Differential Gene Expression of Two Potato Cultivars in Response To Infection With *Ralstonia Solanacearum*

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ABSTRACT: Bacterial wilt, caused by *Ralstonia solanacearum (Rs)*, is one of the most important plant diseases, especially on potato. In this study, the molecular mechanisms of potato resistance against *Rs* were compared between cv. Diamant (susceptible) and cv. Lady Bedford (partially resistant). The results indicated that potato cv. Lady Bedford was significantly more resistant (24.3% wilt) to *Rs* than cv. Diamant (83.8% wilt). The inoculated cv. Diamant plants exhibited the highest decrease in photosynthetic pigment compared to the cv. Lady Bedford. Three differentiated expressed cDNA fragments that present in inoculated plants of the partially resistant cv. Lady Bedford were selected for sequencing and identified as 1-D-deoxyxylulose 5-phosphate synthase gene (*StDXS1*), polyphenol oxidase (*PPO*) gene and WRKY transcription factor (*StWRKY*) from potato genome. The expression level of the *POD* (peroxidase), *PPO*, *StWRKY* and *StDXS1* genes were induced by inoculation with *Rs* compared to the non-infected plants. The highest gene expression level on the two cultivars was recorded at 12, 24, 24 and 12 hpi for the four genes, respectively, and it was significantly higher in the partially resistant cultivar than the susceptible one. POD and PPO, enzymes activity significantly increased After inoculation with *Rs* in the both cultivars. cv. Lady Bedford had a higher enzymes activity and peaked at 12 and 24 hpi for POD and PPO, respectively, while, cv. Diamant peaked at 6 and 12 hpi for the same enzymes. These clear transcriptional responses suggested that these genes and enzymes participates in the basal defense of potato to *Rs.*

Keywords: Antioxidant enzymes, Bacterial wilt, Differential display, Gene expression, *R. solanacearum*

INTRODUCTION

In Egypt, Potato is economic vegetable crops for local consumption, processing, and export, with a production of approximately 5.08 million tons, produced from approximately 175,161 hectares. Egypt is the 15th worldwide and largest producers of potato in Africa (FAO STAT, 2019). Potato can be affected by many pathogens including bacteria, fungi, viruses, and nematodes. Bacterial wilt (Brown rot) disease, caused by *Ralstonia solanacearum* (Smith) is a major limiting factor for potato production worldwide, especially in the tropical and subtropical regions and warm areas such as the Mediterranean region and it causes huge economic losses (Lemaga et al., 2005; Kabeil et al., 2008**;** Khairy et al., 2021).

R. solanacearum is soil borne, gram-negative bacterium that can wilt hundreds of different species including potato, tomato, pepper, tobacco, eggplant, geranium, and banana (Hayward, 2000; OEPP/EPPO, 2004; Denny, 2006). The loss in yield due to the disease in potato has been reported to be 33-90% (Elphinstone, 2005). *Rs* can enter the plant roots *via* wounds or during lateral root emergence. Thereafter it penetrates the xylem and multiply in the xylem vessels, obstructs water flow in the plant then plants quickly wilt and die (OEPP/EPPO, 2004; Tan et al., 2016).

Soil-borne bacteria including *Rs* are difficult to control and planting resistance/tolerant varieties is considered the most effective and cheapest strategy for the control of potato bacterial wilt (Muthoni et al.,2014, 2020). So far, there are no complete resistant commercial varieties against *Rs* has been identified but some degree of resistance/tolerance are available in the potato germplasm (Laferriere et al., 1999; Huet, 2014).

Plants have diverse natural mechanisms to protect themselves against infections by pathogens. Understanding the basic mechanisms of host resistance is necessary for breeding resistant varieties. For study the molecular mechanisms of potato-*Rs* interactions, Li et al. (2010) identified 302 differentially expressed genes using suppression subtractive hybridization (SSH) and microarray techniques. Among them, 81 genes were considered *Rs* resistance related genes, and these genes involved in pathogen recognition, transcription factor functioning, signal transduction, systemic acquired resistance (SAR),

hypersensitive response (HR), and cell protection. On the other hand, more than 140 genes related to pathogenesis-related, lignin biosynthesis, and hormone signaling genes were induced in resistant tomato cultivar, but not in susceptible cultivar after inoculation with *Rs* (Ishihara et al., 2012). Also, expression of b-1,3-glucanase genes and glucanase protein were increased in the resistant cultivar suggested that the ethylene (ET) and jasmonic acid (JA) signaling pathways are involved in signal transduction of resistance to *Rs* (Ishihara et al., 2012). Narancio et al. (2013) suggested a role of both salicylic acid (SA) and ET in the early defense responses to *Rs* when they used *S. tuberosum* cDNA microarrays for determined the transcriptome of the highly-resistant *S. commersonii* accession F100. Eight differentially proteins were identified by the proteome analysis of resistance against *Rs* in potato cultivar CT206-10, includes tomato stress induced-1 (TSI-1) protein, glycine-rich RNA binding protein (GRP), pentatricopeptide repeat containing (PPR) protein and pathogenesis-related (STH-2) protein (Park et al., 2016). More recently, Li et al. (2021a) found an increase in the number of upregulated differentially expressed genes in tobacco. WRKY transcription factors (WRKY6 and WRKY11), ERF transcription factors (ERF5 and ERF15), and PR5 encoding genes were upregulated in the resistant cultivar in response to *Rs*.

In plants, defense-related enzymes consist of an important protective system against pathogen attack. Superoxide dismutase (SOD), phenylalanine ammonia-lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO), are necessary defense-related enzymes and deeply studied in the research of plant- pathogen interaction (Vanitha and Umesha, 2008; Fortunato et al., 2015; Xie et al., 2017). Antioxidant enzymes (SOD, POD, and PPO) are important for defense against oxidative stress and membrane lipid peroxidation during pathogen attack (Foyer and Noctor, 2003; Li et al., 2008). PPO participates in the oxidation of polyphenols into quinones and lignification of plant cells during pathogen invasion (Mohammadi and Kazemi, 2002). POD enzyme involved in oxidation of phenols, and lignification of host plant cells during defense against the pathogen (Chittoor et al., 1999).

The present study was conducted to evaluate the defense mechanisms of two potato cultivars differing in resistance to *Rs*, by analyzing the alteration in gene expression using differential display technique, quantification of the defences gene expression using real-time PCR, and measuring the activities of the peroxidase and polyphenol oxidase enzymes,

MATERIAL AND METHODS

Plant Material and Growth Conditions:

The Potato cultivars, Lady Bedford and Diamant, which are partially resistance and susceptible to *R. solanacearum* (*Rs*), respectively (Elgayar, 2010), were used in this study. Healthy potato tubers were surface sterilized with 1% sodium hypochlorite for five min, washed with sterile water and planted in sterilized 25 cm diameter plastic pots filled with sterile clay, sand and peat moss (2:1:1). The plants were grown in greenhouse (28-30 °C) under natural light condition. Fertilizer solution (N–P–K: 20-20-20) was weekly applied at 0.2 % (w/v) and plants were irrigated with water when necessary. Four-week-old plants were used for inoculations.

