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Differential Expression of Induced Resistance Genes by Abscisic Acid (ABA) Against Potato Early Blight Disease

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Abscisic acid (ABA), a plant hormone involves in various plant physiological processes including, response to biotic and abiotic stress conditions. ABA was tested to study its ability to enhance disease resistance of two potato cultivars *i.e.*, Nicola and Spunta, to the infection by the early blight fungus *Alternaria solani* (*A. solani*) under greenhouse conditions. Treatment with ABA solution (10 µM) reduced the early blight disease severity for both cultivars compared to the untreated infected plants. Differential display gene analysis for treated plants revealed three clear bands. These bands showed significant homology to well-known genes in the GenBank database and identified as superoxide dismutase (CuZnSOD), WRKY transcription factor (StWRKY) and Endoglucanase from potato genome. Real-Time PCR was used to detect the changes in gene expression of CuZnSOD and WRKY-type genes after infection with *A. solani* for both Nicola and Spunta potato cultivars and in different time intervals. The expression level of CuZnSOD and WRKY genes in potato leaves of both cultivars were increased for ABA treated and infected plants and for untreated/infected plants compared to the untreated/uninfected plants. Both genes were over expressed in infected plants treated with ABA than untreated ones and reached the maximum level of expression after two days of inoculation. The highest increase of both genes expression was observed in potato leaves of cv. Nicola compared to cv. Spunta.

Keywords: Abscisic acid (ABA), CuZnSOD gene - WRKY genes, early blight and potato.

Early blight of potato, caused by *Alternaria solani* (Ellis & Martin) Jones & Grout, is one of the most common foliar diseases of potato in Egypt and around the world (Gherbawy, 2005). Yield losses due to early blight disease varied from 5 - 78% (Waals *et al*., 2004). Control of this disease relies on the use of fungicides and resistant cultivars (Secor and Gudmestad, 1999 and Van der Walls *et al*., 2001).

Alternatives to the use of chemical fungicides to control early blight disease were suggested (Abdel-Kader *et al*., 2013 and Burketová *et al*., 2015). This included the use of resistance inducing agents such as jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) for their safe effect on the environment (Odilbekov, 2015). ABA is a plant hormone involves in various plant physiological processes including, control of seed dormancy, regulation of stomatal closure, modifications of gene expression and response to biotic and abiotic stress conditions such drought, salinity,

cold and pathogen attack (Finkelstein *et al*., 2002; Zhu, 2002; Chinnusamy *et al*., 2008 and Danquah *et al*., 2014). It plays a positive role for disease resistance enhancement in plant pathogen interactions. ABA reduced infection of tomato by *Xanthomonas campestris* following exogenous ABA treatment (Ramos and Volin, 1987). Meanwhile, induced resistance of common bean to *Colletotrichum lindemuthianum* was associated with increased content of ABA (Dunn *et al*., 1990). ABA is also known to mediate ABA induced resistance in *Arabidopsis* against *Alternaria brassicicola* (Ton and Mauch-Mani, 2004). Exogenous application of ABA also induced basal resistance of rice against *Cochliobolus miyabeanus* (De Vleesschauwer *et al*., 2010) and effectively reduced early blight (*Alternaria solani*) disease severity in tomato plants (Song *et al*., 2011).

ABA is involved in signal transduction pathways for the activation of defense genes against plant cell membrane damage in the host-pathogen interaction (Adie *et al*., 2007; Lee and Luan, 2012 & Nassar and Adss, 2016). ABA induced the apoplastic H_2O_2 accumulation in maize leaves and increased the activities of antioxidant enzymes such as superoxide dismutase SOD (Hu *et al*., 2005 and 2006 & Zhang *et al*., 2006). Superoxide dismutase (SOD) can repair injured plant cells by catalysing the dismutation of O_2^- to H_2O_2 and O_2. Generally, high SOD activity leads to lower membrane damage and less plant susceptibility (Monk *et al*., 1989 and Zhang *et al*., 2004). Three types of SOD are distinguished by their associating metals: copper/zinc- (Cu/Zn), manganese- (Mn-), and iron- (Fe-) isozymes (Alscher *et al*. 2002). Cu/Zn- and Mn-metalloenzymes are ubiquitously distributed among plants. Cu/Zn-SOD is the most abundant, occurring as two distinct isoforms that are targeted to the cytosol and the chloroplast stroma. Mn-SOD typically appears in the mitochondrial matrix, whereas Fe-SOD is found within the plastids of limited plant species.

