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Full Length Article

Gene Expression of Pathogenesis-Related Proteins and Isozymes in Potato Varieties Resistant and Susceptible to Late Blight Disease

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Abstract

Late blight (LB) disease in potatoes caused by the oomycete *Phytophthora infestans* is considered to be a major disease of Egypt and worldwide. This study aimed to compare the defense mechanisms in resistant and susceptible potato varieties against *P. infestans*. We studied the dynamics of some defense systems such as pathogenesis-related proteins and isozyme activities such as peroxidase (POX) and polyphenol oxidase (PPO) after inoculation with *P. infestans*. In this work, tubers of 11 potato varieties were planted and sprayed with inoculum of *P. infestans* zoospore suspension. The susceptibility of potato varieties to *P. infestans* infection ranged from highly resistant (jelly), moderately resistant (Cara, Diamond, Lady Rosetta, Metro, Mondial and Spunta), to moderately susceptible (Annabelle, Bellini, Deta and Hermes). SDS-PAGE of proteins analysis showed an accumulation of pathogenesis-related proteins (PRP) in highly resistant and resistant potato varieties to *P. infestans* of 12, 22, 30 and 84 kDa. On the contrary, moderately susceptible potato varieties (Annabelle and Bellini) showed a low accumulation of PRPs of 33 and 54 kDa. These bands could be used in potato breeding programs for late blight disease resistance as a marker-assisted selection (MAS). The electrophoretic profiles of the antioxidant enzymes POX and PPO were studied to evaluate the gene expression changes of potato varieties due to the stress-induced by *P. infestans* infection. The highest levels of expression of POX and PPO were recorded in moderately resistant varieties Mondial and Diamond, compared with the susceptible ones, which induced a defense response in plants against LB disease. The increased activity of POX and PPO isozymes in inoculated potato leaves, compared to control might be responsible for the defense response against the fungal infection and the variability in enzyme activity may be due to the different genetic backgrounds of the varieties and their responses to *P. infestans* infection.

Key words: Defense enzymes; *Phytophthora infestans*; SDS-PAGE; Severity of disease; *Solanum tuberosum*

Introduction

The potato (*Solanum tuberosum* L.) is the fourth-most important food crop of Egypt and worldwide, can be influenced by biotic and abiotic factors, involving diseases and environmental stresses (Juskiewicz *et al.* 2005; Rauscher *et al.* 2006; Haverkort *et al.* 2009). The potato crop is susceptible to several microorganisms and the most destructive disease obstructing potato production is the late blight disease caused by the oomycete *Phytophthora infestans*, which destroys the crop in a short time (Hijmans *et al.* 2000). It affects the potato crop at any stage of growth and up to 100% of potato yield production may be lost (Jacobsen and Schouten 2007). It causes losses in potato production in billions of US dollars annually. Furthermore, late blight disease management is very difficult due to the genetic variability of *P. infestans* populations (Fry 2008; Haverkort *et al.* 2008). Late blight control by fungicides became inefficient due to environmental risks and toxic effects on farmers.

The majority of plant pathogens invade the host tissues to absorb the nutrients. Plants have defense systems against pathogens. Some of these defense systems are constitutive, providing constant chemical and physical barriers to prevent infection with the pathogen, while others are stimulated after pathogen invasion. Locally induced host responses involve callose deposition, hypersensitive response (HR), production of pathogenesis-related proteins (PRPs), and generation of reactive oxygen species (ROS). These ROS not only have direct antimicrobial activity (Bolwell and Wojtaszek 1997) but also work as signaling molecules leading to up-or down-regulation of several genes included in defense responses of the host, like the stimulation of defense-related genes and initiation of programmed cell death (PCD) at the infection site (Neill e*t al.* 2002; Apel and Hirt 2004). Production of ROS before HR is reported to be

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induced by *P. infestans* in potatoes and plays a vital role in late blight disease resistance (Yoshioka *et al.* 2003, 2009).