Pathogen Inoculation and Disease Evaluation

A virulent isolate of *Rs*, obtained from Plant Pathology Department, Faculty of Agriculture, Damanhour university, was maintained on tetrazolium chloride (TZC) agar medium (Kelman, 1954) for 48 h at 28 ^{o}C. The bacterial cells were harvested in sterile distilled water and bacterial concentration was spectrophotometrically adjusted to 1 x 10^{8} cfu ml^{-1}.

Plants were inoculated by stem-puncture technique (Prior and Steva, 1990) by forcing sterilized dissecting needle into the stem through a drop of bacterial suspension. Similarly, plants inoculated with sterile distilled water were used as control. The experiment was arranged in a randomized block design with five replicates per treatment and five plants in each replicate.

Disease severity (wilt severity) was recorded 25 days post inoculation (dpi) for individual plants on the scale of 0 to 4 (Kempe and Sequeira, 1983) as follow: 0, no wilting; 1, 1 to 25% of leaves wilted; 2, 26 to 50% of leaves wilted; 3, 51 to 75% of leaves wilted; and 4, 76 to 100% of leaves wilted or dead. The percentage of disease severity (DS%) was calculated according to the formulae:

$$DS\% = \{\Sigma (n \times v) / N \times S\} \times 100$$

Where, Σ = Summation; n = No. of wilted plants in each category; v = Numerical value of wilted plants observed; N= Total number of examined plants and S = Maximum numerical value/grade.

Determination of photosynthetic pigments and growth parameters

Chlorophyll a, b and β-Carotene content on the leaves of the two potato cultivars were determined spectrophotometrically 15 dpi according to Wintermans and De Mots (1965).

Differential display analysis:

Sampling, RNA extraction and cDNA synthesis

Samples of potato leaves (3^{rd}- 4^{th} leaves from the top) from *Rs* infected plants as well as control plants of both cultivars were collected at 3, 6 and 12 hrs post inoculation (hpi), and stored at -80 °C for further analysis. Total RNA was extracted from leave samples using RNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol. The obtained RNA was treated with DNase for 1 h at 37°C to remove any DNA

residues. RNA concentration and purity were assayed spectrophotometrically (Jenway, Model 6305, Bibby Scientific Limited, UK). RNA samples were reverse transcribed into cDNAs using Oligo (dT) primer and reverse transcriptase enzyme (MLV, Fermentas, USA) following manufacturer's instructions.

Differential display real time-PCR (DDRT-PCR)

The synthesized cDNA was used as template for DDRT-PCR. The reaction (25µL) contained: 10× buffer (5µL), 5µL of 10pmol primer (NS2, WRKY1-F and PPO-F, Table 1), 2.5µL of 25mM $MgCl_2$, 2µL of 10mM dNTPs, 0.2µL Taq DNA polymerase (Promega, USA) and 1µL cDNA template. The PCR cycles were one cycle of 95 ˚C for 5 min, then 40 cycles (95˚C for 30 sec, 40°C for 1 min, 72 ˚C for 1min) and final extension of 72 ˚C for 10 min (Chin et al., 2000). The amplified cDNA was visualized on a 1.5% (w/v) agarose gel, stained with ethidium bromide and photographed using gel documentation system.

Table 1. Primers used in the molecular studies

Technique	Primers		Primer sequence 5´ →3´	Annealing (°C)
Differential display PCR	NS2		CAC GAG TGC CCT TTT CTG AC	40
	PPO-F		CATGCTCTTGATGAGGCGTA	40
	WRKY1-F		ACGTTTAAACCATTCTCAGAAATAGC	40
Real time PCR	POD	F	GCTTTGTCAGGGGTTGTGAT	60
		R	TGCATCTCTAGCAACCAACG	
	PPO	F	CATGCTCTTGATGAGGCGTA	60
		R	CCATCTATGGAACGGGAAGA	
	StWRKY	F	ACGTTTAAACCATTCTCAGAAATAGC	60
		R	ACCTCGAGATACATGCCTTACTAGGC	
	StDXS1	F	GCATTTCCTGGGATTTTGAA	60
		R	TTGGCGGTCTCTGTGTGTAG	
	Actin	F	GCTTCCCGATGGTCAAGTCA	60
		R	GGATTCCAGCTGCTTCCATTC	

Sequencing and gene identification:

Selected differentiated fragments were excised from the gel and purified using Gel Extraction Kit (BioLine, UK). The amplified DNA was sequenced by Macrogen Company (Macrogen Inc., Seoul, South Korea). Alignment analysis was done using BLASTN searches at the National Center for Biotechnology Information Web site (http://www.ncbi.nlm.nih.gov). Pair-wise and multiple DNA sequence alignments were carried out using CLUSTALW (1.82) (Thompson et al. 1994). Bootstrap neighbor-joining tree was generated using MEGA version 6 (Tamura et al. 2013).

Quantitative Real-time (qRT-PCR) assay and data analysis

Total RNA was isolated from leaf samples taken at 1, 3, 6, 12 and 24 hpi using total RNA purification kit (Jena bioscience, Germany) according to the manufacturer's protocol. RT-PCR of mRNA was carried out with the same method illustrated in differential display analysis. Two antioxidant *POD* and *PPO* and the differentially expressed *StWRKY StDXS1* genes (Table 1), were confirmed and quantified by comparative qRT-PCR. The potato actin gene was used as internal housekeeping (reference) gene (Nicot et al., 2005).

The PCR Reaction (25 µl) consists of 12.5 µl of 2× Quantitech SYBR® Green Mix (Fermentaz, USA), 1 µl of 25 pM/µl of both forward and reverse primers (Table 1), 1 µl of template cDNA (150 ng), and 9.5 µl of RNase free water. The qRT-PCR was performed on Rotor-Gene 6000 system (Qiagen, ABI System, USA), with one cycle at 95 °C for 10 min followed by 40 cycles (95 °C for 15 sec, 60 °C for 30 sec and 72 °C for 30 sec). Data acquisition performed during the extension step. Relative expression level of each gene was performed using the comparative C_T method ($2^{-\Delta\Delta Ct}$ method, Schmittgen and Livak, 2008). The expression levels of the target genes were normalized relative to actin gene and relative expression of untreated control plants of each cultivar at each time point were set as 1.

Activity of the antioxidant enzymes

Peroxidase (POD) and polyphenol oxidase (PPO) activity were determined in potato leaves after 1, 3, 6, 12 and 24 hpi. The 3rd to 4th leaves from the top of five plants (replicates) per treatment at each specific sampling time were collected for enzyme analysis.

Potato leaf samples (1g) were homogenized in 5 ml of a pre-chilled 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA, and 2% polyvinyl pyrrolidone (PVPP). The homogenate was centrifuged at 12,000×*g* for 20 min at 4 °C (Universal 32R, Hettich Zentrifugen model D-78532, Germany). The collected supernatant was stored at -20 °C and used for enzyme activity assays (Ghareeb et al., 2019).

