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Investigation of Fire Blight Susceptibility and Iron Homeostasis of Pear (Pyrus communis L.) Following Invasion of Tissues by hrpW$^-$, hrpN$^-$ and dspA/E$^-$ Mutants of *Erwinia amylovora*

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Abstract

Erwinia amylovora, the causal agent of fire blight disease in rosaceous plants contains type III secreted effector proteins including DspA/E, HrpN and HrpW which are secreted into host plants during the pathogenicity stages. In order to investigate the role of these effector proteins in the interaction with the host plants, susceptible (Bartlett), tolerant (Harrow Sweet) and resistant (Dargazi) pear cultivars were inoculated with wild-type and mutant strains of *E. amylovora* (hrpW$^-$, hrpN$^-$ and dspA/E$^-$) under *in vitro* conditions. Based on the results, HrpW protein may be involved in pathogenicity in Dargazi cultivar. Different levels of pathogenicity were observed by dspA/E$^-$ mutant in cultivars. The results showed the key role of HrpN, in the defense mechanisms of Dargazi cultivar, and its pathogenic role in Harrow Sweet and Bartlett cultivars. An increase in ferritin levels was observed in all cultivars inoculated with the wild type strain, but resistant and tolerant cultivars showed higher ferritin levels and a decrease in Fe^{2+} was observed only in these cultivars. The obtained data show that the HrpW protein does not affect iron homeostasis. Inoculation of Harrow Sweet and Dargazi cultivars with all strains increased ferritin, which was associated with a decrease in Fe^{2+}. Based on the results, it is not possible to associate any of the effector proteins with changes in ferritin and Fe^{2+}. In general, the ability of resistant pear cultivars to increase ferritin levels and regulation of iron can be one of the reasons for their resistance to fire blight. According

to the results, different mechanisms are employed by pear cultivars to respond to the causative agent of fire blight.

Key words: Bartlett, Harrow sweet, Dargazi, *hrp*W$^-$, *hr*pN$^-$, *dsp*A/E$^-$

Introduction

One of the most important destructive diseases of pear fruits in the world is fire blight, which is caused by the bacterial agent *Erwinia amylovora* (Abdollahi *et al*., 2004). This bacterium is a type of rod-shaped bacterium, and so far, no disease management approach has been definitively effective. (Vanneste, 2000). The use of antibiotics and copper-based compounds, pruning of infected tissues, and the use of resistant cultivars are the most important methods of disease control. (Gusberti *et al*., 2015). According to studies, the most effective and economical method of fire blight disease management is the use of resistant or tolerant cultivars (Vanneste, 2000). The improvement of fire blight resistance on *Cydonia oblonga* using the hybridization breeding showed that the fire blight resistance genes in quince are recessive and Transferring resistance to hybrids is more successful using resistant varieties as pollinators (Shahin et al., 2020).

In non-host plants such as tobacco and resistant hosts plants, the reaction to *E. amylovora* infection is incompatible Which leads to hypersensitive reaction (HR) (Venisse *et al*., 2001). Also, in host plants, the reaction is compatible and leads to infection (Holtappels *et al*., 2018). investigations have shown that in both compatible and incompatible reactions, the production of reactive oxygen species (ROS) and oxidative bursts are the main responses against the attack of *E. amylovora* (Wang et al., 2019). *E. amylovora* produces three types of effector proteins including HrpN, DspA/E and HrpW during pathogenicity in the host plants (Narayanasamy, 2008). A gene cluster in the bacterial genome with a length of about 62 kb, which contains the *hrc*, *hrp* and *dsp* genes, is responsible for producing these effector proteins. Meanwhile, two effector proteins, HrpN and HrpW, are produced by *hrp* genes, and DspA/E is produced by *dsp* genes (Oh and Beer 2005). DspA/E and HrpN proteins have been cited as the main options for compatible interaction in the host, and HrpW protein appears to play a negligible role in this interaction (Taheri Shahrestani *et al*., 2020). According to recent research, the presence of active chloroplasts is necessary for the pathogenicity of HrpN protein in the host (Taheri Shahrestani *et al*., 2020; Abdollahi *et al*., 2015). Hypersensitive reaction, degradation of mitochondrial function and consequent programmed cell death are the results of HrpN protein function in non-host plants (Xie and Chen 2000). The DspA/E effector protein is an essential pathogenicity factor of *E. amylovora* because *dsp*A/E$^-$ mutant strains

