Novel Cucumis Enzymes Associated with Host-Specific Disease Resistance to Phytophthora melonis

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Abstract

The hemibiotrophic oomycetes are significant threats to a wide range of Cucurbitaceae species, causing substantial losses of plant productions. Particularly, *Phytophthora melonis* evokes severe symptoms, thus dramatically limiting yield in cucumber. However, information about cucumber–*P. melonis* interaction is still limited. This study explored changes in the activities of phenylalanine ammonia-lyase (PAL), peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), and polyphenol oxidase (PPO) in cucumber roots of two resistant genotypes (Soheil and Ramezz), one moderately resistant genotype (Baby) and three highly susceptible genotypes (Extrem, Mini 6-23 and Yalda), over the time courses of 7, 14 and 21 days after inoculation (DAI). The results indicated that the activities of defence-related enzymes differed between the resistant and highly susceptible genotypes. Although, the defense-related enzymatic activities were elevated sharply in the resistant and moderately resistant genotypes after inoculation, but no significant correlations were present between the activity trends of PPO, SOD and CAT and resistance characteristics. Moreover, no significant changes in enzyme activities were found in the control plants, non-inoculated plants of the six genotypes during the testing period. Altogether, the resistance of cucumber to *P. melonis* is related to POX and PAL activities, but does not show relationship with PPO, SOD and CAT activities. Studying the physiological metabolic pathways of POX and PAL appears to be an important direction in research to elucidate resistance to *P. melonis* in cucumber genotypes.

1 — INTRODUCTION

Phytophthora melonis , a hemibiotrophic organism belongs to oomycote class and one of the causal agents of damping-off disease, is considered a potentially destructive disease of cucumber (*Cucumis sativus* L.; 2n = 2x = 14) across Iran and other parts of the world, that causes economic losses (Wu et al., 2014; Nazavari et al., 2016). Therefore, it is vital to elucidate the mechanisms of plants in response to damping-off. Typical symptoms of the disease are root and root collar rot, stem lesions, foliar blight, fruit rot and plant death (Hatami et al., 2013; Bagheri et al., 2020). Although current control measures for*P. melonis* are based on the use of fungicides (Lamichhane et al., 2017), identification of disease-resistant genotypes may be an effective strategy and environmentally friendly in modern crop production (Moghaddam et al., 2019; Hashemi et al., 2019). To date, analyses of the interaction between *P. melonis* and *C. sativus* have been limited on screening resistant genotypes to damping-off (Nazavari et al., 2016; Hashemi et al., 2019) and no cucumber genotype immune to*P. melonis* has been reported. In the never ending struggle against pathogens, plants gradually developed a series of complex defense mechanisms involving several factors like defense-related

enzymes and inhibitors which lead to prevent infection of pathogens (Andersen et al., 2018). However, plant response during the *P. melonis* - *C. sativus* interactions or, more specifically, the underlying factors that increase susceptibility and/or resistance of the cucumber plant is not fully understood. So, identification and application of candidate resistance genes and defense enzymes associated with *P. melonis* responses can be an efficient method to build up the host resistance in cucumber genotypes. In 2019, three defense-related genes of *CsWRKY20* , *CsLecRK6.1* and*LOX1* genes were reported to be involved in the resistance to the*P. melonis* infection in cucumber (Hashemi et al., 2019). Complex network of defense-related enzymes, such as peroxidase (POX; EC1.11.1.7), superoxide dismutase (SOD; EC1.15.1.1), catalase (CAT; EC1.11.1.6) and phenylalanine ammonia lyase (PAL; EC4.3.1.5) are promoted the scavenging of ROS and related to resistance inducement in plants (Prasannath and De Costa, 2015; Xie et al., 2017; Khatediya et al., 2018). PAL has a crucial role in flavonoid productions and lignin biosynthesis, which play a key role in the phenylpropanoid biosynthetic pathway (Yusuf et al., 2018). PAL is one of the most extensively studied enzymes with respect to plant responses to environmental stresses, including pathogen infection (Huang et al. 2010; Kim & Huang 2014). Polyphenol oxidase (PPO) and peroxidase (POX) are important oxidative enzymes found in most plant species that catalyze the formation of lignin and other oxidative phenols, thereby contributing to the formation of defense barriers by structural reinforcement, to protect against pathogens (Li & Steffens, 2002; Jiang et al., 2019). In cucumber increasing in the activities of peroxidase were reported following the inoculation with *Fusarium oxysporum* (Zhao et al., 2012). Amaral et al (2019) reported that high activities of antioxidant enzymes played a major role in both basal and induced resistance of cabbage to black rot. Moreover, regarding the oxidative burst, CAT and SOD are useful biochemical indicators of disease resistance (Su et al., 2019). The activities of different defense-related enzymes vary in different plants after pathogen attack and are highly complex (Siddique et al., 2014; Su et al., 2016). To the best of our knowledge, no comprehensive enzymic analyses have yet been accomplished on cucumber plants to compare defence-related enzymes in resistant and susceptible cucumber genotypes upon inoculation with *P. melonis* . Therefore, the present study estimated the activities of PAL, POX, CAT, SOD and PPO quantitatively and investigated their roles in response to *P. melonis* inoculation in the cucumber genotypes. A better insight in cucumber defense responses can help to establish biochemical characteristics for the selection of resistant cucumber sources and provide a theoretical basis for disease control and breeding of cucumber plants with higher resistance toward damping-off.