POD activity was assayed by mixing 100 µl of enzyme extract with 1.5 ml of 0.05 pyrogallol and

reaction was started by adding 0.5 ml of 1% H_2O_2. The increase in the absorbance was measured spectrophotometrically at 420 nm (Jenway, Model 6305, Bibby Scientific Limited, UK). POD activity was expressed as ΔOD_{420} min^{-1} g^{-1} of fresh weight (FW) (Hammerschmidt and Kuc, 1982). PPO activity was measured using the method of Mayer *et al*. (1965). The PPO reaction solution contained 200 µl of enzyme extract, 1.5 ml of 100 mM phosphate buffer (pH 6.5) and 200 µl of 10 mM catechol. The increase in absorbance were recorded at 495 nm. Enzyme activity was presented as ΔOD_{495} min^{-1} g^{-1} FW.

Statistical analysis

Data of disease severity, photosynthetic pigments, gene expression and enzymes activity were analyzed with GraphPad PRISM version 8.4.3 for Windows (GraphPad Software, San Diego, California USA) and Tukey's HSD test was used to assess differences among means at each time point at a $p \leq 0.05$. Data were expressed as mean ± SE.

RESULTS

Susceptibility of the potato cultivars to *R. solanacearum*

Plants were artificially inoculated with *Rs* and wilting symptoms were evaluated at 25 days post inoculations (dpi). Potato cv. Lady Bedford reaching a mean disease severity of 24.3% at 25 dpi and were significantly more tolerant to *Rs* than the susceptible cv. Diamant (83.8% wilt severity) (Fig.1A).

Disease progress on cv. Diamant was more rapid than in cv. Lady Bedford. The cv. Diamant plants inoculated with *Rs* started developing a distinct yellow coloration at 7-10 dpi as compared to the non-infected healthy plants and infected cv. Lady Bedford plants. At 25 dpi the susceptible cv. Diamant plants showed clear wilting symptoms while the plants of partially resistant cv. Lady Bedford remained healthy, although there was occurrence of wilt signs on the lower leaves (Fig.1B).

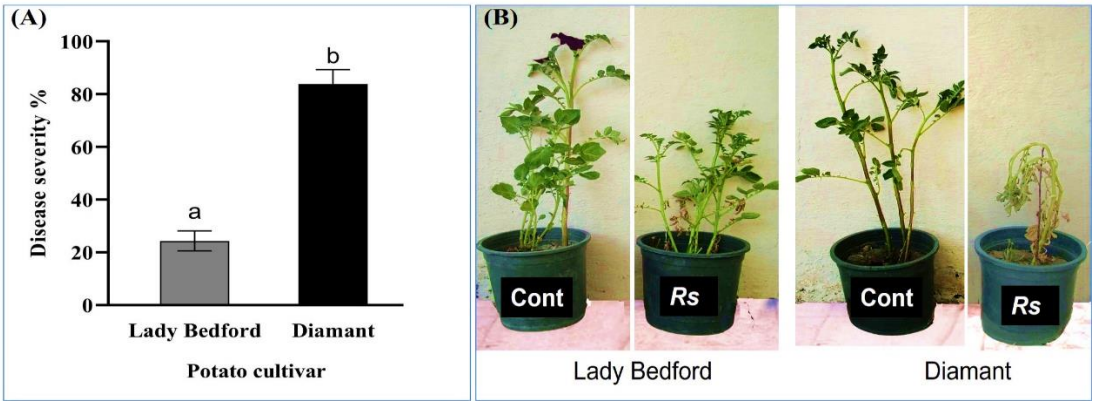

Fig. 1. Comparison of two different potato cultivars (Lady Bedford and Diamant) in response to *R. solanacearum* (*Rs2*) infection.

* Different letters indicate significant differences between cultivars according to the Tuckey HSD test ($P < 0.05$). Values are the mean of five replicates ± SE.

Photosynthetic pigments content:

Figure (2) showed that, there was no significant difference between the non-infected control of both cultivars for total chlorophyll, chlorophyll a, chlorophyll b and the carotene contents. A significant decrease in all pigment contents were observed on *Rs* inoculated plants of the two potato cultivars. The susceptible cv. Diamant exhibited the highest decrease in pigment contents at 15 days after inoculation with approximately 42.02, 42.49, 41.40 and 56.16% decreasing compared with the non-infected control for total chlorophyll, chlorophyll a, chlorophyll b and the carotene, respectively, comparing with 27.11, 20.56, 35.97 and 31.65% decreasing in the same pigments in the partially resistant cv. Lady Bedford.

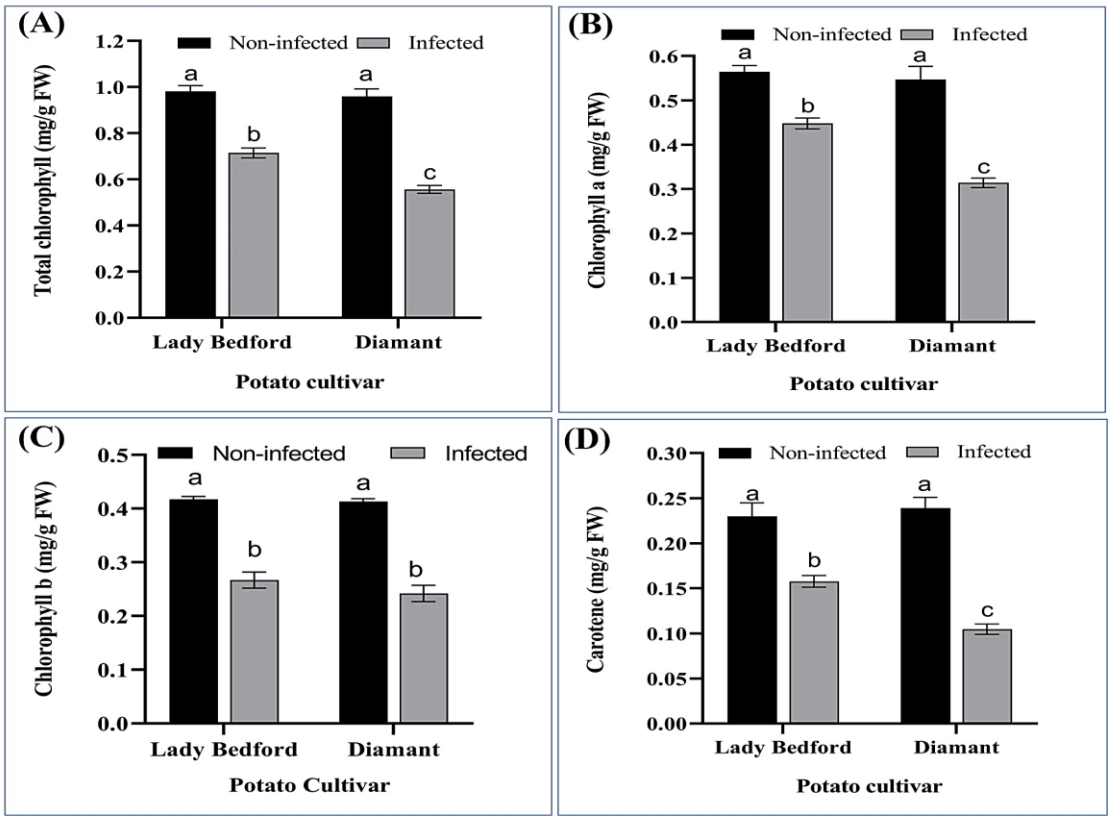

Fig. 2. Total chlorophyll, chlorophyll a, chlorophyll b and the carotene contents in the leaves of two potato cultivars (the partially resistant cv. Lady Bedford and the susceptible cv. Diamant) 15 days after inoculation with *R. solanacearum*.