There are two kinds of plant host proteins included in the interaction host-pathogen: resistance (R) proteins and pathogenesis-related proteins (PRPs). The former is encoded by resistance (*R*) loci. They are identified by a nucleotide-binding site (NBS), they are a big family of proteins and are involved in the detection of different microorganisms such as fungi, oomycetes, bacteria, viruses, insects, and nematodes (McHale *et al*. 2006). In *S. tuberosum* L., R proteins are identified by two regions: NBS and LRR (Leucine-rich repeat). The LRR domain function is the recognition specificity of R proteins, direct interaction with the microorganism proteins has rarely been shown (McHale *et al*. 2006). Jones and Dangl (2006) mentioned that the host PRP proteins might be activated indirectly by effectors that are encoded by the pathogen and not by direct recognition. These PRPs have been divided into 17 families (from PR-1 to -17) depending on their properties and functions. For example, the thaumatin-like PR5 protein family, which has been linked to activity against oomycete fungi, peroxidases (PR-9), which are included in lignification of the cell wall, and glucanases, which break the cell wall and release glucosidal elicitors. PR-1 is excreted in the apoplast and is a generally applied marker for activation of the defense response in the plant host. To avert these host defenses, a microorganism also inhibits protease enzyme that plays a fundamental role in the defense response in the plant.

Polyphenol oxidase (PPO) isozymes are broadly distributed into the host and represented in the plant defense system (Thipyapong *et al*. 2004). The induction of PPO isozymes in tomato susceptible to *Pseudomonas syringae* pv. *Tomato* proposes that these isoforms play the main role in disease resistance (Thipyapong and Steffens 1997). The PPO-overexpressing tomato plants lead to a significant increase in resistance to the pathogen (Li and Steffens 2002). PPO enzymes are responsible for disease resistance by hydroxylizing monophenols to *ortho-diphenols* and oxidizing these components to quinones, which are toxic to the pathogens (Gandia-Herrero *et al*. 2005). Peroxidase (POX) isoenzymes are also linked to defense response in the host, and are known to be involved in the plant cell wall reinforcement (Ascensao and Dubery 2003). In addition, PPO and POX are multifunctional isoenzymes that can prevent chemical and biological attacks by strengthening physical barriers or by counterattacking a microorganism with a high generation of free radicals (Passardi *et al.* 2005).

The aim of this study was to compare the defense mechanisms in resistant and susceptible potato varieties against *P. infestans*. To do that, we studied the dynamics of some defense systems such as pathogenesis-related proteins and isozyme activities such as peroxidase and polyphenol oxidase after inoculation with *P. infestans.*

Materials and Methods

Plant material

In this study, 11 potato cultivars were used, obtained from the Potato Brown Rot Project (PBRP), Ministry of Agriculture, Giza, Egypt. The cultivars were cultivated under greenhouse conditions in pots (25 cm in diameter) filled with a mixture of sterilized sand and soil (1: 1 v/v). The experiment was carried out in a randomized complete block design with three replications, five potato tubers in five pots per each replication (a single potato tuber in each pot). The pots were watered and fertilized as usual. Five tubers of each cultivar were used as a control. All *P. infestans* inoculation experiments were conducted at the Agricultural Research Center, Plant Pathology Research Institute, Giza, Egypt.

Source of *P. infestans* isolate

The *P. infestans* isolate was obtained from the Plant Pathology Department, Faculty of Agriculture, University of Ain Shams.

Inoculation of potato varieties with *P. infestans*

After 30 days of planting, whole plants were sprayed with inoculum with an encysted zoospore suspension from *P. infestans* at a conc. of 5×10^4 sporangia mL^{-1}, until the leaf surfaces were fully saturated with the zoospore suspension (Chen *et al.* 2003). Inoculated potato plants were observed for the development of late blight symptoms on a weekly basis post-inoculation. These varieties were evaluated in the winter season 2020–2021 in the greenhouse.