WRKYs are a large family of regulatory proteins in plants, with 72 members in *Arabidopsis* and close to 100 in rice (Ross *et al*., 2007). The WRKYs genes have been found to play essential roles in various physiological processes and were involved in the defense against phytopathogens such as bacteria (Dong *et al*., 2003), fungi (Zhang *et al*., 2006) and viruses (Youda *et al*., 2002).

Endoglucanase is considered a defense-related protein because it is usually associated with interactions between the plant and fungal pathogens as 1, 3-*β*-glucanase able to degrade fungal cell walls (Simmons, 1994). There are a lot of reports of the induction of 1, 3-*β*-glucanase expression during fungal pathogen infection (Moy *et al*., 2002). Two 1, 3- *β*-glucanases were induced in potato after infection with late blight causal untrue fungus *Phytophthora infestans*. Tomato plants resistant to the fungal pathogen *Cladosporium fulvum* produced l, 3-β-glucanase earlier than susceptible varieties (Young and Pegg 1982; Kombrink *et al*., 1988; Joosten and De Wit 1989 and Neuhaus *et al*., 1992).

The differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) was used to identify differences in gene expression under different environmental conditions as well as the host-pathogen interaction. It is based on the amplification of partial complementary DNA (cDNA) sequences from a pool of messenger RNA (mRNA) (Carginale *et al*., 2004). In addition, Real Time PCR (RT-

PCR) has become an extensively applied technique in molecular plant pathology and it has been extensively used for quantification of resistance gene expression (Nolan *et al*., 2006). RT-PCR proved to be a successful tool for the evaluation of resistance genes in potato (Pasche *et al*., 2013).

The present study therefore aimed to (1) investigate the effect of exogenous application of ABA to enhance resistance of potato plants against *A. solani*, (2) to reveal genes associated with this enhancement, and (3) to study the effect of ABA on expression (activity) of these genes.

Materials and Methods

1. Efficacy of treatment with ABA on early blight development:

1.1. Plant materials:

Two potato cultivars, cvs. Nicola and Spunta were used in current study. These cultivars were obtained from the International Potato Centre, Kafr El-Zayat, Gharbiya Governorate, Egypt. Nicola cv. is resistant, while, cv. Spunta is susceptible to infection by *A. solani* (Nassar and Adss, 2016).

1.2. Fungal culture:

A. solani isolates tested in the present study were isolated from potato leaves showed typical lesions of early blight collected from commercial potato fields in Etay-Elbarood, El- Beheira governorate, Egypt, and identified as *A. solani*, based on the morphological characteristics according to Ellis (1976).

1.3. Inoculum preparation:

To induce sporulation, cultures were grown for 6 days at 23-25^{o}C on PDA medium under near ultraviolet light (310-400 nm) with 16h/day light. Conidia were collected by washing plates with sterile distilled water and the resulting spore suspension was adjusted to a concentration of 10^6 spores/ml using hemocytometer.

Potato tubers of cvs. Nicola and Spunta were surface sterilized with 1% sodium hypochlorite for five minutes, then washed with sterile water, and planted in plastic pots 30 cm diameter filled with sterile peat moss (one tuber per pot). Plants were grown under greenhouse conditions (25±2^{o}C and 60-70% RH), and watered and fertilized as usual for potato cultivation.

1.4. Treatments and inoculation:

At two true leaves stage, plants were sprayed with ABA solution (Bayar products) at concentration of 10 µM until draining. Treatment with ABA was repeated one time weekly until fungal inoculation. After 45 days, potato cultivars were inoculated with *A. solani* by spraying plants with a suspension of 10^6 spores/ml or sterile water as control according to Bokshi *et al*. (2003). The plants were covered with clear plastic bags for 24h to maintain high relative humidity. After 24h, bags were removed and plants were moved to the normal conditions in the greenhouse at 25±2^{o}C. Each treatment was represented by five replicates with 3 pots per replicate.

1.5. Assessment of disease intensity:

Disease index was assessed 15 days after inoculation using a 1-6 scale given by Pandey and Pandey (2002), whereas, 0= Disease free, 1= 1-10%, 2= 11-25%, 3= 26-

50%, 4= 51-75%, 5= 76-100% leaf area infected. The percent of disease intensity (PDI) was calculated according to the following formulae given by Xu *et al*. (2011) as follows:

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

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

2. Molecular effect of ABA against A. solani on potato:

2.1. RNA isolation:

Total RNA from healthy and infected treated and untreated potato leaves was extracted using GStract™ RNA Isolation kit II (Maxim Biotech INC, USA) according to the manufacture protocol.