did not cause disease in the host plants (Taheri Shahrestani *et al*., 2020; Gaudriault *et al*., 1997; Bogdanove *et al*., 1998). Oxidative burst seems to be essential for successful bacterial infection in a compatible interaction (Venisse *et al*., 2001). The report of Venisse *et al* (2003) indicates the combined role of two effector proteins, HrpN and DspA/E, in causing oxidative burst in the interaction of *E. amylovora* with host plants. According to their results, the *dsp*A/E mutant does not cause any symptoms, while the *hrp*N mutant is still able to cause some fire blight symptoms. On the other hand, the *dsp*A/E mutant had a greater ability to activate antioxidant enzymes than the *hrp*N mutant (Venisse *et al*., 2003). Azarabadi *et al* (2016) also reported that tolerance to fire blight is associated with changes in the production pattern of ROS and especially the effect of two major species of hydrogen peroxide (H_2O_2) and hydroxyl ($OH^{\bullet-}$) radical in host tissues. Therefore, considering the key role of DspA/E in the formation of ROS, the levels of effect of this effector protein, and the reaction of the organelles involved in the production of reactive oxygen species, can determine the level of host resistance to fire blight.

E. amylovora elicits a rapid oxidative burst in host plants (Venisse *et al*., 2001, 2003). According to Abdollahi *et al*., (2015), oxidative burst in resistant genotypes of apples and pears inoculated with *E. amylovora* triggered earlier than in susceptible genotypes. Large amounts of ROS such as singlet oxygen (O^2), superoxide anion ($O^{2\bullet}$), H_2O_2 and $OH^{\bullet-}$ are produced as one of the primary responses of plant cells under various abiotic and biotic stresses (Sharma *et al*., 2012). Resistance to disease and destructive activities are among the different roles of ROS in cellular functions. The production and removal of ROS must be tightly controlled in the cell to prevent oxidative damage. Also, due to the numerous roles that ROS have, they should not be completely removed. The expression of disease resistance genes by H_2O_2 has been proven (Hassani *et al*., 2015; Radwan *et al*., 2010; Radwan *et al*., 2006). Another role of H_2O_2 is to act as a substrate for oxidative cross-linking in cell walls (Smirnoff and Arnaud 2018). Therefore, one of the strategies of plants to prevent the spread of pathogens is fine-tuned H_2O_2 generation**.** Thus, H_2O_2 accumulated by the plant is toxic to pathogens but is not toxic to the plant itself. Thus, toxic levels of H_2O_2 act in two ways to limit infection. On the one hand, it directly leads to the elimination of the pathogen, and on the other hand, it induces genes related to plant defense (Dat *et al*., 2000; Smirnoff and Arnaud 2018). Another mechanism of resistance to pathogens is HR, in which plant resistance genes identify pathogenic proteins (Balint-Kurti, 2019). The formation of very high levels of H_2O_2 during the HR response induces programmed cell death and pathogen elimination (Abdollahi *et al*., 2015).

The precursor for the formation of $OH^{\bullet -}$ radicals during the Fenton or Haber-Weiss reaction is H_2O_2. It has been observed that during the infection of resistant pear cultivars with *E. amylovora*, the conversion rate of H_2O_2 to $OH^{\bullet -}$ radical is low, which can be due to the activity of the non-enzymatic antioxidant system of the host cells (Azarabadi *et al*., 2016).

Ferritin is one of the most important metal binding proteins and can stores metal ions (Fe^{3+} and Cu^{2+}) in its core and prevents the formation of $OH^{\bullet -}$ from H_2O_2 (Halliwell and Gutteridge 2015). Iron, as an essential element for plants, firstly participates in the redox reactions and structure of many intracellular enzymes such as peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD), and secondly, through the Fenton or Haber-Weiss reaction, produces ROS (Dat *et al*., 2000). Thus, Fe^{2+} may cause the formation of harmful $OH^{\bullet -}$ radicals from the H_2O_2 precursor through the Fenton reaction. The most important cause of necrosis is $OH^{\bullet -}$ radicals and because they are very toxic to macromolecules, their production must be controlled. According to these statements, it is necessary to regulate the iron content of the cell. In this regard, creating transgenic plants expressing ferritin genes has increased plant resistance against stresses (Yadav *et al*., 2017; Xi *et al*., 2011). In this regard, transgenic tobacco plants expressing ferritin produced more ferritin and showed greater resistance to cold stress (Hegeduse *et al*., 2002). Iron also regulates virulence-related functions in *E. amylovora*. The siderophore desferrioxamine (DFO) is produced by this plant pathogen to sequester iron during the infection process. Also, the protective role of DFO for bacteria during the oxidative burst induced by the defense response of the host plant has been proven (Pandey, 2023). Therefore, the host and the pathogen have developed different and complex strategies to compete with each other for iron resources. So far, there have been no detailed investigations on iron homeostasis in host plants after the attack of the disease agent. However, in our previous study, the levels of active iron in pear cultivars decreased after inoculation with a wild-type strain of *E. amylovora* (Maleki *et al*., 2021). In this research, we tried to investigate the role of *E. amylovora* effector proteins in iron homeostasis and defense mechanisms of pear cultivars.