2 — MATERIALS AND METHODS

2.1 — Plant material

Two cucumber genotypes to damping-off (Soheil and Ramezz), one moderately resistant genotype (Baby) and three susceptible genotypes (Extrem, Mini 6-23 and Yalda) were selected among a collection of thirty-eight commercial genotypes of domestic and exotic hybrids, and inbred lines from different seed companies, we recently investigated and were screened in another study to identify resistant and susceptible genotypes (Hashemi et al., 2019, Nasr Esfahani 2019). The six genotypes are listed from least to most susceptible according to the disease severity index in greenhouse investigations in Table 1. A number of 100 seeds per genotypes were grown in seedling nursery trays filled with forced-air oven sterilized mix of sand-peat moss in equal parts at 200°F for 30 minutes. Cucumber seedlings were grown in a greenhouse condition (26±2°C), provided with 16 h photoperiod and 65% relative humidity. The experiment was arranged in a completely randomized factorial design with three replications (Nasr Esfahani 2012, 2014).

2.2 — Pathogen culture and plant inoculation

Aggressive isolate of *P. melonis* , obtained from naturally infected cucumber plants exhibiting post-emergence damping-off and root rot symptoms and identified as *P. melonis* (MH924841). Inoculum was obtained by growing of *P. melonis* isolate in 500 mL flasks containing water soaked wheat seed, which had been autoclaved at 120degC for 30 min on 2 consecutive days. The flasks were inoculated with 5 cm disks cut, from 10-day-old culture of *P. melonis* isolate, and placed in the dark for 2 weeks at 25+-2degC. 45-day-old seedlings were inoculated by 10-g of wheat seed (106 sporangia/ml) and incubated for two days under saturated moist condition in the greenhouse (Nasr Esfahani et al., 2018, 2020, hashemi et al., 2019). In seedling stage,

inoculated and control root samples were harvested in three biological replicates over the time courses of 7, 14 and 21 days after inoculation (DAI) and then immediately transferred to liquid nitrogen and kept in -80degC. All experiments were repeated 3 times.

2.3 — Antioxidant enzyme extraction and activity assays

The pre-weighed infected and non-infected roots were homogenized in 4 mL buffer (50 mM Tris pH = 8.5, 14.4 mM 2-mercaptoethanol) and 1% (w/w) insoluble polyvinylpolypyrrolidone and centrifuged at 6000g for 15 min at 4 degC to determine the SOD, CAT, POX, PPO and PAL (Jung et al., 2011). Total protein content of each enzyme extract was determined using bovine serum albumin as the standard (Bradford, 1976). The CAT activity was determined by measuring the rate of H2O2 conversion to O2 in absorbance at 240 nm and expressed as U mg^{-1} protein (Gratao et al., 2012). The POX activity was determined by the method described by Dazy et al. (2008). The photometric intensity of the reaction was measured using a spectrophotometer (470 nm) in a 40 mM hydrogen peroxide solution and expressed as units of enzyme activity per mg of soluble protein per min (U mg-1 protein). The activity of PPO was determined using the method of Constabel et al. (1998). Supernatant was added to substrate consisted of 5mgmL^{-1}L-3,4-dihydroxyphenylalanine (L-Dopa). The assay solution consisted of 100mM NaPO4 (pH 7), 0.015% (w/v) sodium dodecyl sulfate, and catalase (280 U mL^{-1}). The absorbance was measured at 490 nm and the PPO activity expressed as U mg^{-1}protein. The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Van Rossum et al. (1997) and expressed as units of enzyme activity per mg of soluble protein per min (U mg^{-1}protein). The PAL activity was assayed following the method of Beaudoin-Eagan and Thorpe (1985). The photometric intensity of the reaction was measured using a spectrophotometer (290 nm) with 15 mM phenylalanine as the substrate. PAL specific activity was expressed in U mg^{-1}.

2.4 — Statistical analysis

The data collected from all experiments were analyzed separately for each parameter and subjected to two-way analysis of variance (ANOVA) SAS (ver. 9.4, SAS Institute, Cary, NC), The means were compared for significance using least significant differences (L.S.D.) at P [?] 0.05 and the values presented are mean of three replicates.