Reverse transcription reactions were performed to transform mRNA to first-strand cDNA using oligo dT primer (5′-TTTTTTTTTTTTTTT-3′). Each 25 µl reaction mixture contained 2.5 µl (5x) buffer with $MgCl_2$, 2.5 µl (2.5 mM) dNTPs, 1 µl (10 pmol) primer, 2.5 µl RNA (2 µg/µl) and 0.5 unit reverse transcriptase enzyme (M-MLV, Fermentas, USA). PCR amplification was performed in a thermal cycler (Eppendorf, Germany) programmed at 42°C for 1 hr. Synthesis reactions were stopped at 72°C for 10 min incubation. The product was stored at 4°C until use (Chin *et al*., 2000 and Ramadan *et al*., 2015).

2.2. Differential display real time-PCR (DDRT-PCR):

The differential display real time-PCR (DDRT-PCR) (Liang and Pardee, 1992) was performed on single stranded-cDNAs (ss-cDNA) using different primers, PR2, NS2, EzA1A13, WRKY1, PR1 and SOD, specific for segment amplification of gene-encoding enzymes and factors related to resistance as random primers (Table 1). PCR amplification was performed in a thermal cycler (Eppendorf, Germany) programmed for one cycle at 95°C for 5 min, then 34 cycles as follows: 30 s at 95°C for denaturation, 1 min at 45°C for annealing and 2 min at 72°C for elongation. Reaction was then incubated at 72°C for 10 min for final extension (Chin *et al*., 2000 and Ramadan *et al*., 2015). Two µl of loading dye were added prior to loading of 10 µl of the differential PCR product per gel slot. Electrophoresis was performed at 80 Volt with 0.5 x TBE as running buffer in 1.5 % agarose/0.5x TBE gels and then the gel was stained in 0.5 $\mu g/cm^3$ (w/v) ethidium bromide solution and distained in deionized water. Finally, the gel was visualized and photographed using a gel documentation system.

2.3. Purification and partial sequencing of differentiated bands:

The target clear differentiated bands of PCR products for each primer were excised from the gel and purified using Gel Extraction Kit (BioLine, UK). The amplified DNA was sequenced by Macrogen Company (Seoul, South Korea).

2.4. Alignment of sequenced PCR products and phylogenetic analysis:

Comparison with sequences in the GenBank database was achieved in BLASTN searches at the National Centre for Biotechnology Information site (http://ncbi.nlm.nih.gov). Bootstrap neighbour-joining tree was generated using MEGA version 6 (Kumar *et al*., 2013) from CLUSTALW alignment.

2.5. Quantitative Real-time qRT-PCR assay:

Two genes fragments identified as differentially expressed, StWRKY and CuZnSOD genes, were confirmed and quantified by comparative quantitative Real-time (qRT-PCR). Total RNA was extracted from leaves samples collected 0, 1, 2, 4, 8 and 15 days post inoculation using GStract™ RNA Isolation kit II (Maxim Biotech INC, USA). Reverse transcription-polymerase chain reaction (RT-PCR) of mRNA was carried out with the same method illustrated in differential display technique.

Real-time PCR was performed using SYBR Green PCR Master Mix (Fermentas, USA). Each reaction was performed in a 25 µl mixture, which contains 12.5 µl of 2× SYBR Green PCR Master Mix, 1 µl of 10 pmol/µ1 of each primer, 1 µl of template cDNA (50 ng), and 9.5 µl of RNase free water. The amplification program proceeded 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 step. The reaction was performed using a Rotor-Gene 6000 (Qiagen, ABI System, USA).

Table 1. Sequence of primers used in the study

Primers			Primer sequence 5′→3′	Annealing (°C)
Differential PCR	PR2		GGACACCCTTCCGCTACTCTT	40
	NS2		GGC TGC TGG CAC CAG ACT TGC	40
	EzA1A13		CAG GCC CTT CCA GCA CCCAC	40
	WRKY1 (F)		ACGTTTAAACCATTCTCAGAAATAGC	40
	PR1		GCCAAGCTATAACTACGCTACCAAC	40
	SOD F		TGTTGCAAATGCTGAGGGCATAGC	40
Real time PCR	StWRKY	F	ACGTTTAAACCATTCTCAGAAATAGC	60
		R	ACCTCGAGATACATGCCTTACTAGGC	
	CuZnSOD	F	ATGGTGAAGGCTGAAGCTGTTCTT	60
		R	CTATCCTCGCAAACCAATACCG	
	ITS	F	TCCGTAGGTGAACCTGCGG	60
		R	TCCTCCGCTTATTGATATGC	

2.6. Quantitative Real-time qRT-PCR data analysis:

Comparative quantification analysis was conducted using Rotor-Gene-6000 Series Software according to Rasmussen (2001). The ratio of a target gene is expressed in a sample versus a control in comparison to a reference gene. The sample and control dataset of real-time PCR data were analysed with appropriate Bioinformatics and Statistical program for the estimation of the relative expression of genes using real-time PCR and the result normalized to ITS housekeeping gene (Reference gene). The data were statistically evaluated, interpreted and analysed using Rotor-Gene-6000 version 1.7.