Materials and Methods

Bacterial strain

The characteristics of *E. amylovora* strains used in this study are given in Table 1. The effect of each of the effector proteins of HrpW, HrpN and DspA/E proteins on the pathogenicity of *E. amylovora* was investigated using *hrp*W^-, *hrp*N^- and *dsp*A/E^- mutants, respectively and compared

with the wild-type strain. The bacterial strains were cultured in LB (Luria-Bertani) liquid medium at 28ºc. For the pathogenicity assay of the bacteria, each bacterial strain was cultured overnight then turbidity of the bacterial inoculum was measured via the spectrophotometer at λ600nm and adjusted to OD=1 using sterilized potassium phosphate buffer (pH = 7) and used for inoculation of *in vitro* shootlets of pear cultivars (Abdollahi *et al*., 2004).

Plant material and growth conditions

Three pear cultivars with different susceptibility levels to fire blight, including Bartlett (susceptible), Harrow Sweet (tolerant) and Dargazi (resistant), were used for *in vitro* inoculation. Establishment and proliferation of pear cultivars were done on QL medium (Quoirin and Lepoivre 1977) enriched with 1 mg/L BAP, 1 mg/L 2ip, and 0.1 mg/L NAA (pH=5.7). For all media, 8 g/L agar, 30 g/L sucrose and 5 g/L pectin were used. The presence of a carbon source in the culture medium in *in vitro* conditions causes the inactivation of the electron transport chain (ETC) of chloroplast (Yabuta *et al*., 2007; Oswald *et al*., 2001; Fuentes *et al*., 2005). Therefore, in all experiments, the ETC activation was perform by removing sucrose from the culture medium. Pear shootlets were grown *in vitro* at a constant temperature of 23 ± 1 °C under 16 h light photoperiod using white fluorescent lamps (Sylvania, Germany) at 40 $\mu molm^{-2}\ s^{-1}$ photon flux and subcultured every 45 days (Abdollahi *et al*., 2015). Pear shootlets with an approximate length of 3 cm were used for inoculation using 200 microliters of each bacterial strain (Abdollahi *et al*., 2004). for this purpose, basal inoculation was carried out by adding 200 µl of the overnight grown bacterial suspension (OD= 1) on the surface of the proliferation medium. Subsequently, 4–5 mm of the basal ends of the shootlets were removed, and transferred to the test tubes. Five replications were considered for each treatment. The percentage of shootlets necrosis was calculated using the following formula: % necrosis = necrosis length/shootlets length × 100.

Electrolyte leakage determination

Electrolyte leakage assay was used to evaluation of membrane stability of pear cultivars. In this method, 0.1 g pear shootlets were rinsing with distilled water and cut into 5 mm disks. Then it was transferred to 50 ml tubes and 10 ml of sterilized distilled water was added to them and incubated at room temperature for 24 hours in a shaker (110 rpm). Using an EC meter (WalkLAB conductivity pro meter), the electrical conductivity of distilled water containing suspended sample discs was read as EC1. EC2 was measured after immersing the test tubes for 45 min in a boiling

water bath (110 °C). the relative electrolyte leakage (EL) was calculated using the following equation (Sairam and Srivastava, 2001):

$EL\% = [EC1/EC2] \times 100$

Ferritin levels measurement

The ferritin assay kit (Eliza kit, Pishtaz Teb, Tehran, Iran) was used to measure the ferritin levels of pear cultivars according to the manufacturer's protocol. First, 1 gram of the pear shootlets was ground in the ice-cold extraction buffer (100mM sodium chloride, 10 mM sodium phosphate buffer, 1mM PMSF and 2% W/V PVP, pH = 7.2) and then centrifuged at 15,000g for 4 minutes at 4°C (Lukac *et al*., 2009). The supernatant was used to measure ferritin using the kit. Finally, the absorbance of the samples was read using an ELISA reader (Stat Fax 2100, Awareness) at 450 nm.

Estimation of Active iron (Fe^{+2})

The method of Katyal and Sharma (1980) with slight modifications was used to estimate the amount of active iron in pear cultivar shootlets. First, one gram of fresh peer shootlets was taken, washed by distilled water and the moisture was removed by absorbent papers. Then the leaves were separated and chopped into fine bits. The samples were treated with 0.4 g/L ortho-phenanthroline extract (pH 3.0) for 20 hours then centrifuged at 5000 rpm and room temperature. The supernatant was used to estimation of Fe^{+2} by reading the transmittance at 510 nm by spectrophotometer.