Evaluation assays of potato late blight resistance

The foliar disease was assessed as a percentage of total foliage twice each week (Runno-Paurson *et al.* 2019). Late blight infection was evaluated according to the 0–100% scale (EPPO Bulletin 1989). Two-year disease severity data were scored weekly during the growing season of 2020–2021. To measure the percentage of disease severity, the following formula was used.

$$\text{Severity of disease (\%)} = \frac{\text{Number of infected leaves/plant}}{\text{Total number of leaves/plant}} \times 100.$$

Data recording

The data on disease severity were recorded on weekly basis after disease prevailing using 0–9 Henfling scale till the end of growing season (Henfling 1979). Where 0

indicated that there was no disease (immune) and 9 indicated that all the leaves and stems were drying and dead due to disease (highly susceptible). The host status was assessed by HS: Highly susceptible (8–9 grade on rating scale), S: Susceptible (7 grade on rating scale), MS: Moderately susceptible, (5–6 grade on rating scale), MR: Moderately resistant (3–4 grade on rating scale), R: Resistant (2 grade on rating scale) and HR: Highly resistant (1 grade on rating scale).

Electrophoretic analysis of protein by SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done according to (Laemmli 1970) as modified by (Studier 1973) by using 15% SDS gel for total protein profiling. After, the electrophoresis gel was stained with Coomassie Brilliant Blue dye and then was de-stained to visual the protein bands.

Polyphenol oxidase (PPO) and peroxidase (POX) isoforms

For the assay of antioxidant enzymes, POX and PPO were extracted depending on the method described in Stagemann *et al.* (1985). PPO and POX isozymes were separated by Native-polyacrylamide gel electrophoresis (Native-PAGE). The activities of POX and PPO were determined according to Brown (1978); Baaziz *et al.* (1994). Relative mobility (*Rf*) values were calculated for each band based on the migration of the band relative to the front or tracking dye. The gels were scored as presence (+) or absence (-) of isozyme bands.

Results

Pathogenicity test

All potato varieties were colonized by the *P. infestans* isolate used. The symptoms were recorded on the 7th day after inoculation (Fig. 1). The percentage of infection (% PI) was assessed weekly during the growing seasons. The mean performance of 11 tested potato varieties for late blight disease resistance has been shown in Table 1. Eleven potato varieties varied in susceptibility to *P. infestans* infection from highly resistant (Jelly; PI= 2.2%), moderately resistant [Cara (PI=7%), Lady Rosetta (PI=7.8%), Metro (PI=8.2%), Mondial (PI=9%), Diamond (PI=23%) and Spunta (PI=28.9%)] to moderately susceptible [Deta (PI=37.5%), Hermes (PI=44.2%), Bellini (PI=57%) and Annabelle (PI=61.5%)] (Table 1).

Protein expression of potato varieties inoculated with *P. infestans*

Inoculation of potato leaves of each variety with *P. infestans* isolate led to induction and accumulation of PRPs in inoculated leaves.

SDS-PAGE analysis revealed a drastic change in protein patterns between inoculated plants and un-inoculated controls, which differed in their response to late blight disease infection. These changes depend on a number of the bands on the gel and molecular weights (MWs) (Fig. 2). The genetic variability among potato varieties inoculated with *P. infestans* revealed outstanding differences in the banding profiles represented by their presence and absence of bands as are shown in Fig. 2. Analysis of total soluble proteins showed accumulation of PRPs in potato varieties resistant to *P. infestans e.g*., Lady Rosetta, Spunta, Cara, Mondial, Jelly, Diamond, and Metro, compared with healthy control. The molecular weights of these proteins were 12, 22, 30, 33, 54, 60 and 84 kDa. In addition, moderately susceptible potato varieties (Annabelle, Bellini, Deta, and Hermes) recorded a low accumulation of PRPs with MWs of 33 and 54 kDa.

Some of the moderately resistant varieties, such as Lady Rosetta, Spunta, Mondial, and Metro, induced an increase in protein content depending on a number of bands when compared to the control. In contrast, it was observed that some of the moderately resistant potato varieties *e.g*., Diamond and moderately susceptible like Annabelle, Deta, and Herms scored a decrease in the content of the proteins, compared with the control.