Statistical analysis:

Statistical analyses were performed using the SAS ver. 8.1 (SAS, 2000). Least Significant Difference (LSD) test was used to assess differences among means at 0.05 levels.

Results

1. Effect of abscisic acid on the development of early blight:

Data in Table 2 show that potato, cv. Nicola was most resistant to *A. solani* as exhibited 42.6% disease intensity compared to 61.0 % for cv. Spunta. Meanwhile, treatment with abscisic acid (ABA) solution at 10 µM reduced the early blight disease intensity by 55.4 and 47.1% on the tested cvs. Nicola and Spunta, respectively, compared to the untreated inoculated potato plants.

Table 2. Early blight disease intensity on potato plants of two cultivars treated with abscisic acid (ABA) (10 µM) and inoculated by *Alternaria solani* under greenhouse conditions

Treatment	cv. Nicola		cv. Spunta	
	Disease intensity (%)	Reduction (%)	Disease intensity (%)	Reduction (%)
Inoculated- untreated	42.6 a	----	61.0 a	----
Inoculated- treated	19.0 b	55.4	32.3 b	47.1

2. Molecular effect of ABA to induce potato resistance against A. solani:

2.1. Differential display, Nucleotide sequence and phylogenetic analysis:

The messenger RNA (mRNA) differential display was used to study the response of the two potato cvs. treated with ABA to the infection by *A. solani*. Leaf samples were collected two days post inoculation with *A. solani* from ABA treated plants as well as the untreated inoculated plants and healthy untreated plants (control). The extracted mRNA was used to synthesis complementary DNA (cDNA) and subsequently subjected to differential display PCR (DD-PCR) analysis using different six primers, PR2, NS2, EzA1A13, WRKY1-F, PR1, and SOD-F.

Results of the differential display (Fig. 1) revealed that new differentiated bands were visualized in the electrophoretic pattern of the ABA treated potato plants compared to the untreated ones particularly in the more tolerant cv. Nicola (Fig. 1 A, B, C).

Three of these differentiated visualized bands were randomly excised from gels and sequenced. Band 696 bp (Fig. 1A) amplified from cv. Nicola plants treated with ABA was identified as superoxide dismutase and showed 98% similarity to *Solanum tuberosum* superoxide dismutase gene [Cu-Zn], chloroplastic (LOC102605869), transcript variant X3, mRNA (NCBI Reference Sequence: XM_006348977.2). The phylogenetic tree constructed for the identified superoxide dismutase (SOD) gene, with other 17 different genes from GenBank showed similarity with 15 out of the 17 listed genes tested in gene bank with different similarity coefficient (Fig. 2).

Meanwhile, band 550 bp (Fig. 1B), was shown with WRKY1-F primer, and showed 99% similarity to *Solanum tuberosum* StWRKY mRNA gene (*Solanum tuberosum* WRKY1 (AY615273.1) and other related species in Gene bank with different similarity coefficients (Fig. 3), and so identified as WRKY transcription factor. Also, band 960 bp amplified using PR2 primer (Fig. 1C) from cv. Nicola was identified as endoglucanase and showed 98% similarity to *Solanum tuberosum* endoglucanase gene 8-like (LOC102592181), mRNA NCBI Reference Sequence: XM_006341014.2. On the other hand, Fig. (4) shows that endoglucanase gene of potato and the others 13 genes presented in GenBank exhibited of related species with different similarity coefficients. The gene was grouped with *Solanum tuberosum* and *Solanum lycopersicum* genes.