3 — RESULTS

3.1 — Disease response

P. melonis -inoculated plants of all tested genotypes revealed a wide range of symptoms depending on their genetic makeup and none of the inoculated plant was symptomless. The genotypes responded as resistant showed minor symptoms, moderately resistant ones indicated moderate, while highly susceptible genotypes revealed severe symptoms as earlier reported by hashemi et al. (2019). The defense response in the resistant genotypes were able to stop pathogen growth and infection, whereas in the susceptible genotypes, necrosis continued to progress along the roots and stem until the plant dies (Fig 1).

3.2 — Peroxidase activity (POX)

Analysis of variance and changes in POX activity in the roots of the six cucumber genotypes with different levels of resistance after inoculation with *P. melonis* was represented in Table 2 and 3 and Fig 2. In Ramezz and Baby, POX activity indicated a significant increase in 14 DAI, and increased to maximum at 21 DAI (Fig 2.B and C). Highest POX activity of 80.8 units was recorded in resistant Ramezz after 21 DAI which was 6 folds higher than that of the untreated control which recorded 13.8 units POX activity after 21 DAI (Fig 2.C). Soheil increased at 7 and 14 DAI had a decrease of SOD activity at 28 DAI and showed no significant difference between control and inoculated at 28 DAI. (Fig 2.A). Pattern study of POX activity in inoculated susceptible samples of Mini 6-23 and Extrem were similar to the uninoculated samples, but, the amount of enzyme activity was significantly higher in inoculated susceptible samples of Yalda at 21 DAI. Interestingly, the POX activity in infected plants of Extrem was lower than controls (Fig 2.D). The increase

in POX activity caused by inoculation varied among genotype and has a strong negative correlation with damping-off susceptibility at 14 DAI (Supplemental Table 1).

3.3 — Superoxide dismutase (SOD)

Analysis of variance and the activities of SOD in roots of susceptible and resistant cucumber genotypes subjected to pathogen infection are presented in Table 2 and 4 and Fig 3. The SOD activity in the control of each genotype showed little change from day 7 to day 21 (Table 4). However, the SOD activity in the inoculated cucumbers changed dramatically. Before inoculation, the SOD concentrations of all genotypes were relatively low. In resistant and moderately resistant genotypes, the SOD activity increased after inoculation by *P. melonis* at 14 DAI, remained high in Soheil and Baby and decreased in Ramezz at 24 DAI (Fig 3. A, B and C). The levels of SOD in inoculated Soheil ranged from 0.42 U mg-1 (7 DAI) to 1.68 U mg^{-1}(21 DAI), in Ramezz, it ranged from 0.21 U mg-1 (7 DAI) to 0.35 U mg^{-1} (21 DAI) and in Baby from 2.19 U mg^{-1} (7 DAI) to 0.79 U mg^{-1} (21 DAI). The genotype with the highest activities was the moderately resistant Baby. The SOD activity in 'Baby' was 4.09 times greater compared to control at 14 DAI. The induction of SOD in all genotypes found little to no significant differences compared to control plants at 7 DAI, except Baby and Yalda (Table 4). The SOD activity of the susceptible Mini 6-23 also showed increases of 1.47 U mg^{-1} on day 21 after inoculation, but the susceptible Yalda began to decline on day 21 after inoculation. The maximum amount of SOD activity between the three susceptible genotypes in inoculated plants was observed in Mini 6-23 at 21 DAI (Fig 3.E). The increase in SOD activity caused by inoculation did not correlate with damping-off susceptibility (Supplemental Table 1, varied between -0.56 and -0.14).

3.4 — Catalase activity (CAT)

The changes in CAT activity of the six cucumber genotypes after inoculation with *P. melonis* are shown in Table 2 and 5 and Fig 4. Before inoculation, the CAT activities in resistant and moderately resistant genotypes (Soheil and Baby), were significantly higher than another resistant genotype (Ramezz) (Table 5). After inoculation, the CAT activity in Soheil increased sharply to 20.3 U mg-1 at 14 DAI and then decreased at 21 DAI (Fig 4. A). A similar pattern was also observed in Ramezz. However, the CAT activity in another moderately resistant genotype (Baby) showed a different reaction, with a non-significant decrease after inoculation at 14 DAI and a significant increase at 21 DAI. The maximum amount of CAT activity in roots of inoculated plants was observed in Ramezz and Soheil (Table 5). No significant difference was found in two susceptible genotypes, Extrem and Mini 6-23 (Table 5 and Fig 4.D and E). In another susceptible genotype (Yalda) CAT activity never exceeded the activity before inoculation at 14 and 21 DAI (Fig 4. F). However, no correlation was observed between damping-off susceptibility and CAT activity changes (Supplemental Table 1).