Genetic changes in POX and PPO isozyme activities of potato varieties under *P. infestans* infection

The objective is to evaluate the gene expression profiling of the oxidative enzymes due to the stress-induced by infecting the potato varieties with *P. infestans*. The electrophoretic profiles of the antioxidant enzymes POX and PPO of 11 potato varieties inoculated with *P. infestans* are presented in Fig. 3.

POX isozymes splayed 11 isoforms with different relative mobility (*Rf*) values varying from (0.35 to 0.91). The POX enzyme activities were increased in resistant and susceptible varieties to late blight disease, except moderately resistant Lady Rosetta and moderately susceptible Herms induced a decrease in POX activities, compared to healthy leaves. The highest POX enzyme activities were induced in moderately resistant Mondial (four loci), followed by the moderately resistant Diamond variety (three isozymes). However, the lowest isoform activities were observed in the highly resistant Jelly variety and moderately resistant Spunta, Cara and Metro varieties and moderately susceptible Bellini, Annabelle, and Deta varieties (two isoforms). On the other hand, it has been observed that Lady Rosetta and Herms have not induced any an increase in POX activities (0 loci) (Fig. 3**)**.

PPO isozymes scored 13 loci with *Rf* values ranging from 0.14 to 0.95. The PPO activities were increased in inoculated leaves compared to healthy leaves. The PPO isozyme activities were increased in moderately resistant

Table 1: Disease rating scale for late blight on the potato

Degree of resistance	Potato varieties	Symptoms	Late blight infection (%)	Scale value*
Highly resistant	Jelly	No symptoms	2.2	1
Moderately	Cara	Dark brown blotches and yellowing.	7.00	3
resistant	Lady Rosetta	Dark brown blotches, yellowing, necrosis on stem and dead of leaves.	7.80	3
	Metro	Dark brown blotches on leave edges.	8.20	3
	Mondial	Dark brown blotches and brown on stem.	9.00	3
	Diamond	Dark brown blotches surrounded by a yellow green ring and yellowing.	23.0	4
	Spunta	Dark brown blotches on leave edges, yellowing and death of leaves.	28.9	4
Moderately	Deta	Dark brown blotches on leaves and brown on stem.	37.5	5
susceptible	Bellini	Dark brown blotches surrounded by a yellow green ring.	57.0	5
	Hermes	Dark brown blotches surrounded by yellowish green ring	44.2	5
	Annabelle	Dark brown blotches surrounded by a yellow green ring and yellowing.	61.5	5

*The disease scoring scale (0–9) was 0 = No late blight, 1 = a few lesions; 2 = < 5; 3 = 5–15; 4 = 16–35; 5 = 36–65; 6 = 66–85; 7 = 86–95; 8 = 91–100; 9 = 100