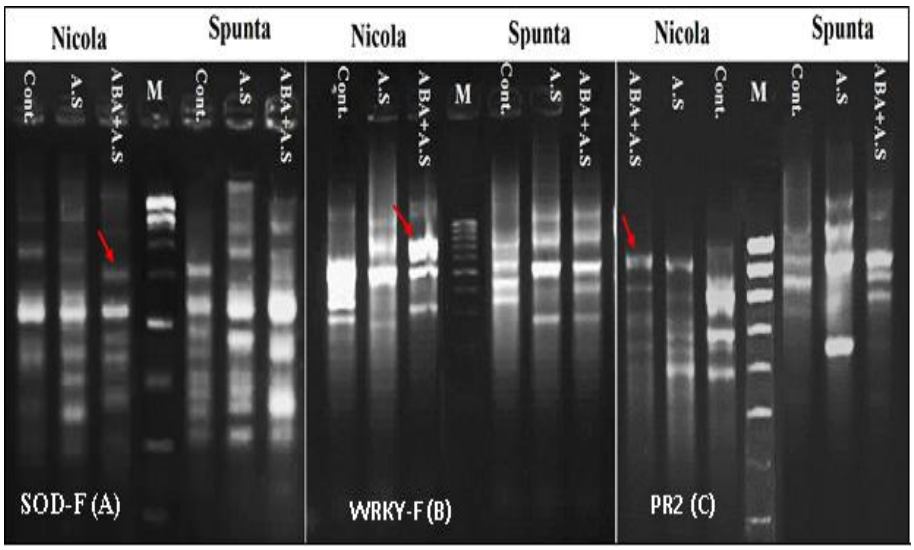

Fig. 1. Electrophoretic pattern of PCR for detection of genes involved in resistance in two potato cultivars cvs. Nicola and Spunta, treated with ABA and inoculated with *A. solani* using three primers, SOD-F (A), WRKY-F (B) and PR2 (C). Cont.: untreated control; A.S: *A. solani*; ABA+A.S: ABA+ *A. solani*; M: 1000 bp DNA marker (1000, 900, 800,700, 600, 500, 400, 300, 200 and 100bp); arrows indicate the selected up regulated gene.

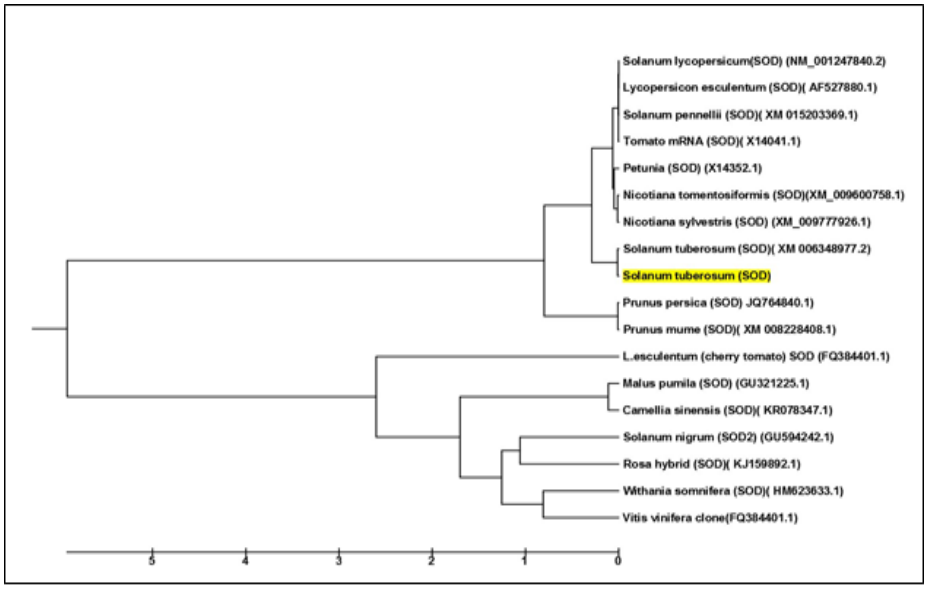

Fig. 2. Phylogenetic tree showing evolutionary relationship between superoxide dismutase (SOD) gene of potato plants and the others presented in GenBank. The Neighbor-Joining method was used to construct the tree using the Mega 6 program. Names and accession number of the nucleotide sequences encode the corresponding SOD genes

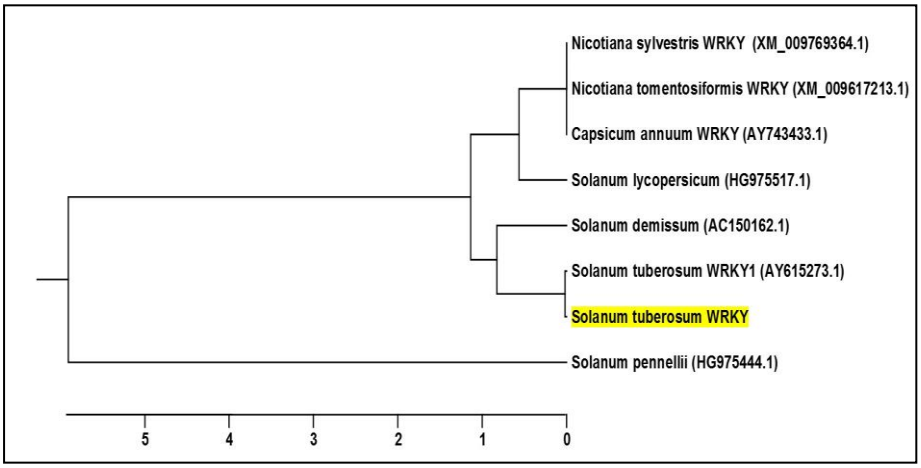

Fig. 3. Phylogenetic tree showing evolutionary relationship between WRKY gene of potato plants and the others presented in GenBank. The Neighbor-Joining method was used to construct the tree using the Mega4 program. Names and accession number of the nucleotide sequences encode the corresponding WRKY genes.