3.5 — Activity of phenylalanine ammonia-lyase (PAL)

In each of the six cucumber genotypes, the PAL activities of the control plants indicated no significant changes from day 7 to day 21 (Table 6). In the resistant and moderately resistant genotypes, PAL activities showing significant difference from the control at time point after inoculation. After inoculation, PAL activity in the resistant genotype Soheil increased, reaching its first peak on day 7 then, it decreased on day 14 and increased to 1.86 U mg^{-1} on day 21 after inoculation (Fig 5. A). The CAT activity in resistant Ramezz increased sharply to 2.17 U mg^{-1} at 7 DAI and then decreased at 14 and 21 DAI (Table 6). The moderately resistant genotype, Baby, showed behavior similar to that of the Soheil, but it increased sharply and reached its maximum on day 21(5.20 U mg-1). The two highly susceptible genotypes Extrem and Mini 6-23 showed completely different patterns of PAL activity, that in the both genotypes increased on day 7 and 14 and then decreased on day 21 (Fig 5.D and E). Whereas on the other genotype Yalda increased on day 7 and then decreased on day 14 and 21 (Fig 5.F). Therefore, changes in PAL activity showed differences among different genotypes and it was significantly higher in inoculated plants of both moderately resistant and resistant genotypes, as compared to susceptible genotypes. The significant negative correlations between activity of PAL and damping-off susceptibility were only observed at 7 and 14 DAI (Supplemental Table 1, r= -0.94 and r= -0.82, respectively), in the highly susceptible genotypes, the increase of PAL activity was lower than

in the resistant genotypes.

3.6 — Activity of polyphenol oxidase (PPO)

As shown in Table 7, comprehensible difference in PPO activity was not observed in the control of each genotype from day 7 to day 21. After inoculation with *P. melonis* , the PPO activity in the cucumber plants changed notably. Changes in the PPO activity of the roots of the six cucumber genotypes with different levels of resistance after inoculation with *P. melonis* are shown in Table 7 and Fig 6. The PPO activities in the resistant and moderately resistant genotypes were significantly higher than those in the three highly susceptible genotypes. In Soheil and Baby, the activity of PPO continuously displayed high activity until 21 DAI (Fig 6.A and B). In the resistant genotype Ramezz, the significant increase in the activity of POX occurred at 7 DAI and then began to decrease at 14 and 21 DAI after inoculation (Fig 6. C). The maximum amount of PPO activity was found to be 6-fold higher in roots of inoculated Ramezz at 7 DAI than the respective control plants. The CAT activity of the three highly susceptible genotypes Extrem, Mini 6-23 and Yalda revealed a similar trend after inoculation: a slow increase at 7 DAI followed by a decrease at 14 and 21 DAI (Table 7). In the present study, PPO activity showed no significant differences in inoculated susceptible genotypes, as compared to non-inoculated plants. There was no correlation between damping-off susceptibility and PPO activity in the cucumber genotypes (Supplemental Table 1). Furthermore, the activity of PPO significantly was correlated with the POX (r = 0.23) and PAL (r = 0.53) activities in the six genotypes of cucumber with different susceptibilities to damping-off (Supplemental Table 2).

4 — Discussion

Early and elevated levels of the expression of various defense enzymes are an important feature of plant disease resistance to different pathogens (Andersen et al., 2018). Although defense-related enzymes constitute an important protective system for plants against pathogen invasion but the underlying mechanism of defense reactions and their relationships with damping-off diseases in cucumber remain unclear. Using uninoculated cucumber genotypes and time points before inoculation as controls, this study analyzed the changes in the activities of PPO, PAL, SOD, POX, and CAT in the roots of six cucumber genotypes with different susceptibilities to damping-off to identify indices that allow relevant information on damping-off resistance, which suggests that diverse mechanisms contribute to the specific levels of resistance to*P. melonis* in genotypes. Although oxidative stress caused by*P. melonis* led to increased activities of defence-related enzymes, the overall enzyme activity patterns were distinct by enzyme and genotype.

Generally, POX activity in plant tissues are induced by pathogen infection and a greater increase was recorded in resistant plants compared to the susceptible ones (Mydlarz & Harvell 2006; Bharathi et al., 2019). Similar increase in POX activity, as observed in the resistant genotypes in this study was observed in castor infected with*Fusarium oxysporum* (Bharathi et al. 2019), cucumber infected by*Pythium aphanidermatum* (Sabbaghi et al., 2018), and zucchini and cucumber infected by mosaic virus (Riedle-Bauer, 2000). The increased activity of POX in the resistant genotypes after inoculation at 14 DAI was correlated with damping-off susceptibility in the cucumber genotypes and related to plant disease resistance. Su et al. (2016) found that the POX activity levels could potentially be used as a genetic marker for resistant evaluation during the early phase of infection. Our results support this indication that POX may also play an important role in cucumber resistance to damping-off. POX and POD is widely distributed in higher plants and protects cells against damaging effects of H2O2 by catalyzing its decomposition (Fernandes et al., 2006).