* Different letters indicate significant differences between cultivars according to the Tuckey HSD test ($P < 0.05$). Values are the mean of five replicates ± SE

DDRT-PCR analysis

In the present experiments leaves of 3, 6 and 12 hpi were selected for analysis to isolate expressing genes involved in potato wilting defense. The DDRT-PCR profiles of the potato samples revealed considerable variation of gene expression in response to the infection with *Rs*. Many up-regulated and down-regulated genes were observed in both cultivars at different times post inoculation with *Rs* (see Fig. 3). Three randomly clear differentiated cDNA fragments that are present in inoculated plants of the partially resistant cv. Lady Bedford but not in the susceptible cv. Diamant or control plants were selected for sequencing and identification. The partial nucleotide sequences of the selected genes were aligned, compared with other genes available in the GenBank database and the phylogenetic trees were constructed.

cDNA fragment No.1 (446 bp, Fig. 3A) was up-regulated and amplified by NS2 primer from cv. Lady Bedford after 12 hpi and identified as 1-D-deoxyxylulose 5-phosphate synthase gene and showed 93% similarity to *Solanum tuberosum* 1-D-deoxyxylulose 5-phosphate synthase (DXS1) mRNA, complete cds, from *S. tuberosum* (GenBank accession No. NM_001288201). The cDNA fragment No.2 (475 bp, Fig. 3B) amplified from cv. Lady Bedford by PPO-F primer was highly expressed after 3 hpi and showed 98% homologous to *S. tuberosum* tuber polyphenol oxidase PPO *(POT33 allele)* mRNA (GenBank accession No. U22922.1). The cDNA fragments No.3 (630 bp, Fig. 3C) amplified by WRKY1-F primer was boosted up in cv. Lady Bedford after 3 hpi and showed 97% homologous to *S. tuberosum* StWRKY mRNA (GenBank accession No. AY615273, NM_001288675 and AJ278507).

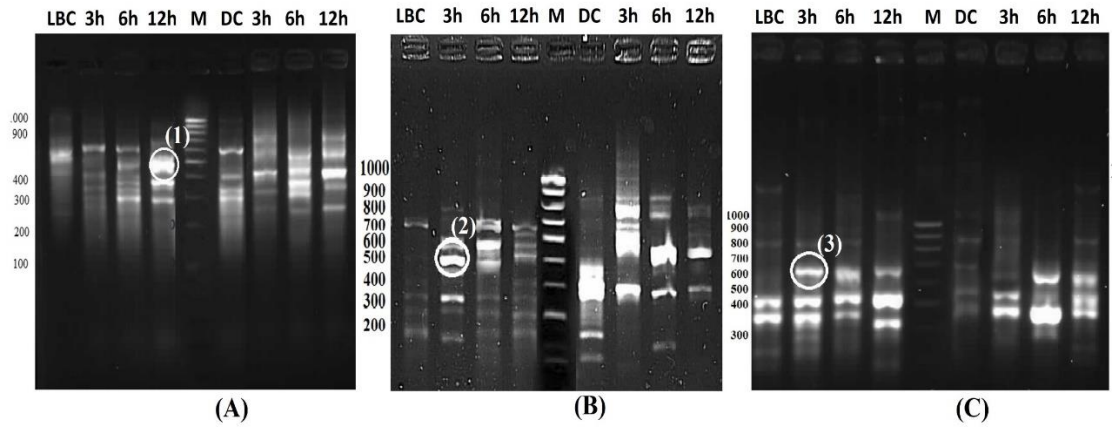

Fig. 3. Differential display (DD-PCR) using primers NS2 (A), and PPO-F (B) and WRKY-F (C) of two potato cultivars infected with *R. solanacearum* and non-infected plants (controls). M:1000bp DNA marker, LBC: Lady Bedford control, lanes (3h, 6h and 12h) samples collected after 3, 6 and 12 hpi respectively of cv. Lady Bedford; DC: Diamant control, lanes (3, 6 and 12h) samples collected after 3, 6 and 12 hpi respectively, of cv. Diamant.

Phylogenetic analysis

The phylogenetic analysis of the three sequenced genes including *StDXS1*, *PPO* and *StWRKY* of *S. tuberosum* and other plant species was performed to estimate the evolutionary distances between these genes and other orthologs. Neighbor-Joining tree was performed using Mega 6 software. *StDXS1* showed similarity with the other ones isolated from *S. tuberosum*, *S. lycopersicum*, *S. pennellii* and *S. pennatisectum*, with different identity (Fig. 4). Data in Figure 5 revealed that *PPO* gene showed similarity with their alleles listed in gene bank isolated from *S. tuberosum*, *S. lycopersicum*, *S. pennellii*, *Capsicum annuum* and *S. melongena*. The gene was grouped with *S. tuberosum* genes. *StWRKY* gene showed similarity with their alleles listed in gene bank isolated from *S. tuberosum*, *S. pennellii*, *S. lycopersicum*, *Coffea eugenioides*, *Nicotiana tomentosiformis*, *Citrus sinensis*, *Chrysanthemum morifolium*, *Santalum album*, *S. pinnatisectum*, *Gossypium turneri* and *G. raimondii* with different identity (Fig. 6). The gene was grouped with *Solanum tuberosum* genes.