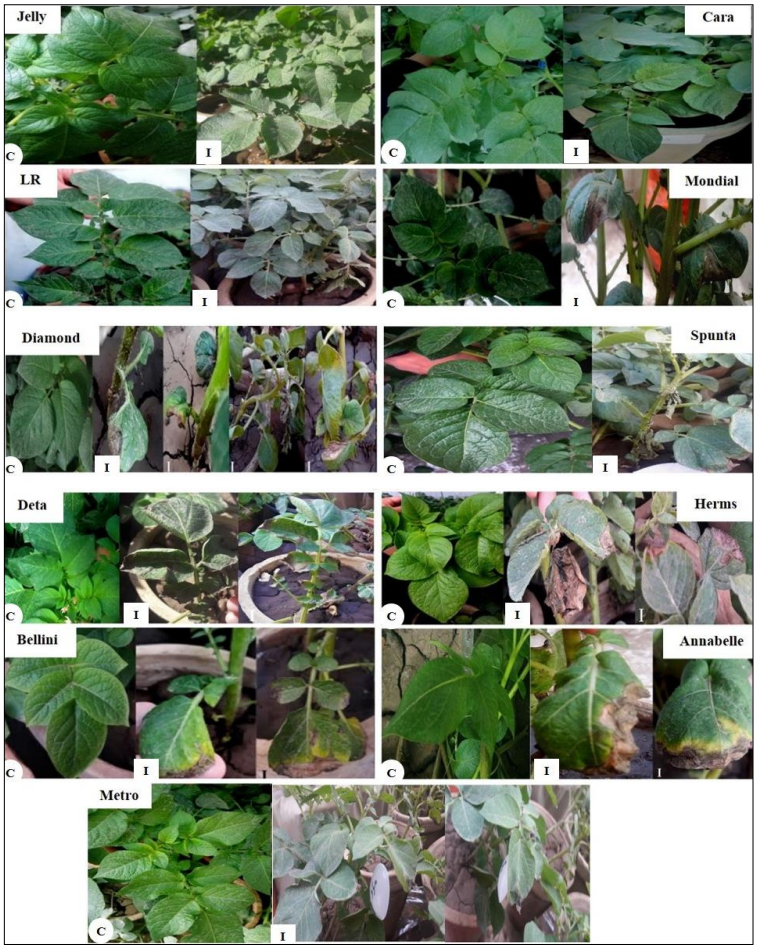


Fig.1: Phenotypes of differential resistance response of 11 potato varieties at ten days post inoculation with *P. infestans* on different potato varieties. C = control, I = potato leaves inoculated with *P. infestans* isolate, LR = Lady Rosetta variety

Lady Rosetta, Spunta and Diamond and moderately susceptible Bellini and Deta (two isoenzymes), followed by moderately resistant Mondial and highly resistant Jelly (one locus). On the other hand, it has been observed a decrease in PPO isozyme activities in moderately resistant Cara and Metro and moderately susceptible Annabelle and Herms. On the contrary, it has been shown that moderately resistant varieties Cara and Metro and moderately susceptible Annabelle and Herms have not recorded any changes in PPO activities (0 Loci) (Fig. 3).

From the previous results, it can be concluded that the highest POX and PPO activities were recorded in moderately resistant Mondial and Diamond (five loci), followed by moderately resistant Spunta and moderately susceptible Bellini and Deta (four isoforms), while the lowest POX and PPO activities were found in moderately resistant Lady

Table 2: Peroxidase (POX) and polyphenol oxidase (PPO) activities induced in 11 potato varieties inoculated by *P. infestans*

Rf	Lady Rosetta (MR)	Bellini (MS)	Spunta (MR)	Cara (MR)	Annabelle (MS)	Mondial (MR)	Jelly (HR)	Diamond (MR)	Metro (MR)	Deta (MS)	Herms (MS)
Peroxidase (POX)											
0.35								+			
0.40		+		+			+		+		
0.63						+				+	
0.82			+		+	+					
0.87			+		+	+	+	+			
0.91		+		+		+		+	+	+	
Total number of POX bands= 6	0	2	2	2	2	4	2	3	2	2	0
Polyphenol oxidase (PPO)											
0.14										+	
0.19								+		+	
0.31	+	+									
0.45			+								
0.65								+			
0.80	+										
0.89		+	+				+				
0.95						+					
Total number of PPO bands= 8	2	2	2	0	0	1	1	2	0	2	0
Total number of POX and PPO bands= 14	2	4	4	2	2	5	3	5	2	4	0

Rf = Relative mobility, + = Presence of bands, MR = Moderately resistant, MS = Moderately susceptible, HR = Highly resistant