After inoculation with *P. melonis* , the SOD activity increased in the resistant genotypes. Although the SOD activity also increased in the highly susceptible genotypes Mini 6-23 and Yalda, the response time and range of increase were far lower than those in the resistant genotypes. Therefore, the differences in the activity of SOD between the six genotypes may be due to the complex activity patterns of SOD and appeared to be an important physiological basis for resistance to disease. However, the SOD activities did not correlate with damping-off susceptibility. Other researchers have indicated an increase in the activity of SOD after inoculation in cucumber (Nostar et al., 2013; Moradi et al., 2016) and other plants in a way similar to our study (Su et al., 2019).

The CAT enzyme activity displayed different patterns in different genotypes under stress and mainly affects plant disease resistance through two physiological pathways (Jiang et al., 2019). This seems to be an important H202-scavenging enzyme in plants and be localized in the peroxisomes. Catalase can be induced and then be consumed as a result of oxidative stress (Garg & Manchanda, 2009). In this study, there was a difference in the CAT activity between the resistant and susceptible genotypes, which means the alternative of CAT showed intraspecific genotypic variation in response to *P. melonis* inoculation and suggested that altered CAT activity also had an important effect on damping-off resistance. However, the physiological pathway by which CAT affects resistance to *P. melonis* remains unclear and further investigations are needed. This result is consistent with other studies for Lilium hybrids (Su et al., 2019), walnut (Jiang et al., 2019) and*Brassica juncea* (Pandey et al., 2017).

Due to the importance of PAL in the phenylpropanoid pathway and the relationship between PAL and plant disease resistance, it has always been a hot research topic. Several previous studies using different plant species have shown that the PAL activity is increased after fungal infection and played an important role in plant defense against pathogenic fungi (Saunders and O'neill, 2004; Zhang et al., 2017). In this study, significant changes in the PAL activity occurred after inoculation with *P. melonis* in resistant and moderately resistant genotypes. There were also some differences among the genotypes. Numerous studies also revealed that the activation of PAL and subsequent increase in phenolic content in plants is a general response associated with disease resistance. In black rice, PAL contributes to the resistance mechanism against *Xanthomonas oryzae* pv. *oryzae*(Solekha et al., 2019). In tomato, PAL activity was enhanced in roots by a biotic elicitor *Fusarium mycelium* extract (Mandal and Mitra, 2007). In cucumber roots, high levels of PAL were induced after inoculation with *P. aphanidermatum* (Chen et al., 2000). In transgenic soybean, overexpression of GmPAL2.1 increases resistance to*Phytophthora sojae* (Zhang et al., 2017). Therefore, increase in level of PAL in the infected root tissue of cucumber genotype showed that phenyl propanoid pathway accumulate phenolics might have prevented the pathogen invasion, and thus, the activity maintained at higher levels during the infection period.

Our findings show that, although the PPO activity increased in roots of infected plants in comparison to the control in the resistant and moderately resistant genotypes, but the activities varied among the three genotypes. Several studies have indicated induction of PPO in plants in response to infection by different pathogens (Vanitha et al., 2009; Khodadadi et al., 2020). In present study, systemic induction of PPO expression in the resistant and moderately resistant genotypes in response to *P. melonis* might provide an additional line of defense to protect cucumber plants against further attack by pathogens. The PPO appear to play a role in resistance to *P. melonis* , since this compound was present in considerably higher levels in roots of inoculated plants of resistant genotypes which is consistent with observations made for cucumber, walnut and common bean plants in which PPO was demonstrated to be induced by *Fusarium oxysporum,Xanthomonas arboricola* and arbuscular mycorrhizal fungi, respectively (Moghbeli et al., 2017; Jiang et al., 2019; Abdel-Fattah et al., 2011). Additionally, a significant correlation between the antioxidant enzymes indicate the role of antioxidant enzymes system under *P. melonis* infection. The role of antioxidant enzymes in eliciting systemic resistance against *Fusarium oxysporum* ,*Pyricularia oryzae* , *Alternaria* sp. and *Sclerotium*sp. has been reported earlier (Rais et al., 2017). These findings indicated the complex interaction between both oxidative-burst and response to damping- off. Tt seems that the oxidativeburst and H2O2 accumulation also happened in resistant genotypes as an adaptive response to *P. melonis* infection, and resistant genotypes may handle stress better than susceptible genotypes during the infection due to other mechanisms. However, the results of the changes in defensive enzyme activity are far from sufficient to explain damping-off resistance in cucumber. To understand cucumber resistance physiology more clearly, other physiological indicators should be considered.

The results of this study indicated that the disease defense response is more pronounced in the resistance genotypes as demonstrated by an earlier defense response through increasing the content of defense-related enzymes. In addition, the results suggest the PAL and POX have active roles in disease resistance against damping-off; however, there was no direct connection between damping-off resistance and PPO, SOD and CAT activities. Meanwhile, the varied response of defense-related enzymes suggests that the oxidative-burst

response after inoculation by *P. melonis* , and the response may have differed depending on the genotype and inoculation period. These findings can help us to understand the resistance physiology of damping-off and provide indicators for cucumber breeding. However, diverse mechanisms contribute to the specific levels of resistance to *P. melonis* in genotypes and the mechanisms by which defense-related enzymes accumulation contribute to resistance in cucumber remain to be explored in future studies.