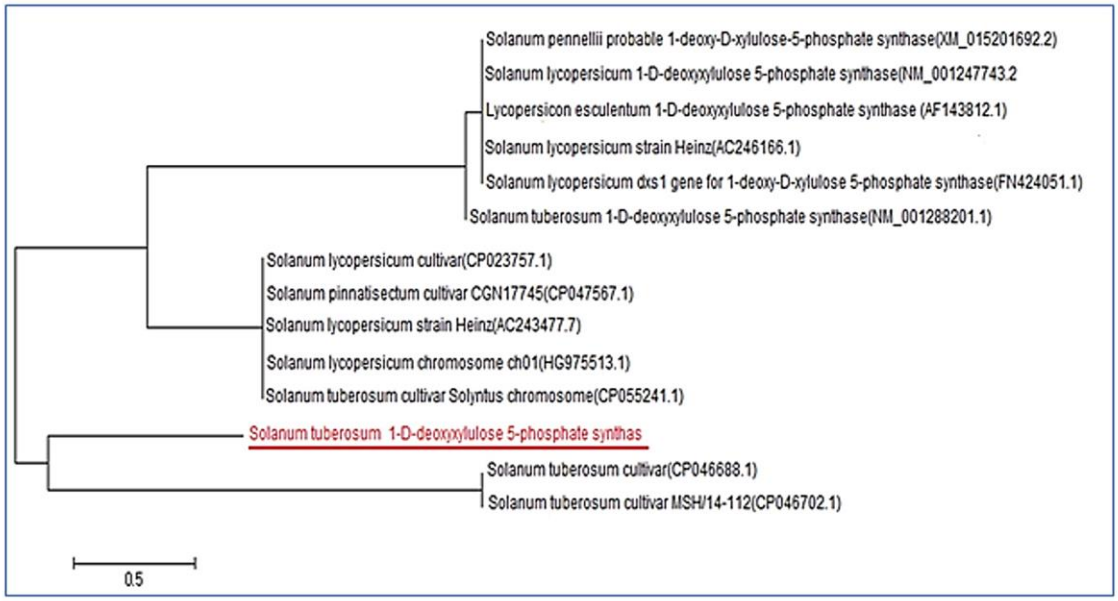

Fig. 4. Phylogenetic tree showing evolutionary relationship of Solanum tuberosum 1-D-deoxyxylulose 5-phosphate synthase (*StDXS1*) mRNA of potato plants and the others presented in GenBank.

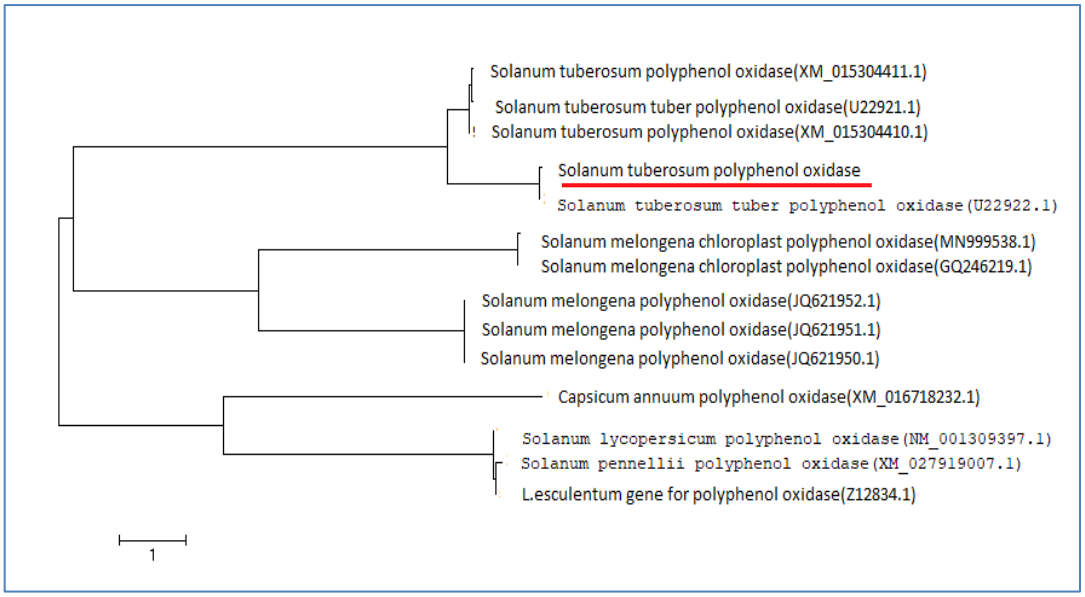

Fig. 5. Phylogenetic tree showing evolutionary relationship of *Solanum tuberosum PPO* gene of potato plants and the others presented in GenBank.

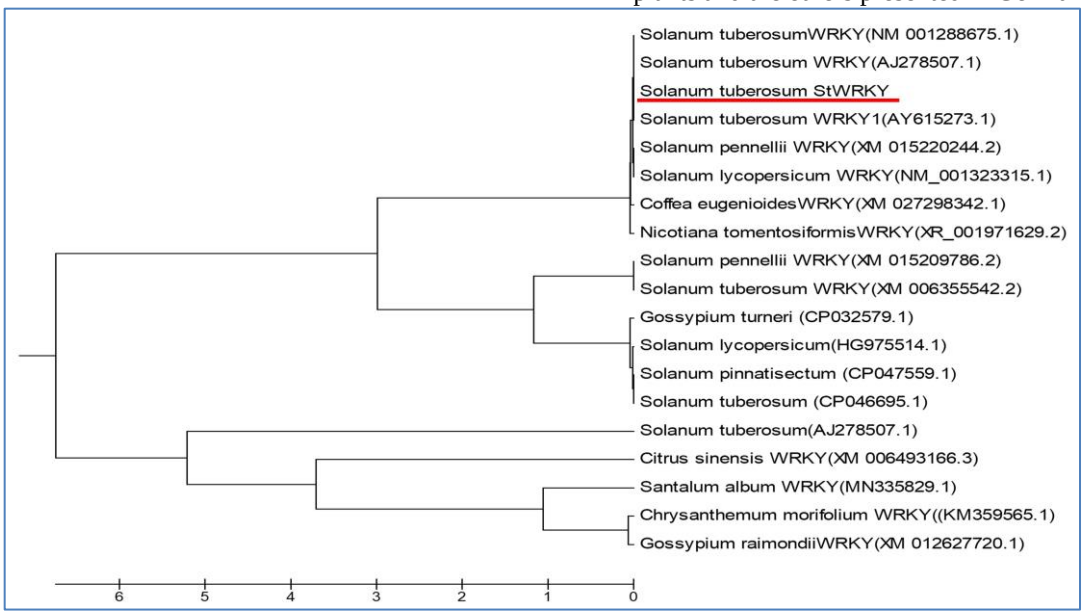

Fig. 6. Phylogenetic tree showing evolutionary relationship of Solanum tuberosum StWRKY gene of potato plants and the others presented in GenBank.

Expression analysis of candidate defense response genes against *R. solanacearum*

Expression timing patterns of peroxidase (*POD*), polyphenol oxidase (*PPO*), *StWRKY* and *StDXS1* genes were analyzed using RT-qPCR in potato leaf samples of partially resistant cv. Lady Bedford and susceptible cv. Diamant at 1, 3, 6, 12, and 24 hrs post inoculation (hpi) with *Rs*.

The obtained results in Fig. (7) revealed that the infected plants of both cultivars showed a significantly higher expression level of *POD* and *PPO* genes compared to the non-infected control plants. Expression level of *POD* increased significantly from 1 hpi in the partially resistant cultivar Lady Bedford (1.41-fold), and from 3 hpi in the susceptible cultivar Diamant (1.28-fold). Levels of *POD* activity were gradually increased in both cultivars until 12 hpi and decline at 24 hpi. The highest expression level was recorded at 12 hpi in both cultivars, with approximately 3.96-fold and 2.35-fold in the resistant and susceptible cultivars, respectively (Fig. 7A).

On the other hand, *PPO* expression level was not significantly different between infected and non-infected plants of the two cultivars at 1 hpi. A significant increase was observed in both cultivars from 3 hpi. *PPO* activity gradually increased in both cultivars until reaching a maximum expression of 4.36-fold and 2.46-fold, at 24 hpi, respectively, in the resistant and susceptible cultivars (Fig. 7B). A significant difference in *POD* and *PPO* activity were observed between

both cultivars when they compared at each sampling point of the experiment.