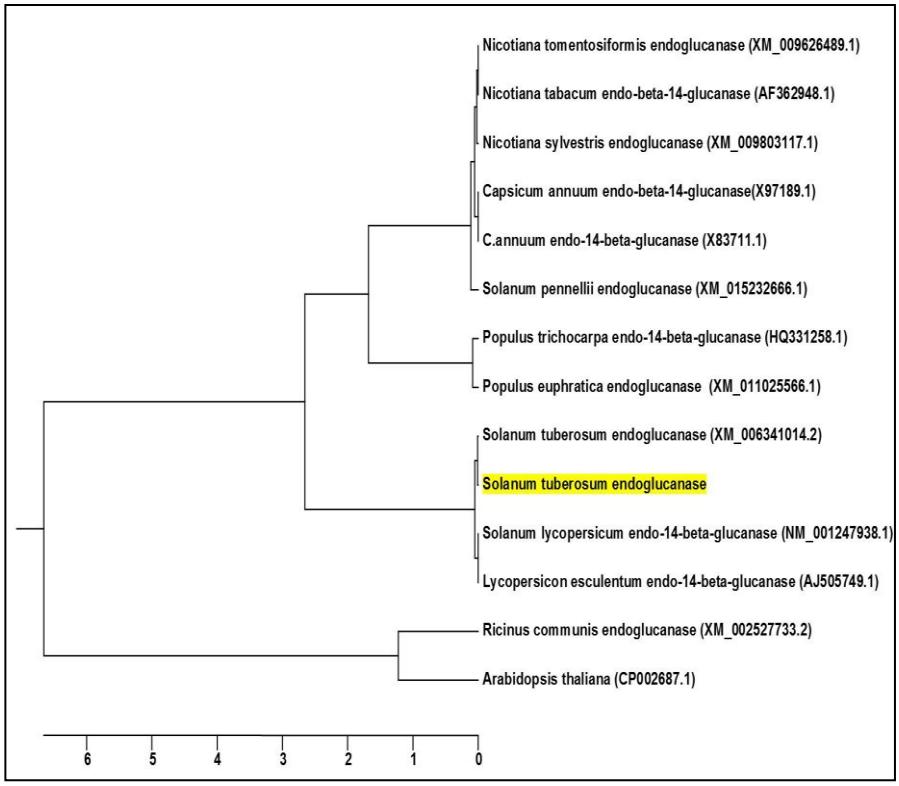

Fig. 4. Phylogenetic tree showing evolutionary relationship between endoglucanase gene of potato plants and the others presented in GenBank. The Neighbor-Joining method was used to construct the tree using the Mega4 program. Names and accession number of the nucleotide sequences encode the corresponding endoglucanase genes.

3. Gene expression (activity):

Data illustrated in Fig. 5 show that the expression of CuZnSOD and StWRKY genes analysed by real-time PCR was higher in the ABA treated and inoculated potato leaves of both potato cultivars than the untreated and inoculated plants over the 15 days of the investigation with more evident for higher expression in the more tolerant cv. Nicola compared to the susceptible cv. Spunta. Meanwhile, a noticeable increase in gene expression was revealed in potato plants of both cvs. inoculated with *A. solani* compared to the uninoculated plants (Fig. 5). However, the highest gene expression was detected two days after inoculation, for both genes then a decline was occurred to reach the lowest level after 15 d of infection but remained greater than control (Fig.5).