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CONFLICTS OF INTEREST

There is no conflict of interest relating to this article

REFERENCES

Abdel-Fattah, G.M., El-Haddad, S.A., Hafez, E.E., & Rashad, Y.M. (2011). Induction of defense responses in common bean plants by*arbuscular mycorrhizal* fungi. *Microbiological Research* , 166, 268–281.

Andersen, E. J., Ali, S., Byamukama, E., Yen, Y., & Nepal, M. P. (2018). Disease Resistance Mechanisms in Plants. *Genes* , 9(7), 339.

Amaral, L.S., Debona, D., Costa, L.C., Luiza, A., Jose, R.S., Fabricio, R.O., & Rodrigues, A. (2019). Biochemical insights into basal and induced resistance in cabbage to black rot. *Journal of Phytopathology* , 167, 390–403.

Bagheri, L.M., Nasr-Esfahani, M., Abdossi, V., & Naderi, D. (2020). Analysis of candidate genes expression associated with defense responses to root and collar rot disease caused by *Phytophthora capsici* in peppers *Capsicum annuum* , *Genomics* , 112(3), 2309-2317.

Beaudoin-Eagan, L.D & Thorpe, T.A. (1985). Shikimate pathway activity during shoot initiation in tobacco callus cultures. *Plant Physiology* , 73,228-232.

Bharathi, E., Santha Lakshmi Prasad, M., Yadav, P., & Bee, H. (2019). Defense responses to *Fusarium oxysporum* f. sp. *ricini*infection in castor (*Ricinus communis* L.) cultivars. *Indian Phytopathology* , 72, 647-656.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* , 72,248-254.

Chen, C., Belanger, R.R., Benhamou, N., & Paullitz, T.C. (2000). Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR). *Physiological Molecular Plant Pathology* , 56, 1323.

Constabel, C.P., & Ryan, C.A. (1998). A survey of wound-and methyl jasmonate-induced leaf polyphenol oxidase in crop plants,*Phytochemistry* , 47 (4), 507–511.

Dazy, M., Jung, V., Ferard, J.F., & Masfaraud, J.F. (2008). Ecological recovery of 712 vegetation at a former coke-factory industrial wasteland: Role of plant 713 antioxidant enzymes and possible implications in site restoration. *Chemosphere* , 74,57-63.

Erwin, D. C., & Ribeiro, O. K. (1996). Phytophthora Disease Worldwide. APS Press. St. Paul, MN, pp, 562.

Fernandes, C.F., Moraes, V.C.P., Vasconcelos, L.M., Silveira, J.A.G., & Oliveira, J.T.A. (2006). Induction of an anionic peroxidase in cowpea leaves by exogenous salicylic acid. *Journal of Plant Physiology* , 163, 1040-1048.

Garg, N., & Manchanda, G. (2009). ROS generation in plants: Boon or bane? *Plant Biosystems* , 143, 81-96.

Gratao, P.L., Monteiro, C.C., Carvalho, R.F., Tezotto, T., Piotto, F.A., Peres, L.E., & Azevedo, R.A. (2012). Biochemical dissection of diageotropica and Never ripe tomato mutants to Cd-stressful conditions,*Plant Physiology and Biochemistry* , 56, 79-96.

Hashemi, L., Golparvar, A.R., Nasr Esfahani, M., & Golabadi, M. (2019). Correlation between cucumber genotype and resistance to damping-off disease caused by *Phytophthora melonis* , *Biotechnology & Biotechnological Equipment* , 33(1), 1494-1504.

Hatami, N., Aminaee, M.M., Zohdi, H., & Tanideh, T. (2013). Damping-off disease in greenhouse cucumber in Iran. *Archives of Phytopathology and Plant Protection* , 46(7), 796–802.

Huang, J., Gu, M., Lai, Z., Fan, B., Shi, K., Zhou, Y. H., Yu, J.Q., & Chen, Z. (2010). Functional analysis of the Arabidopsis PAL gene family in plant growth, development, and response to environmental stress.*Plant Physiology* , 153, 1526–1538.

Jiang, S., Han, S., He, D., Cao, G., Fang, K., Xiao, X., Yi, J., & Wan, X. (2019). The accumulation of phenolic compounds and increased activities of related enzymes contribute to early defense against walnut blight. *Physiological and Molecular Plant Pathology* , 108, 101433.

Jung, W.J., F. Maboodb, A. Souleimanovb, & D.L. Smith. (2011). Induction of defense-related enzymes in soybean leaves by class IId bacteriocins (thuricin 17 and bacthuricin F4) purified from Bacillus strains. *Microbiological Research* , 167, 14-19.