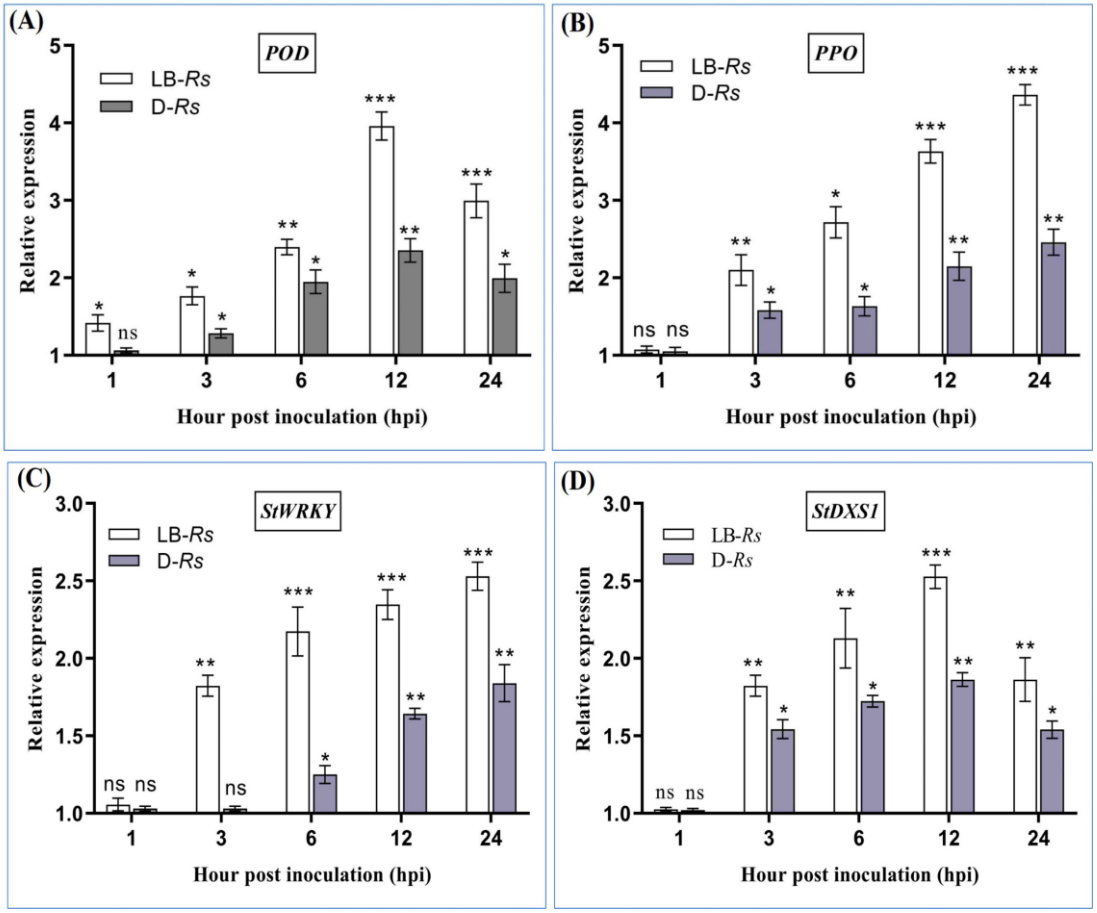

Fig. 7. Relative gene expression of *POD* (A), *PPO* (B), *StWRKY* (C) and *StDXS1* (D) genes in the leaves of two potato cultivars (the partial partially resistant cv. Lady Bedford and the susceptible cv. Diamant) after 1, 3, 6, 12 and 24 hours post inoculation (hpi) with *R. solanacearum*. LB-*Rs* and D-*Rs* are plants inoculated with *R. solanacearum* of cv. Lady Bedford and cv. Diamant, respectively. The expression level of the target genes were normalized relative to actin gene and relative expression of untreated control plants at each time were set as 1.

* Each value represents mean ± SE (n = 3).

* Asterisks indicate significant differences with control using Tuckey HSD test (ns, non-significant, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Compared with the non-infected control plants, a significant Increase of *StWRKY* transcripts was observed early as 3 hpi in partially resistant cv. Lady Bedford (1.82- fold) and from 6 hpi in the susceptible cv. Diamant (1.25-fold). The maximal expression level was recorded at 24 hpi in the resistant cultivar (2.53-fold) and in the susceptible cultivar (1.84-fold) (Fig. 7C). high expression levels of *stWRKY* were maintained between 3 and 24 hpi.

For *StDXS1* gene, a significant raise in the expression level was recorded in the two cultivars from 3 hpi. The highest expression level of *StDXS1* gene was recorded at 12 hpi in the partially resistant cv. Lady Bedford (2.52-fold) and in the susceptible cv. Diamant (1.86-fold). Levels of *StDXS1* transcripts were decrease on both cultivars at 24 hpi to reach values similar to those recorded at 3 hpi (Fig. 7D). Generally, the expression level of the four studied genes was significantly lower in the susceptible cultivar than that recorded in the resistant cultivar.

POD and PPO enzyme activities:

The results in Fig. 8 showed that the differences in peroxidase (POD) and polyphenol oxidase (PPO) enzyme activities between the uninoculated plants of the two tested cultivars were not significant ($P < 0.05$). After inoculation with *Rs*, the POD and PPO enzyme activities significantly increased in the both cultivars during 1 to 24 hpi. POD activity in the partially resistant cv. Lady Bedford was increased gradually from 1 hpi and the maximum activity (2.38-fold compared with control plants) was recorded at 12 hpi, then reduced at 24 hpi but still maintained a relatively higher level than control. While, POD activity in the susceptible cv. Diamant was raised from 3 hpi and peaked at 6 hpi

(1.67-fold) and decline at 12 and 24 hpi to reach values close to those recorded in the control plants (Fig. 8A).

PPO enzyme activity was continuously increased in both cultivars and reaches a peak of 2.16-fold higher than that in un-inoculated control plants in the partially resistant cv. Lady Bedford at 24 hpi, while in the susceptible cv. Diamant, maximum activity was observed at 12 hpi and it was approximately 1.70-fold that in the control plants and decline later (Fig. 8B).