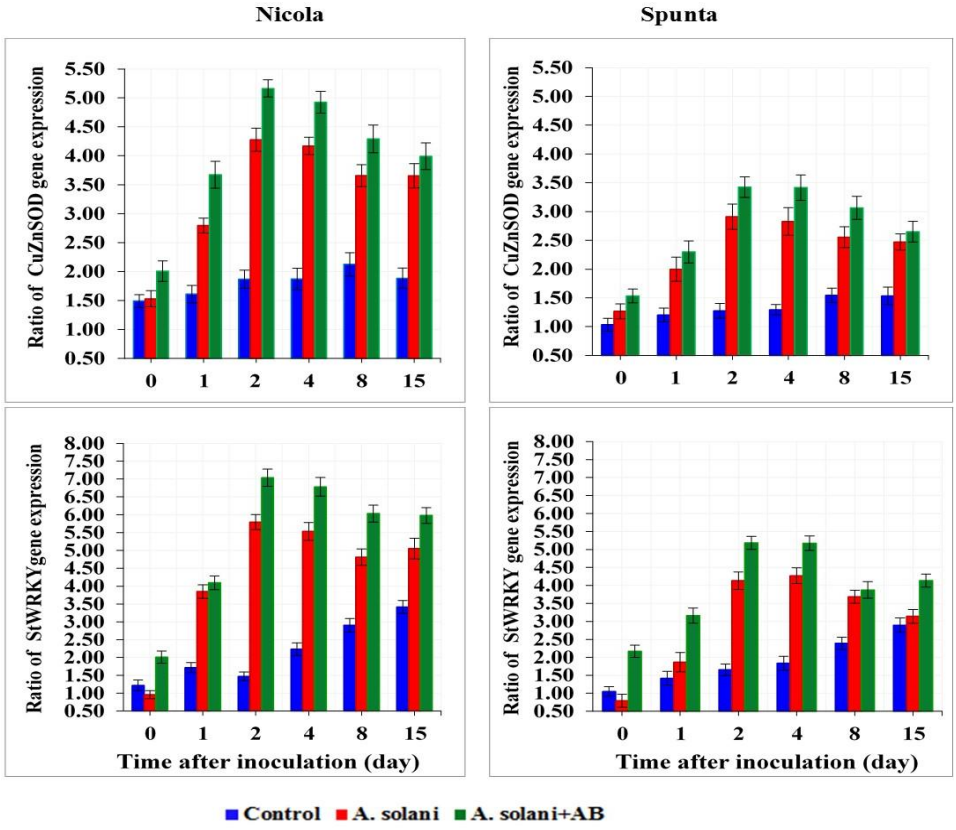

Fig. 5. Superoxide dismutase CuZnSOD and WRKY genes expression in cvs. Nicola and Spunta, of potato leaves treated with ABA and inoculated with *A. solani* up to 15 days after inoculation.

Discussion

Early blight is one of the major diseases affected potato plants in many parts of the world (Binyam, 2014). Control of this disease mainly relies on the use of fungicides and using resistant cultivars (Patel *et al.*, 2004; Mustafee *et al*., 2007 and Abo El-Abbas *et al*., 2010). Resistance inducing agents such abscisic acid (ABA) was suggested to be used as alternative for the use of fungicides to control early blight disease (Song *et al*., 2011).

The results obtained in the present study showed that potato cv. Nicola was more resistant to *A. solani* as exhibited 42.6% disease intensity compared to the susceptible cv. Spunta which showed 61% disease intensity. These results are in harmony with those detected by Abo El-Abbas *et al*. (2010). Meanwhile, abscisic acid (ABA) treatment increased the tolerance of cvs. Nicola and Spunta and reduced

the disease intensity by 55.4% and 47.1%, respectively. Such finding is consistent with that obtained by Song *et al*. (2011) on tomato plants treated with ABA (7.58 mM) and showed a significant reduction in infection development by *A. solani*. In plant-pathogen interactions, ABA is a part of a complex transduction network of synergistic and antagonistic interactions (Mauch-Mani and Mauch, 2005 and Derksen *et al*., 2013). The generation of reactive oxygen species (Guan *et al*., 2000 and Laloi *et al*., 2004), Calcium signaling (Blume *et al*., 2000), production and accumulation of phytoalexins (Henfling *et al*., 1980 and Ward *et al*., 1989), synthesis of PR proteins and defence-related enzymes and triggering gene expression (Song *et al*., 2011 and Danquah *et al*., 2014) were important keys for ABA signalling and activation of disease resistance responses.

Differential display analysis has been carried out to detect genes and changes in gene expression of two potato cvs. treated with ABA to control *Alternaria solani*, the causal agent of potato early blight, using different primers. The study revealed the appearance of differentiated bands in the electrophoretic pattern of PCR in the ABA treated plants compared to the untreated ones. Three of these bands were excised from gels and sequenced. The sequences of these bands showed significant homology to known genes in the GenBank database and identified as superoxide dismutase (SOD), WRKY transcription factor (StWRKY) and Endoglucanase from potato genome. The SODs are all enzymes that play a primary role in the protection against oxidative stress in plants. The SODs dismutate superoxide radicals (O_2^-) to O_2 and H_2O_2 thus, perform a primary line of defence against oxidative damage (Scandalios, 1993 and Bowler *et al*., 1994). The exogenous application of ABA appeared to cause a rapid induction of SOD. ABA-pre-treated plants and challenged with *A. solani* showed a more significant induction of CuZnSOD gene expression than *A. solani* untreated infected plants (Song *et al*., 2011).