Statistical analyses

Comparison of all data was done using Microsoft Excel (Microsoft, USA-Version 2007) by drawing curves and Microsoft SigmaPlot (Sigma-Aldrich, USA-Version 11.5) was used to one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT).

Results and Discussion

Necrotic lesion development

In this study, our aim was to investigate the role of *E. amylovora* effector proteins including HrpN, DspA/E, and HrpW in interaction with different pear cultivars including susceptible (Bartlett), tolerant (Harrow Sweet) and resistant (Dargazi) cultivars. The necrosis assay was successful in all shootlets of *in vitro* pear cultivars inoculated with mutant and wild-type strains of *E. amylovora*, while no disease symptoms appeared in any of the non-inoculated shootlets (Fig. 1). The effect of plant cultivars and bacterial strains on disease development was evaluated as

significant ($P < 0.01$). The appearance time and progression rate of necrosis in pear cultivars inoculated with mutant strains of *E. amylovora* were different from those inoculated with the wild-type strain (Fig. 1). Inoculation of resistant pear cultivar (Dargazi) with wild-type and *hrp*W$^-$ strains of *E. amylovora*, showed slower necrosis progress and a lower percentage of necrosis compared with those of susceptible and tolerant cultivars (Fig. 1 and Fig. 2)**.** It indicates that the susceptibility behavior of cultivars to *E. amylovora* can be evaluated using *in vitro* system (Abdollahi *et al*., 2004). Harrow Sweet and Bartlett cultivars showed signs of necrosis three days after inoculation with the wild-type strain of *E. amylovora* and the appearance of symptoms in Dargazi cultivar was delayed for up to seven days post-inoculation (Fig. 1 and Fig. 2a). On the other hand, the development rate of necrotic lesions in Harrow Sweet cultivar was slightly lower than Bartlett. Interestingly, the first signs of necrosis in Dargazi cultivar were observed after 7 dpi and the final percentage of necrosis lesions in this cultivar was completed after 13 dpi. The late appearance and slow progress of the disease in the resistant cultivar Dargazi is consistent with the previous results of Abdollahi *et al*. (2004). Therefore, the delay in the appearance and progression of disease symptoms is one of the signs of disease resistance in vitro condition. In other words, in *in vitro* conditions, due to the absence of wood tissues and lignin barriers of tissues, as well as high humidity and favorable conditions for the growth of the disease agent, resistance to the disease manifests itself as a delay in the development of the disease. Almost, the appearance and progression rate of necrosis lesions in pear cultivars inoculated with the *hrp*W$^-$ mutant strain were similar to those of pear cultivars inoculated with the wild-type strain. Accordingly, in the necrosis assay, no significant difference ($P > 0.05$) was observed in the pathogenicity of the *hrp*W$^-$ mutant and the wild-type strain (Fig. 1). Based on these results and the results of previous studies (Taheri *et al*., 2020; Kim and Beer 1998; Venisse *et al*., 2003), it is concluded that this protein has no effect on the pathogenicity of *E. amylovora*. The ROS produced in the host plant during the pathogenesis of *E. amylovora* causes lipid peroxidation, resulting in electrolyte leakage from the cells (Foyer *et al*., 1994; Venisse *et al*., 2001). Mock-inoculated *in vitro* shootlets showed electrolyte leakage of about 10% (Fig. 1 and 4). A small percentage of electrolyte leakage has already been reported in a number of healthy plants (Krasuska and Gniazdowska 2012; Filek *et al*., 2012; Brisset and Paulin 1991). The efficiency of using the two indicators of the appearance of disease symptoms as well as the necrosis progression to evaluate the resistance of different pear cultivars was not exactly corresponded to previous research (Abdollahi and Salehi 2017; Abdollahi *et al*., 2015). In our

study, the appearance and progression of necrosis occurred with a delay of several days, depending on the variety. According to our experiments in active chloroplast condition and the results of previous reports in this regard (Abdollahi *et al*., 2015; Taheri *et al*., 2020), the delay in the appearance and progression of the disease could be due to the interaction of bacterial effector proteins with host cell chloroplasts and the key role of chloroplasts during systemic acquired resistance (SAR) (Debroy *et al*., 2004).