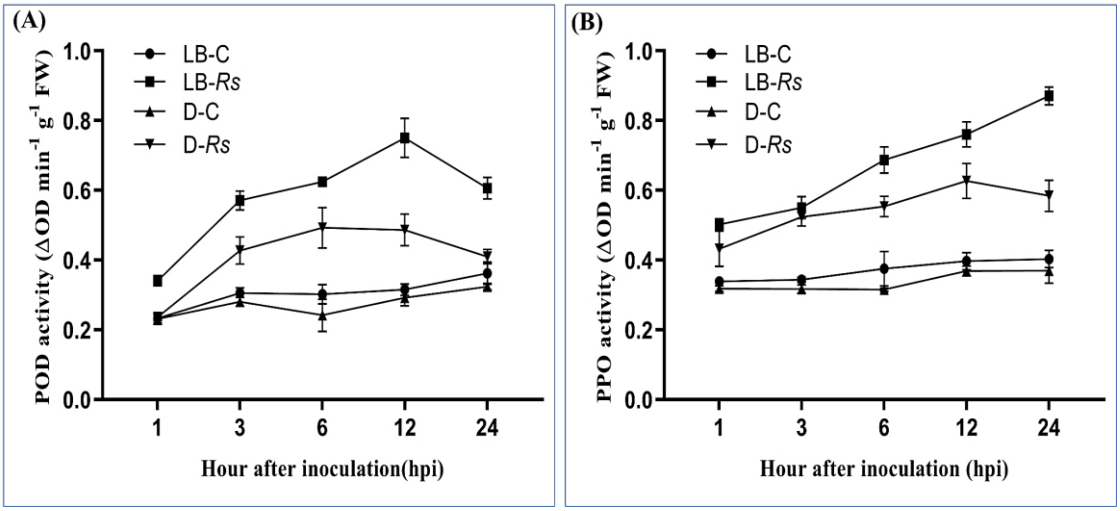

Fig. 8. **Activities of peroxidase, POD, (**A**) and polyphenol oxidase, PPO, (**B**) enzymes in the leaves of two potato cultivars** after inoculation with *R. solanacearum*. **(LB-C), cv.** Lady Bedford **non-infected plants; (LB-*Rs*), cv.** Lady Bedford **infected plants; (D-C), cv.** Diamant **non-infected plants; (D-*Rs*), cv.** Diamant **infected plants.**

*** Values are the mean of five replicates ± SE.**

DISCUSSION

Disease control for Bacterial welt is difficult because the pathogen is soil-borne and has a wide host range (Champoiseau et al., 2009). Planting resistance genotypes is considered the most effective, successful, economical and environmentally safe for controlling the disease. Unfortunately, there are no available commercial potato varieties with complete resistant against *Rs* (Huet, 2014). Therefore, while waiting for development of durable resistance potato cultivar, thus its deemed to understand more about the mechanisms that control the interaction between potato and *Rs*. In the present study, the ability of two potato cultivars in resistance to *Rs* infection were investigated. Current results indicated that the Lady Bedford cultivar has a much higher resistance to *Rs* infection than the Diamant cultivar and can be considered as a partially tolerant cultivar. The Diamant cultivar is a susceptible variety because it has a high disease severity. These results are, to some extent, in agreement with the data obtained by Elgayar (2010) and Muthoni et al. (2014) who reported different response of potato cultivars to infection by *Rs***.** It is worth noting that the resistance to *RS* shown to be unstable because it is affected by the highly variation of the pathogen and the environmental conditions (Muthoni et al., 2014).

The data cleared that the rate of photosynthetic pigments reduction due to infection with *Rs* was negatively correlated with tolerance of potato cultivars to *Rs.* The susceptible cv. Diamant inoculated with *Rs* exhibited a much reduction in total chlorophyll, chlorophyll a, b and carotene compared with the partial resistant cv. Lady Bedford. Such finding coincides with that obtained by El-Argawy and Adss (2016).

Differential display reverse transcription PCR (DDRT-PCR) has been used in the study of differential gene expression in plant-pathogen interactions (Kim et al., 2000; EL-Argawy et al., 2017) In this study, we use DDRT-PCR to detect genes that differentially expressed in plants of two potato cvs differed in resistance to *Rs*. Three randomly clear differentiated cDNA fragments that are present in inoculated plants of the partially resistant cv. Lady Bedford but not in the susceptible cv. Diamant or control plants were excised from gels and sequenced. The sequences of these cDNA fragments show significant homology to known genes in the GenBank database and identified as 1-D-deoxyxylulose 5-phosphate synthase gene (*StDXS1*), polyphenol oxidase (*PPO)* gene and WRKY transcription factor (*StWRKY*).

The DXS catalyzes the initial step in the methyl-D-erythritol-4-phosphate (MEP) pathway and considered the key enzyme in isoprenoid (terpenoids) biosynthesis (Henriquez et al., 2016; Li et al., 2021b). Isoprenoid had a highly diverse family of natural products which involved in photosynthetic pigments, redox or hormones cofactors and plant defense against pathogens

(Bouvier et al., 2005; Gershenzon and Dudareva, 2007; Henriquez et al., 2016). The complete potato *StDXS1* cDNA encodes for 719 amino acid residues, present in single copy in the potato genome and highly conserved among species (Henriquez et al., 2016). Suppression of *StDXS1* that leads to a reduction in the accumulation of isoprenoids could be correlated with the increase in the late blight symptoms (Henriquez et al., 2016). In the present study StDXS1 were upregulated at 12 hpi in the partially resistant cv. Lady Bedford plants infected with *Rs* which may be associated with accumulation of isoprenoids that contribute to plant protection against *Rs*.

In higher plants, WRKY is one of the largest families of transcription factors (TF) that participate in regulation of a wide range of signal transduction and in biotic and abiotic stress response pathways (Dong et al., 2003; Ramamoorthy et al., 2008; Giacomelli et al., 2010; Rushton et al., 2010; He et al., 2016; Li et al, 2020). WRKY TFs are zinc-finger-type protein (Eulgem et al., 2000) specifically bind to the W-box in the promoter region of pathogenesis-related proteins (PR), to regulate the expressions of these genes (Maleck et al., 2000; Bi et al., 2016). Some WRKY proteins are required in the SA and JA/ET signaling pathways crosstalk that modulate defecne system in plant (Li et al., 2006; Mao et al., 2007; Liu et al., 2016). WRKY TFs has been well known to play vital roles in plant defense against fungal, bacterial and viral pathogens (Eulgem and Somssich, 2007; Giacomelli et al. 2010; Cheng et al., 2015; Jiang et al., 2016; Liu et al., 2017). EL-Argawy et. al., 2017 reported differentially expressed of *StWRKY* gene after infection with *A. solani* in the resistance potato cultivar, which were consistent with the results of the present study.

PPO is an important phenol-oxidizing enzyme that involved in response to defense reaction and hypersensitivity against pathogens (Mohammadi and Kazemi, 2002; Agrios, 2005). PPO catalyze the oxidation of phenolic compounds into quinones (antimicrobial toxic compounds), lignification of plant cells and participate in the oxidative polymerization of flavonoids (Schijlen et al., 2004; Constabel and Barbehenn, 2008). PPO is one of the main systemic acquired resistance (SAR) related enzymes, and their activities are related to resistance in plant. Expression of the PPO has been found to be associated with the resistance to bacteria, fungi and insect pathogens (Campos et al., 2004; Constabel and Barbehenn, 2008). Over-expression of PPO in transgenic tomato plants decreased the susceptibility to *Pseudomonas syringae* (Li and Steffens, 2002). In contrast, downregulation of *PPO* increased disease susceptibility (Thipyapong et al. 2004). The inoculation with *Rs* results in up-regulated of *PPO* gene expression in the resistant tomato cultivar and down-regulated in the susceptible one (Jayanna and Umesha, 2017) which was in harmony with the results in the present study.