Khatediya, N.K., Parmar, D.V., Mahatma, M.K., & Pareek, M. (2018). Increased accumulation of phenolic metabolites in groundnut (*Arachis hypogaea* L.) genotypes contribute to defense against *Sclerotium rolfsii* infection, *Archives of Phytopathology and Plant Protection* , 51(9-10), 530-549, DOI: 10.1080/03235408.2018.1490519.

Kim, D.S., & Huang, B.K. (2014). An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic aciddependent signalling of the defense response to microbial pathogens. *Journal of Experimental Botany* , 65, 2295-2306.

Khodadadi, F., Tohidfar, M., Vahdati, K., Dandekar, A.M., & Leslie, C.A. (2020). Functional analysis of walnut polyphenol oxidase gene (JrPPO1) in transgenic tobacco plants and PPO induction in response to walnut bacterial blight. *Plant pathology* , 69(4), 756-764.

Lamichhane, J.R., Durr, C., Schwanck, A.A., Robin, M.H, Sarthou, J.P., Cellier, V., Messean, A., & Aubertot, J.N. (2017). Integrated management of damping-off diseases. A review. *Agronomy for Sustainable Development* , 37, 10. Li, L., & Steffens, J. (2002). Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta* , 215, 239-47. pmid: 12029473.

Mandal, S., & Mitra, A., (2007). Reinforcement of cell wall in roots of*Lycopersicon esculentum* through induction of phenolic compounds and lignin by elicitors. *Physiological and Molecular Plant Pathology* , 71, 201-209.

Mydlarz, L.D., & Harvell, C.D. (2006). Peroxidase activity and inducibility in the see fan coral exposed to a fungal pathogen.*Comparative Biochemistry & Physiology* . 10, 1016.

Moghbeli, E., Nemati, S.H., Aroiee, H., & Olfati, J.A. (2017). Evaluation of Resistance, Enzymatic Response, and Phenolic compounds in roots of F1 cucumber hybrids to *Fusarium oxysporium* F. SP.*Radicis-Cucumerium* . *Journal of Horticultural Research* , 25(1), 117–124. DOI: 10.1515/johr-2017-0012.

Moghaddam, G.A, Rezayatmand, Z., Nasr Esfahani, M. & Khozaei, M. (2019). Genetic defense analysis of tomatoes in response to early blight disease, *Alternaria alternata* . *Plant Physiology and Biochemistry* , 142, 500-509.

Moradi, N., Rahimian, H., Dehestani, A., & Babaeizad, V. (2016). Cucumber Response to Sphaerotheca fuliginea: Differences in antioxidant enzymes activity and pathogenesis-related gene expression in susceptible and resistant genotypes. *Journal of Plant Molecular Breeding* , 4(2), 33- 40.

Nasr Esfahani, M. (2018). Analysis of virulence and genetic variability of *Alternaria alternata* associated with leaf spot disease in potato plants in Iran. *Acta Mycologica* , 53, 1-9.

Nasr Esfahani, M., Chatraee, M., Shafizadeh, S., Jalaji, S. (2012). Evaluation of resistance of cucurbit and cucumber cultivars to*Phytophthora drechsleri* in Greenhouse. *Iranian Seed and Plant Improvement Journal* , 28,407-417.

Nasr Esfahani M, Nasehi A, Rahmanshirazi P. (2014). Susceptibility assessment of bell pepper genotypes to crown and root rot disease.*Arch Phytopathol Plant Protect* ; 47: 944–953.

Nasr Esfahani, M. (2019). Morphological, virulence and genetic variability of *Ulocladium atrum* causing potato leaf blight disease in Iran. *J Plant Prot Res* 59: 41-49.

Nasr Esfahani, M. (2020). Genetic variability and virulence of some Iranian *Rhizoctonia solani* isolates associated with stem canker and black scurf of potato (*Solanum tuberosum* L.). J. *Plant Prot. Res* . 60 (1): 21–30.

Nazavari, K., Jamali, F., Bayat, F., & Modarresi, M. (2016). Evaluation of resistance to seedling damping-off caused by *Phytophthora drechsleri* in cucumber cultivars under greenhouse conditions.*Biological Forum* , 8, 54-60.

Nostar, O., Ozdemir, F., Bor, M., Turkan, I., & Tosun, N. (2013). Combined effects of salt stress and cucurbit downy mildew (*Pseudoperospora cubensis* Berk. and Curt. Rostov.) infection on growth, physiological traits and antioxidant activity in cucumber (*Cucumis sativus* L.) seedling. *Physiological and Molecular Plant Pathology* , 83, 84-92.

Pandey, V., A.K. Tewari, & Saxena, D. (2017). Activities of defensive antioxidant enzymes and biochemical compounds induced by bioagents in Indian mustard against *alternaria blight* . *Proceedings of the National Academy of Sciences,* 2, 1–10.