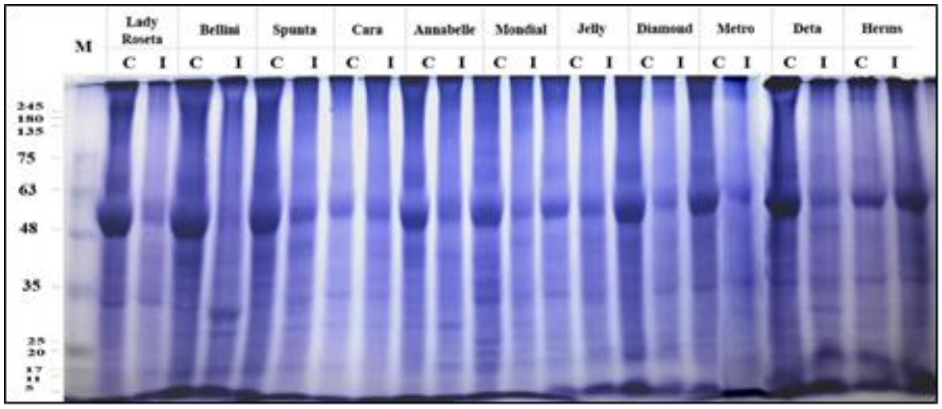


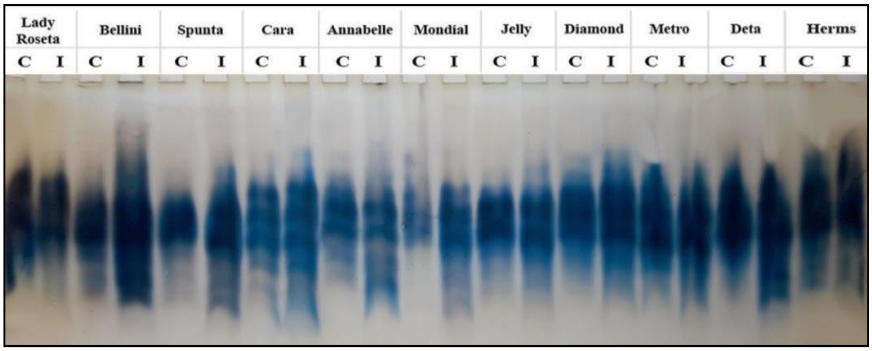
Fig. 2: 12% PAGE showing protein profiling ten days after inoculation with *P. infestans* isolate on different potato varieties. Accumulation of pathogenesis-related proteins (PRPs) in potato leaves inoculated with *P. infestans* (I) compared with the un-inoculated control (C)

Rosetta, Cara and Metro and moderately susceptible Annabelle (two loci). In contrast, Herms has not scored any increment in POX and PPO activities (0 loci) (Table 2).

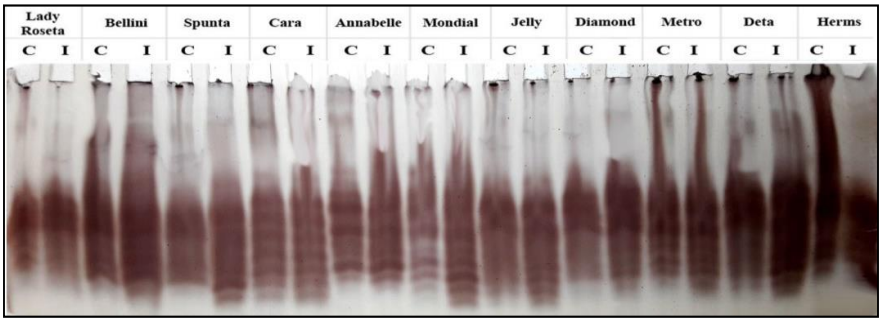
Discussion

The potato crop is susceptible to several pathogens, *e.g*., late blight disease caused by the oomycete *P. infestans*, which destroys the crop in a short time (Hijmans *et al.* 2000).

In this study, 11 potato varieties displayed different levels of resistance to late blight disease. Jelly variety was highly resistant, while six varieties were moderately resistant *i.e*., Cara, Diamond, Lady Rosetta, Metro, Mondial, and Spunta (Abou-Taleb *et al*. 2010). On the contrary, four varieties were moderately susceptible to late blight *e.g*., Annabelle (Duarte *et al*. 2012), Bellini, Deta, and Hermes (Iqbal *et al*. 2013). Genetic diversity for resistance to infection has formerly been reported in different crops and wild species (Roux *et al*. 2010; Newton 2016). In *S. tuberosum*, 20 *R*-genes (*R*1–*R* 20) have been indicated (Jiang *et al.* 2018) and four loci have been located on the genetic map of *S. tuberosum* (El-Kharbotly *et al*. 1994; Meksen *et al*. 1995). However, their immune response can be easily overcome by the occurrence of novel virulent factors. Stewart *et al*. (1983) discovered that evaluating potato species to late blight disease in the field and the greenhouse was similar, although the glasshouse estimations are suggested only as a preliminary selecting. Several ways of selecting for late blight disease resistance have been reported depending on detached leaves whole-plant or field experiments. Trials suitable for selecting would be sufficiently controlled to record reproducible results and be able to precisely identify both resistant and susceptible potato varieties, as was the whole plant test performed in the