In the present study, gene expression levels in the leaves of a partially resistant cultivar, Lady Bedford, and a susceptible cultivar, Diamant, using qRT-PCR technique was analyzed. The level expression of *POD, PPO, StWRKY* and *StDXS1* genes were increased on potato infected with *Rs* compared to the control. However, Lady Bedford cultivars had a higher gene expression than Diamant cultivar. This clear transcriptional response suggested that these genes participate in the basal defence of potato to *Rs*. Several studies reported that POD and PPO gene expressions were induced in host-pathogen interaction for example, *Vicia faba- Uromyces fabae* (Walters et al., 2006), hybrid poplar- *Melampsora medusae* (Miranda et al., 2007), mung bean- *Xanthomonas hortorum or X. axonopodis* (Farahani and Taghavi, 2016) and potato- *R. solanacearum* (EL-Argawy and Adss., 2016; Jayanna and Umesha, 2017) pathosystems. The results of *StWRKY* gene expression obtained on this study confirmed the importance of these transcription factors in the modulation of resistance to plant pathogens. Various studies reported the upregulation of *WRKY* genes in potato in response to pathogen invasions. For example, *WRKY* gene was upregulated after infection by *P. infestans* (Yogendra et al., 2015, 2016), *E. carotovora* (Mabrouk et al., 2008), Mexican potato purple top phytoplasma (Longoria-Espinoza et al., 2013), *A. Solani* (El-Argawy et al., 2017). Furthermore, the potato resistance cultivars had a higher *StWRKY* gene expression and peaked earlier than the susceptible cultivars (Mabrouk et al., 2008; El-Argawy et al., 2017). On the other hand, *StDXS1* gene was upregulated after infection by *RS* and reach the maximum expression at 12 hpi. Such finding coincides with that obtained by Henriquez et al., 2016 who found that *StDXS1 transcripts* in plants infected by aggressive isolate of *P. infestans* were induced until 48 hpi, decline drastically thereafter and the highest induction occurred at 12 hpi.

Disease resistance in plants is associated with activation of a wide range of defense mechanisms that slow down or prevent infection at particular stages of the host-pathogen interaction. This defense mechanisms that activated upon infection include the induction of defense-related enzymes, particularly PAL, POD, PPO, LOX, and SOD, which reduced or prevent pathogen infection (Li and Steffens, 2002; Ebrahim, 2012; Vanitha et al., 2009 and Nisha et al., 2012). In the present study, we report the induction of POD and PPO during *Rs*-potato interaction. POD and PPO enzyme in non-infected resistant and susceptible plants recorded lesser activities compared with infected plants, indicating a possible role of these enzymes

in potato resistance to *Rs*. POD and PPO activity was higher in partially resistant cv. Lady Bedford plants than in susceptible cv. Diamant plants indicates that they might have an important role in the development of host resistance. Our findings are in harmony with the earlier studies of EL-Argawy and Adss (2016), where they reported an increase in POD, PPO and catalase enzyme activities in the resistance potato cultivar compared to susceptible cultivar after *Rs* infection. The activity of POD, LOX and PAL in the resistant tomato cultivars inoculated with *Rs* was higher when compared to the uninoculated seedlings and with the susceptible cultivars (Vanitha and Umesha, 2008; Vanitha et al., 2009). Similarly, PAL and PPO activities were increased in the resistant tomato cultivars inoculated with *X. axonopodis* pv. *vesicatoria* compared to susceptible cultivars (Kavitha and Umesha, 2008). The increase in the antioxidant enzymes activities, PAL, POD, PPO, SOD and catalase, were associated with resistance to *E. carotovora* (Mustafa and Alawami, 2012 and Ngadze *et al*., 2012). In plant, POD and PPO involved in the formation of lignin, strengthening the cell wall, wound responses, pathogen attack, growth regulation, and synthesis of phytoalexins and phenolics (Ramamoorthy et al. 2002; De Ascensão and Dubery, 2003), which enhance plant resistance to pathogens.

CONCLUSION

The findings of this study may contribute to the understanding of resistance of potato plants to *Ralstonia solanacearum* the causative agent of bacterial welt disease. We have successfully identified three differentially expressed genes in partially resistant potato cultivar challenged by *Rs* using differential display technique. 1-D-deoxyxylulose 5-phosphate synthase (*StDXS1*), polyphenol oxidase (*PPO)* and WRKY transcription factor (*StWRKY*) were induced in early responses in potato leaves with *Rs* infection. Peroxidase and polyphenol oxidase genes and enzymes also involved in the potato defense response.

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الملخص العربى

الإختلاف في التعبير الجيني لصنفين من البطاطس إستجابة للعدوي ببكتيريا *Ralstonia solanacearum*

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مرض الذبول البكتيري المتسبب عن بكتيريا *Ralstonia solanacearum* (*RS*) يعتبر من أهم الأمراض النباتية وخاصة علي نباتات البطاطس. في هذه الدراسة تم مقارنة الأساس الجزيئي لمقاومة صنفين من البطاطس، صنف دايمونت (حساس) وصنف ليدي بيدفورد (مقاوم جزئياً)، للإصابة بالبكتيريا. حيث أظهرت النتائج أن صنف ليدي بيدفورد كان أكثر مقاومة (24.3% ذبول) من صنف دايمونت (83.8% ذبول) وحدث أعلي خفض في الكلوروفيل والكاروتين في صنف دايمونت بالمقارنة مع صنف ليدي بيدفورد. تم إختيار 3 شظايا من cDNA من الجل والتي ظهرت في الصنف المقاوم وتم فك التتابعات الوراثية لها وتم تعريفها علي أنها 1-D-deoxyxylulose 5-phosphate synthase gene (*StDXS1*) و polyphenol oxidase (*PPO*) gene و WRKY transcription factor (*StWRKY*) من جينوم البطاطس. أظهرت النتائج أنه تم إستحثاث التعبير الجيني لجينات (peroxidase) *POD* و *PPO* و *StWRKY* و *StDXS1* بعد العدوي بالمقارنة مع الكنترول الغير معدي. وكان أعلي مستوي للتعبير الجيني في الصنفين عند 12 و24 و24 و12 ساعة بعد العدوي للأربعة جينات علي التوالي. نشاط إنزيمات البيروكسيديز POD والبولي فينول أوكسيديز PPO زاد معنويا في كلا الصنفين بعد العدوي. صنف ليدي بيدفورد المقاوم جزئياً أعطي أعلي نشاط إنزيمي وكان أعلي قيمة له عند 12 و24 ساعة بعد العدوي لكل من POD و PPO علي التوالي بينما صنف دايمونت الحساس أعطي أعلي قيمة نشاط إنزيمي له عند 6 و 12 ساعة بعد العدوي لكلا الصنفين. تشير هذه الإستجابات إلي إحتمالية مشاركة هذه الجينات والإنزيمات في الدفاع عن البطاطس ضد الإصابة ببكتيريا *R. solanacearum*.