Electrolyte Leakage

E. amylovora elicits an oxidative burst in host plants during plant defense responses in which ROS are produced (Shetty *et al*., 2008). Lipid peroxidation and consequent electrolyte leakage from cells are the results of ROS activity (Venisse *et al*., 2001). In this study, electrolyte leakage was measured as the main indicator of the severity of disease damage to cells. The studied cultivars significantly differed in their electrolyte leakage values ($P < 0.01$). Electrolyte leakage of all *in vitro* shootlets of the studied cultivars before inoculation with *E. amylovora* strains was estimated to be about 10% (Fig. 1 and 3) which is consistent with previous researches (Filek *et al*., 2012; Krasuska and Gniazdowska 2012). Based on the results, after the appearance of necrosis symptoms in pear cultivars, the electrolyte leakage rate was estimated to be more than 70%. Harrow sweet and Bartlett cultivars showed the first major changes in electrolyte leakage after inoculation with *E. amylovora* (Fig. 3). Despite the electrolyte leakage of these two cultivars from the first days after inoculation, the progression of electrolyte leakage in Harrow sweet genotype was slightly faster than Bartlett genotype. Considering these results and comparing them with the results of necrosis, it is found that Harrow sweet cultivar, despite more fire blight resistance, has less membrane stability than Bartlett cultivar. Unlike susceptible and tolerant cultivars, electrolyte leakage in the resistant cultivar started about two days post inoculation with wild-type strain of *E. amylovora* and then progressed at a slower rate. According to the results of this study, membrane damage in resistant cultivar (Dargazi), does not start from the first days after inoculation, unlike the sensitive and tolerant pear cultivars (Fig. 3a). As a result, membrane stability in Dargazi cultivar is higher than other cultivars and also Bartlett cultivar has higher membrane stability than Harrow sweet cultivar (Fig. 3a). Therefore, it seems that the membrane stability of tolerant cultivar (Harrow sweet) could not be the reason for its relative resistance to disease.

Interaction of pear cultivars with *hrp*N$^-$ strain

appearance of necrosis was observed in Dargazi, Bartlett and Harrow sweet pear cultivars after 4, 7 and 14 days after inoculation with *hrp*N$^-$ mutant strain, respectively (Fig. 2b). Thus, in the Harrow sweet and Bartlett cultivars, symptoms appeared later than when they were inoculated with the wild-type strain of *E. amylovora*. On the other hand, symptoms of necrosis appeared earlier in the resistant cultivar (Dargazi). Thus, Dargazi cultivar acted like a sensitive cultivar after inoculation with *hrp*N$^-$ mutant strain. These results indicate the role of HrpN on induction of plant defense mechanisms in Dargazi cultivar and as pathogenicity factor in Harrow sweet and Bartlett cultivars. Also, the rate of necrosis progression in all studied cultivars did not show a significant difference with the control ($P > 0.05$). initiation of electrolyte leakage after inoculation of cultivars with *hrp*N$^-$ mutant strain was observed first in Dargazi cultivar, then in Bartlett and Harrow sweet (Fig. 3b). However, electrolyte leakage in Dargazi and Bartlett cultivars progressed rapidly but lasted up to 21 days in Harrow sweet cultivars (Fig. 3b). These results were consistent with the results of necrosis studies (Fig. 1). Previous studies have shown that HrpN protein has two roles, including induction of the defense mechanisms and pathogenicity factor in the host tissue (Dong *et al*., 1999; Taheri *et al*., 2020; Norliza *et al*., 2018; Qiu *et al*., 1997). Early appearance of necrosis symptoms and electrolyte leakage in Dargazi cultivar indicates that the role of HrpN effector protein in induction of defense mechanisms was more likely than its pathogenic role in this cultivar. Unlike Dargazi cultivar, the pathogenic role of HrpN was more prominent in Harrow sweet and Bartlett cultivars, because a significant delay in electrolyte leakage and necrosis symptoms appearance were observed ($P < 0.01$). Dong *et al* (1999) Showed that HrpN induces pathogenesis-related (PR) protein genes in plants, and also in Arabidopsis transgenic plants, which had lost their ability to accumulate salicylic acid, HrpN protein neither elicited resistance nor activated SAR gene expression. Therefore, HrpN protein induces resistance through the SAR signal transduction pathway in a SA-dependent manner.

Interaction of pear cultivars with *hrp*W$^-$ strain

Symptoms of necrosis were observed in Dargazi, Harrow sweet and Bartlett cultivars 9, 3- and 2-days post-inoculation with the *hrp*W$^-$ strain, respectively (Fig. 2c). The rate of necrosis progression in Bartlett cultivar was higher than other cultivars and was completed after three days. Harrow sweet and Dargazi cultivars showed complete necrosis at a slower rate. The results of electrolyte leakage in pear cultivars inoculated with *hrp*W$^-$ mutant strain were almost consistent with the results of inoculation with non-mutant strain (Fig. 1 and 3). These results are consistent with a

previous report by Venisse *et al* (2003). The results of necrosis and electrolyte leakage experiments in Dargazi cultivar inoculated with wild type strain and hrpW$^-$ mutant strain show slight differences. Therefore, it seems that the effector protein HrpW may have slight effect on the pathogenicity of *E. amylovora* in Dargazi cultivar and no effect on Harrow sweet and Bartlett cultivars. According to previous results using the *hrp*W$^-$ mutant strain, the HrpW protein had no effect on induction of hypersensitive reaction and pathogenicity of *E. amylovora*. However, according to previous reports by Taheri *et al*., (2017) And Abdollahi (2003) this effector protein may have little effect on induction of plant defense mechanisms, which requires further research.