Prasannath, K. & De Costa, D.M. (2015). Induction of peroxidase activity in tomato leaf tissues treated with two crop management systems across a temperature gradient. Proceedings of the International Conference on Dry Zone Agriculture 2015. Faculty of Agriculture, University of Jaffna, Sri Lanka. 15th & 16th October 2015. 34-35.

Rais, A., Jabeen, Z., Shair, F., Hafeez, F.Y., & Hassan, M.N. (2017). Bacillus spp., a bio-control agent enhances the activity of antioxidant defense enzymes in rice against *Pyricularia oryzae* . PLoS ONE, 12(11), e0187412. https://doi.org/10.1371/ journal. pone.0187412.

Riedle-Bauer, M. (2000). Role of reactive oxygen species and antioxidant enzymes in systemic virus infections of plants. *Journal of Phytopathology* . 148, 297–302.

Sabbaghi, E., Sabbagh, S.K., Panjehkek, N., & Bolokyazd, H.R. (2018). Jasmonic Acid Induced Systemic Resistance in Infected Cucumber by*Pythium aphanidermatum* . Journal of agricultural science, 24, 143-152. DOI: 10.15832/ankutbd.446416.

Siddique, Z., K.P. Akhtar, A. Hameed, N. Sarwar, I.U. Haq, & S.A. Khan. (2014). Biochemical alterations in leaves of resistant and susceptible cotton genotypes infected systemically by cotton leaf curl Burewala virus. *Journal of Plant Interactions* , 9,702–711.

Su, Y., Wang, Z., Xu, L., Peng, Q., Liu, F., Li, Z., & Que, Y. (2016). Early selection for smut resistance in sugarcane using pathogen proliferation and changes in physiological and biochemical indices.*Frontiers in Plant Science* , 7, 1133.

Su, X., Guan, L., & Hu, F. (2019). Comparison of Defensive Enzyme Activities in the Leaves of Seven Oriental Lily Hybrids after Inoculation with *Botrytis elliptica* . Journal of the American Society for Horticultural Science, 144(1), 55-62.

Solekha, R., Susanto, F.A., Joko, T., Nuringtyas, T.R., & Purwestri, Y.A. (2019). Phenylalanine ammonia lyase (PAL) contributes to the resistance of black rice against *Xanthomonas oryzae* pv.*oryzae* . *Journal of Plant Pathology* , https://doi.org/10.1007/s42161-019-00426-z.

Saunders, J., & O'neill, N. (2004). The characterization of defense responses to fungal infection in alfalfa. *Biological control* , 49, 715–728.

Vanitha, S.C., Niranjana, S.R., & Umesha, S. (2009). Role of phenylalanine ammonia lyase and polyphenol oxidase in host resistance to bacterial wilt of tomato. *Journal of Phytopathology* , 157, 552–557.

Van Rossum, M.W., Alberda, M., & van der Plas, L.H. (1997). Role of oxidative damage in tulip bulb scale micropropagation, *Plant Science* . 130 (2), 207–216.

Wu, T., Wang, R., Xu, X., He, X., Sun, B., Zhong, Y., Liang, Z., Luo, S., & Lin, Y. (2014). *Cucumis sativus* L-type lectin receptor kinase (CsLecRK) gene family response to *Phytophthora melonis* ,*Phytophthora capsici* and water immersion in disease resistant and susceptible cucumber cultivars. *Gene* , 549, 214-222.

Xie, J.H., Chai, T.T., Xua, R., Liu, D., Yang, Y.X., Deng, Z.C., Jin, H., & He, H. (2017). Induction of defense-related enzymes in patchouli inoculated with virulent *Ralstonia solanacearum* . Electronic Journal of Biotechnology, 27, 63-69.

Yusuf, C.Y.L. Abdullah, J.O. Shaharuddin, N.A. Abu Seman, I., & Abdullah, M.P. (2018). Characterization of promoter of EgPAL1, a novel PAL gene from the oil palm *Elaeis guineensis* Jacq. *Plant Cell Reports* , 37, 265–278.

Zhang, C.Z., Wang, X., Zhang, F., Dong, L., Wu, J., Cheng, Q., Qi, D., Yan, X., Jiang, L., Fan, S., Li, N., Li, D., Xu, P., & Zhang, S. (2017). Phenylalanine ammonia-lyase2.1 contributes to the soybean response towards *Phytophthora sojae* infection. *Scientific reports* , 7(1), 7242. https://doi.org/10.1038/s41598-017-07832-2.

Zhao, S., Du, C.M., & Tian, C.Y. (2012). Suppression of *Fusarium oxysporum* and induced resistance of plants involved in the biocontrol of cucumber Fusarium wilt by Streptomyces bikiniensis HD 087. *World Journal of Microbiology & Biotechnology* , 28, 2919–2927.

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