Plant Pathol. 20 (1) (1982) 61–71.
[47] A. Mayer, E. Harel, R. Ben-Shaul, Assay of catechol oxidase—a critical comparison of methods, Phytochemistry 5 (4) (1966) 783–789.
[48] N. Zieslin, R. Ben-Zaken, Peroxidase activity and presence of phenolic substances in peduncles of rose flowers, Plant Physiol Biochem 31 (3) (1993) 333–339.
[49] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method, Methods 25 (4) (2001) 402–408.
[50] S. Sobhy, K. Allah, E. Kassem, E. Hafez, N. Sewelam, Seed priming in natural weed extracts represents a promising practice for alleviating lead stress toxicity, Egypt J Exp Biol 15 (2019) 453.
[51] A.A. Khedr, Management of soft rot disease, caused by Erwinia carotovora subsp. carotovora in potato tubers, African Journal of Biological Sciences 15 (2019) 211–218.
[52] M.A. Samy, M.A. Abbassy, E.E. Hafez, E.I. Rabea, D.G. Aseel, Biosynthesis and characterization of silver nanoparticles produced by plant extracts and its antimicrobial activity, South Asian Journal of Research in Microbiology 3 (2019) 1–14.
[53] M. Pérombelon, G. Salmond, Bacterial soft rots, in: Prokaryotes, Elsevier, 1995, pp. 1–20.
[54] A. Motyka, S. Zoledowska, W. Sledz, E. Lojkowska, Molecular methods as tools to control plant diseases caused by Dickeya and Pectobacterium spp: a minireview, New biotechnology 39 (2017) 181–189.
[55] M.U. Akbar, M. Aqeel, M.S. Shah, G. Jeelani, N. Iqbal, A. Latif, R.O. Elnour, M. Hashem, O.M. Alzoubi, T. Habeeb, Molecular regulation of antioxidants and secondary metabolites act in conjunction to defend plants against pathogenic infection, South Afr. J. Bot. 161 (2023) 247–257.
[56] K.K. Rai, Revisiting the critical role of ROS and RNS in plant defense, J. Plant Growth Regul. 42 (2023) 6202–6227.
[57] Q. Gong, Y. Wang, L. He, F. Huang, D. Zhang, Y. Wang, X. Wei, M. Han, H. Deng, L. Luo, Molecular basis of methyl-salicylate-mediated plant airborne defence, Nature 622 (2023) 139–148.
[58] B. Nandi, S. Babu, Mechanism of action of salicylic acid during defense response of plant, Indian J. Sci. Res. 4 (2013) 101–104.
[59] Y. Guttman, J.R. Joshi, N. Chriker, N. Khadka, M. Kleiman, N. Reznik, Z. Wei, Z. Kerem, I. Yedidia, Ecological adaptations influence the susceptibility of plants in the genus Zantedeschia to soft rot Pectobacterium spp, Horticulture Research 8 (2021).
[60] P.R. Davidsson, T. Kariola, O. Niemi, E.T. Palva, Pathogenicity of and plant immunity to soft rot pectobacteria, Front. Plant Sci. 4 (2013) 191.
[61] S. Neerja, B. Sohal, J. Lore, Foliar application of benzothiadiazole and salicylic acid to combat sheath blight disease of rice, Rice Sci. 20 (2013) 349–355.
[62] N. Woch, S. Laha, P. Gudipalli, Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of Givotia moluccana (L.) Sreem, In Vitro Cellular & Developmental Biology-Plant (2023) 1–22.
[63] S.K. Wilson, T. Pretorius, S. Naidoo, Mechanisms of systemic resistance to pathogen infection in plants and their potential application in forestry, BMC Plant Biol. 23 (2023) 404.
[64] S.H. Spoel, X. Dong, Salicylic acid in plant immunity and beyond, Plant Cell (2024), https://doi.org/10.1093/plcell/koad329 koad329.
[65] A. Hajhamed, W. Sayed, A. Yazied, N. Ghaffar, Suppression of bacterial soft rot disease of potato, Egypt. J. Phytopathol. 35 (2007) 69–80.
[66] R.A. Creelman, J.E. Mullet, Biosynthesis and action of jasmonates in plants, Annu. Rev. Plant Biol. 48 (1997) 355–381.
[67] H.G. Boman, Antibacterial peptides: basic facts and emerging concepts, J. Intern. Med. 254 (2003).

[68] F. Gozzo, Systemic acquired resistance in crop protection: from nature to a chemical approach, J. Agric. Food Chem. 51 (2003) 4487–4503.
[69] A. Koornneef, A. Leon-Reyes, T. Ritsema, A. Verhage, F.C. Den Otter, L.C. Van Loon, C.M. Pieterse, Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation, Plant Physiol 147 (2008) 1358–1368.
[70] J.S. Thaler, B. Owen, V.J. Higgins, The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles, Plant Physiol 135 (2004) 530–538.