Interaction of pear cultivars with *dsp*A/E$^-$ strain

Symptoms of necrosis were appeared in Harrow sweet and Dargazi pear cultivars 12 and 9 days after inoculation with *dsp*A/E$^-$ mutant strain, respectively and the rate of necrosis progression was higher in Harrow sweet cultivar than Dargazi cultivar. (Fig. 1 and 2d). However, Bartlett cultivar did not show Symptoms of necrosis even after 30 days (Fig. 2d). Electrolyte leakage initiated later in pear cultivars inoculated with the dspA/E$^-$ mutant strain (Fig. 3d). Also, electrolyte leakage in these cultivars reached 100% in a longer period of time**.** Harrow sweet cultivar started electrolyte leakage before Dargazi cultivar but reached maximum electrolyte leakage in a longer period of time. In Bartlett cultivar, even after 30 days from inoculation, no significant increase in relative electrolyte leakage was observed ($P < 0.01$). It seems that the increase in relative electrolyte leakage of this cultivar after 30 days to about 27% was due to plant stresses in *in vitro* condition**.** According to these results, the effector protein DspA/E has a significant effect on the pathogenicity of *E. amylovora*, so that in Bartlett cultivar, even after 30 days post-inoculation, no symptoms of necrosis and significant electrolyte leakage were observed (Fig. 2 and 3). These results confirm the previous results regarding the non-pathogenicity of *dsp*A/E$^-$ mutant strain in pear (Bogdanove *et al*., 1998 ؛ Gaudriault *et al*., 1997). Thus, according to the results of electrolyte leakage and necrosis experiments, the effector protein DspA/E can be considered as the main pathogenicity factor of *E. amylovora*.

Ferritin levels

In this study, by measuring Fe^{2+} and plant ferritin, we investigated the role of iron in the resistance of different pear cultivars to fire blight. Inoculation of pear cultivars by wild-type strain of *E. amylovora* caused significant differences ($P < 0.01$) in ferritin level in all pear cultivars (Fig. 4).

Thus, two days after inoculation with wild-type strain of *E. amylovora*, the ferritin content of Bartlett, Harrow sweet and Dargazi cultivars increased by 27%, 47% and 46%, respectively (Fig. 4). Resistant and tolerant cultivars in our experiment had higher levels of ferritin even before inoculation with *E. amylovora*. According to the results, all cultivars used in this experiment have the ability to increase ferritin levels, but the rate of this increase is much higher in resistant and tolerant cultivars. The results of changes in ferritin content in pear cultivars inoculated with wild-type and *hrp*W$^-$ mutant strains of *E. amylovora* were consistent with each other (Fig. 4). These results indicate that HrpW protein has no effect on increasing the expression of ferritin genes in the cultivars used in our study. Inoculation of Harrow sweet cultivar with *dsp*A/E$^-$ mutant strain increased ferritin levels, which could indicate the possible role of DspA/E protein in inhibiting ferritin gene expression. In Bartlett cultivar, *hrp*N$^-$ and *dsp*A/E$^-$ mutant strains reduced ferritin content compared to the time of inoculation with wild-type strain of *E. amylovora* (Fig. 4). This indicates that the increase in ferritin observed in this cultivar is due to the interaction of two effector proteins, HrpN and DspA/E. Ferritin is one of the important proteins that is considered during various stresses in plants (Briar *et al*., 2010). it can store and oxidize up to 4,500 Fe^{2+} atoms in its core, thus preventing the formation of destructive free radicals $OH^{\bullet-}$ during the Fenton reaction (Ong *et al*., 2006). Recent research has shown that the expression of exogenous ferritin genes in transgenic plants has led to resistance to pathogens and abiotic stresses (Yadav *et al*., 2017; Malnoy *et al*., 2003; Xi *et al*., 2011; Xang *et al*., 2017; Deak *et al*., 1999). In view of the above, it seems that one of the characteristics of resistant and tolerant pear cultivars used in this study, is their ability to increase ferritin levels after infection with *E. amylovora*. Therefore, the sensitive cultivar Bartlett lacks sufficient ability in this regard.