(a)



(**b**)

Fig. 3: Isoenzyme patterns of peroxidase (**a**) and polyphenol oxidase (**b**) identified by native –PAGE ten days after inoculation with *P. infestans* isolate on different potato varieties. C = Control, I = Potato leaves inoculated with *P. infestans* isolate

glasshouse under controlled conditions. It is also a precondition that the resistance characterized in the greenhouse experiment is also reflected in field performance (Caligari *et al*. 1985; Zhang *et al*. 2020). Forbes *et al*. (2005) reported that resistance to foliar late blight in *S. tuberosum* varieties may be either unstable or stable. Unstable resistance is due to three factors: the pathogen population or the environment (as photoperiod) and a combination of these. Haynes *et al*. (2002) showed that many potato clones resistant or moderately resistant to late blight disease were stable. Duarte *et al*. (2012) suggested that the potato clones with the highest levels of resistance to late blight disease displayed later maturity.

In the current investigation, it has been shown that PRPs with MWs ranging from 12–84 kDa linked to defense response to late blight disease. Accumulation of PRPs ascribed to resistance or susceptibility of *S. tuberosum* L. varieties. Besides, the accumulation of these proteins was higher in resistant varieties, compared with susceptible ones. We also recorded four unique bands with MWs 12, 22, 30 and 84 kDa, which were induced only in resistant potato varieties but absent in susceptible ones. These protein bands could represent as the marker-assisted selection in potato breeding programs for late blight disease resistance. These results were similar to those obtained by Van-Loon and Van-Strien (1999); Graham *et al*. (2003) found that pathogenesis-related proteins (PRPs) accumulate in the plant cells under biotic and abiotic stress conditions. The proteomic analysis showed a significant increase in the amount of proteins in potato varieties susceptible and resistant to late blight, compared with the healthy ones. Hong and Hwang (2002); Hong *et al*. (2004) observed a significant increase in the content of proteins in the resistant potato varieties, compared with the susceptible ones. These results were in agreement with Tonon *et al*. (2002) who found that the accumulation of PRPs was higher in incompatible interaction than the compatible ones. PRPs involved; PRP-1 type protein (14 kDa) Hong and Hwang (2002), two osmotin isoform (22 and 24 kDa) (Takemoto *et al*. 1997), four chitinase (26, 27, 30 and 32 kDa) (Lawrence *et al.* 1996), two β-1,3- glucanase (33 and 35 kDa) (Tonon *et al*. 2002), a 45 kDa protein (Fischer *et al*. 1989) and the alkaline end proteinase (69 kDa) (Lawrence *et al*. 1996). El-Komy *et al.* (2010) recorded nine proteins with MWs of 12, 14, 20, 22, 24, 26, 30, 34 and 45 kDa in the resistant potato varieties Hanna and Cara. Also, it has observed the accumulation of PRPs with MWs of 56 and 60 kDa after *P. infestans* infection. These proteins are linked to defense response against late blight disease in potatoes. On the other hand, late blight susceptible potato varieties showed a weak accumulation of proteins post-inoculation with MWs of 12, 14, 20, 22, 24, 26, 30, 34 and 45 kDa.