The WRKY proteins are superfamily of transcription factors (TFs) that involved in the plant abiotic and biotic stress responses including pathogen defence. Liu *et al*. (2014) demonstrated that tomato WRKY transcriptional factor is required for disease resistance against *Botrytis cinerea* and tolerance to oxidative stress. Meanwhile, endoglucanase was found to be associated with ABA effect to induce potato resistance against *A. solani*, the endo-*β*-1, 3-glucanases play an important role in plant defence, directly, by degradation of β-1, 3/1,6-glucans in the pathogen cell wall, or indirectly, by releasing oligosaccharides which act as elicitors that induce additional plant defences (Leubner-Metzger and Meins, 1999 and Rose *et al*., 2002).

In our study, expression of SOD (CuZnSOD) and StWRKY genes in potato leaves of cvs. Nicola and Spunta were increased in ABA treated plants compared to the untreated ones indicating that ABA may be an important signal in the activation of defences against *A. solani*. Data also, indicated that the increase in expression of CuZnSOD and StWRKY genes reached the maximum level in ABA treated plants after two days of treatment. Meanwhile, the level of expression of both genes was always higher in potato leaves of the most tolerant cv. Nicola compared to the susceptible Spunta cultivar. These findings are in harmony with Wang *et al*. (2005) and Siddappa *et al*. (2014) on potato- *P. infestans* and Song *et al*. (2011) on tomato- *A. solani* pathosystems.

In conclusion, the exogenous application of ABA can be used in the integrated disease management of early blight disease of potato to reduce frequency of fungicides application and to save the environment as ABA proved efficacy to enhance resistance of potato plants against *A. solani*. Meanwhile, the incorporation of these genes into the local potato cultivars in the breeding programs could help to induce and/or enhance resistance of potato plant against early blight disease.

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التعبير التفريقي لجينات المقاومة المستحثة باستخدام حامض الأبسيسك ضد مرض اللفحة المبكرة في البطاطس.

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حامض الأبسيسك هرمون نباتي يتدخل في العديد من وظائف النبات الفسيولوجية التي من ضمنها رد الفعل تجاه الأجهادات البيئية والمرضية. في هذه الدراسه تم اختبار حامض الأبسيسك لمعرفة مدي قدرته على زيادة مقاومة صنفين من البطاطس هما نيكولا و أسبونتا لمرض اللفحة المبكرة المتسبب عن الفطر *ألترناريا سولاني* وذلك تحت ظروف الصوبة الزجاجية. وقد أظهرت المعاملة بحمض الأبسيسك بتركيز ١٠ ميكرومول بانخفاض شدة الإصابة في كلا من الصنفين المختبرين وذلك مقارنة بالنباتات المعداة من الصنفين وغير المعاملة بحمض الأبسيسك، وقد أظهر تحليل الجين التفريقي للنباتات المعداة والمعاملة ثلاثة باندات واضحة وقد أظهرت هذه الباندات مطابقة واضحة مع جينات سابقة التعريف في قاعدة بيانات الجين بنك وتم تعريف هذه الباندات كالتالي سوبر أوكسيد دسميوتيز، ويركي ترانسكربشن فاكتور، أندوجلوكانييز وذلك باستخدام قاعدة البيانات الخاصة بجينوم البطاطس. تم استخدام تقنية الريل تايم بي سي أر لقياس التغيرات في التعبير الجيني في كلا من سوبرأوكسيد دسميوتيز والويركي تايب جينز وذلك بعد الإصابة بفطر *ألترناريا سولاني* لصنفين البطاطس نيكولا وأسبونتا وتم قياس تلك التغيرات علي فترات زمنية مختلفة. كان التعبير الجيني لكلا من الجينين في أوراق البطاطس المعداة/ الغير معاملة و المعداة/ المعاملة بحمض الأبسيسك أعلى من النباتات غير المعداة وغير المعاملة وذلك في كلا من الصنفين. وقد كان التعبير الجيني لكلا من الجينين أكثر تعبيرا في النباتات المعداة/المعاملة مقارنة بالنباتات المعداة/غير المعاملة وقد وصلت قمة التعبير الجيني للجينين بعد يومين من المعاملة وقد كان التعبير الجيني في أوراق نباتات البطاطس من صنف نيكولا أعلى مقارنة بصنف سبونتا.