Active iron (Fe^{2+})

The concentrations of Fe^{2+} in all pear cultivars before inoculation with wild-type strain of *E. amylovora* was not significantly different ($P > 0.05$) (Fig. 5). Two days after inoculation with wild-type strain of *E. amylovora*, the amount of Fe^{2+} in Dargazi and Harrow sweet cultivars decreased by 28% and 33%, respectively, and no significant change was observed in Bartlett cultivar ($P > 0.05$). The results of variation in Fe^{2+} concentration in pear cultivars inoculated with the hrpW$^-$ mutant strain and the wild type strain of *E. amylovora* were almost similar (Fig. 5). This also indicates that HrpW effector protein has no effect on pathogenicity or induction of defense mechanisms of pear cultivars. Inoculation of Dargazi cultivar using wild-type strain and *hrp*W$^-$ and

*hrp*N$^-$ mutant strains, reduced Fe^{2+} concentration. However, the use of the dspA/E$^-$ mutant strain to inoculate the Dargazi cultivar did not cause a significant change in Fe^{2+} concentration ($P > 0.05$) (Fig. 5). Prior to this experiment, inoculation of Dargazi cultivar with all strains of *E. amylovora* had increased ferritin levels. Thus, ferritin is not an essential regulator of iron homeostasis in Dargazi cultivar and DspA/E effector protein play a key role in the control of iron by other pathways. Inoculation of Harrow sweet cultivar with each strains of *E. amylovora* reduced Fe^{2+} concentration almost equally. Therefore, changes in Fe^{2+} in this cultivar cannot be attributed to any of the effector proteins of *E. amylovora*. However, in the previous experiment, inoculation of this cultivar with all strains of *E. amylovora* increased ferritin levels. Thus, it is possible that the interaction of the *E. amylovora* effector proteins caused regulation of iron in this cultivar. Unlike Dargazi and Harrow sweet cultivars, inoculation of Bartlett cultivar with wild-type and *hrp*W$^-$ mutant strains did not cause significant change in active iron concentration ($P > 0.05$). Based on the Fig. 5, it can be concluded that in Bartlett cultivar, the interaction of two effector proteins, HrpN and DspA/E, prevented the change of iron content, but the separate effect of each of these two proteins led to a decrease in active iron. According to the results of the ferritin test, this decrease in active iron is not related to ferritin. Because in similar conditions ferritin has decreased. Therefore, the decrease in the amount of active iron in this cultivar could be due to other iron storage proteins or other cell methods to regulation of iron. As a result, this genotype does not have the ability to control and regulation of iron in the face of wild-type strain of *E. amylovora*.

Previously, the role of iron in the virulence of plant pathogens in only a limited number of pathogens has been investigated. However, so far, no information is available on the role of effector proteins in plant iron homeostasis. The issue of iron homeostasis in plants is a very complex issue that is affected by many factors. In our recent study in greenhouse conditions, depending on the susceptibility of pear cultivars, fire blight spread to a certain part of the stem length and then stopped (Maleki *et al*., 2021). In this regard, Aznar *et al*., (2015) Showed that strong iron depletion occurs in leaf tissues colonized by *D. dadantii*, while ahead of colonial areas, healthy plant cells still have accumulated ferritin and iron. On the other hand, the production of ferritin and siderophores during infection in host tissues by *E. amylovora* complicates the competitive situation much more. Zhao *et al*. (2005) found that the Ftn gene encoding ferritin is induced in *E. amylovora* during infection in pear tissues. Siderophores are the virulence factors of *E. amylovora* that are produced in iron-limited environments and enable the pathogen to overcome the condition of iron

limitation (Franza and Expert 2013). They can also protect bacteria against reactive oxygen species produced by the Fenton reaction (Venisse *et al*., 2003). Several reports have shown that siderophores can trigger plant defense responses (Aznar *et al*., 2014; Dellagi *et al*., 2009). Thus, iron starvation by the production of siderophores leads to the accumulation of antimicrobial compounds and other plant defense responses. Together these data show that iron deficient plants may be more resistant to *E. amylovora* than non-deficient plants. For instance, iron starved *A. thaliana* plants were more resistant to the *Dickeya dadantii*. Given the conditions of this study in a culture medium with sufficient amounts of iron, competitive iron conditions may show other interesting results.

Based on the presented results, it seems that DspA/E has the most role in pathogenicity of *E. amylovora* and the role of HrpN in induction of plant defense mechanisms is more important and HrpW has little effect on the pathogenicity of *E. amylovora* in Dargazi cultivar. Also, regardless of the possible role of DspA/E effector protein in iron homeostasis in Dargazi cultivar, it seems that iron homeostasis in pear cultivars is the result of the interactions of effector proteins, especially HrpN and DspA/E. Previously, Venisse *et al*. (2003) showed that the elicitation of oxidative burst in the interaction of *E. amylovora* and pear is the result of the combined action of two effector protein DspA/E and HrpN. However, in general, the ability of resistant pear cultivars to increase ferritin and iron homeostasis can be one of the reasons for their resistance to fire blight. According to the results, refraining from excessive consumption of iron sources before and after the attack of the disease agent can prevent severe damage.