In our study, the increased activity of POX and PPO isozymes in inoculated leaves, compared to un-inoculated control, might be responsible for defense response in plants against the pathogen. The highest level of expression of POX and PPO was shown in moderately resistant Mondial and Diamond varieties, compared to the susceptible ones. The results also reported that varieties with higher enzyme activities gave resistance against late blight disease. However, the increase in these enzyme activities in moderately susceptible potato varieties was not enough to avert the development of the disease in the leaves of the infected plants. These results are in harmony with those obtained by Kumar *et al*. (2010); Arnnok *et al*. (2010) showed that an increase in total phenols, o-dihydroxy phenols, and defense-related enzymes such as phenylalanine ammonia-lyase (PAL), POX, and PPO in plants inoculated with *P. infestans* over control. Shahbazi *et al*. (2010) found that potato varieties differed in disease severity due to variation in the genotypes. Although POX activities appear to contribute to resistance against *Alternaria solani* in *S. tuberosum* L., other factors such as PRP gene expression are involved. These results were supported by the work of Li and Steffens (2002) who found that an over-expression of PPO is over a 10-fold increase in PPO isozymes, which led to improved *P. syringae* disease resistance in transgenic tomatoes. Kumar *et al*. (1991) also found that potato varieties resistant to *Pectobacterium carotovorum* scored the highest PPO activities and contained large amounts of phenolic components. PPO isozymes are an important tool in the early stage of a host plant defense response, where membrane degradation causes the release of phenolic components like chlorogenic acid. Phenol compounds and their oxidation products such as quinones were shown to inhibit *P. carotovorum* (Sequeira 1983; Lyon and McGill 1989; Weber *et al*. 1996). Lojkowska and Holubowska (1992) found that potato varieties that had a high content of phenolic components scored a low level of tolerance to soft rot disease. Lojkowska and Holubowska (1992) observed that there was no relation between resistance to soft rot disease and isozyme activities. The difference in soft rot susceptibility between potato varieties could be the result of differences in the conditions under which the trials were performed. There are several agents, such as size and maturity of the tubers and physiological state, which can affect the response of the potato tubers to soft rot disease (Marquez-Villavicencio *et al*. 2011). Other authors also found that resistance to soft rot pathogen could be linked to the weight of the tuber, small potato tubers being more resistant to *P. carotovorum*. They also showed that differences in responses among potato tubers of the same variety harvested from different fields (Ngadze *et al*. 2012).

During the evaluated period, POX and PPO activities were similar in some potato varieties resistant and susceptible to late blight, such as moderately resistant Lady Rosetta, Cara, and Metro and moderately susceptible Annabelle (two isoforms) and moderately susceptible Bellini and Deta and moderately resistant Spunta (four isozyme markers). These results support the findings of Balbi-Peña *et al*. (2014) catalase activity was similar in early blight resistant and susceptible tomato plants and has not recorded any significant increases. This proposes that catalase was not working as H_2O_2-scavenging enzyme due to the low accumulation of reactive oxygen species (ROS).

Conclusion

Screening of potato varieties resistant to late blight disease will reduce the cost of the fungicide and reduce the environmental pollution of fungicide residues on the potato tubers. It is also a precondition that evaluation of potato varieties to late blight resistance under glasshouse conditions will reflect the field performance. In the present study, 11 potato varieties varied in susceptibility to *P. infestans* infection from highly resistant (jelly), moderately resistant (Cara, Diamond, Lady Rosetta, Metro, Mondial, and Spunta), to moderately susceptible (Annabelle, Bellini, Deta, and Hermes). We recognized four unique bands with MWs of 12, 22, 30, and 84 kDa, which induced only in resistant varieties. These protein bands could act as marker-assisted selection in potato breeding programs for late blight disease resistance. On the other hand, the highest level of expression of POX and PPO isozymes was shown in moderately resistant Mondial and Diamond varieties, compared to the susceptible ones, which induce defense response in plants against *P. infestans*.

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Author Contributions

HAM designed the experiments, SDS and isozyme activities, HZA carried out pathogenicity test and OZE performed data analysis, contributed in writing the manuscript.

Conflicts of Interest

All authors declare no conflicts of interest.

Data Availability

Data presented in this work will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable.

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