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References

1. Abdollahi, H. 2003. Molecular biology of interaction between *Erwinia amylovora* and pear (*Pyrus communis* L.) genotypes with different susceptibility to fire blight. Ph.D. Thesis, Faculty of Agriculture, University of Florence, Italy.
2. Abdollahi. H., Ghahremani, Z. and Erfani Nia, K. 2015. Role of electron transport chain of chloroplasts in oxidative burst of interaction between *Erwinia amylovora* and host cells. *Photosynth. Res*., **124:** 231-242.

418 3. Abdollahi, H. and Salehi, Z. 2017. Histology of Oxidative Stress and Generation of
419 Reactive Oxygen Species Against Progress of Fire Blight Causal Agent in Pear Cultivars.
420 *Seed and Plant Production Journal* 33 (2) 139-162.
421 4. Abdollahi, H., Rugini, E., Ruzzi, M. and Muleo, R. 2004. *In vitro* system for studying the
422 interaction between *Erwinia amylovora* and genotypes of pear. *Plant Cell, Tissue and*
423 *Organ Culture* 79:203–212.
424 5. Aznar, A., Patrit, O., Berger, A. and Dellagi, A. 2015. Alterations of iron distribution in
425 Arabidopsis tissues infected by *Dickeya dadantii*, *Mol. Plant Pathol*. 16 521–528.
426 6. Aznar, A., Chen, N. W. G., Regault, M., Riache, N., Joseph, D., Desmaele, D., Mouille,
427 G., Boutet, S., Soubigou- Taconnat, L., Renou, J-P., Thomine, S., Expert, D. and Dellagi,
428 Alia. 2014. Scavenging iron: a novel mechanism of plant immunity activation by
429 microbial siderophores1C W, Plant Physiol. 164(4): 2167–2183.
430 7. Azarabadi, S., Abdollahi, H., Torabi, M., Salehi, Z. and Nasiri, J. 2016. ROS generation,
431 oxidative burst and dynamic expression profiles of ROS-scavenging enzymes of
432 superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in response
433 to *Erwinia amylovora* in pear (*Pyrus communis* L). *European Journal of Plant Pathology*
434 147:279-294.
435 8. Balint-Kurti. P. 2019. The plant hypersensitive response: concepts, control and
436 consequences. Molecular Plant Pathology 20:1163–1178.
437 9. Briat, J. F., Ravet, K., Arnaud, N., Duc, C., Boucherez, J., Touraine, B., Cellier, F. and
438 Gaymard, F. 2010. New insights into ferritin synthesis and function highlight a link
439 between iron homeostasis and oxidative stress in plants. *Annals of Botany* 105:811-822.
440 10. Bogdanove, A. J., Kim, J. F., Wei, Z., Kolchinsky, P., Charkowski, A. O., Conlin, A. K.,
441 Collmer, A. and Beer, S. V. 1998. Homology and functional similarity of an *hrp*-linked
442 pathogenicity locus, *dsp*EF, of *Erwinia amylovora* and the avirulence locus *avr*E of
443 *Pseudomonas syringae* pathovar tomato. *National Academy of Sciences* 95:1325–1330.
444 11. Brisset, M. N. and Paulin, J. P. 1991. Relationships between electrolyte leakage from
445 *Pyrus communis* and virulence of *Erwinia amylovora*. *Physiological and Molecular Plant*
446 *Pathology* 39:443-453.

12. Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inze, D. and Van Breusegem, F. 2000. Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57:779–795.
13. DebRoy, S., Thilmony, R., Kwack, Y. B., Nomura, K. and He, S. Y. 2004. A family of conserved bacterial effectors inhibits salicylic acid–mediated basal immunity and promotes disease necrosis in plants. *PNAS USA* 101:9927–9932.
14. Deak. M., Horvath, G. V., Davletova, S., Torok, K., Sass, L., Vass, I., Barna, B., Kiraly, Z. and Dudits, D. 1999. Plants ectopically expressing the iron-binding protein, ferritin, are tolerant to oxidative damage and pathogens. *Nat Biotechnol* 17:192–196.
15. Dellagi, A., Segond, D., Rigault, M., Fagard, M., Simon, C., Saindrenan, P. and Expert, D. 2009. Microbial siderophores exert a subtle role in Arabidopsis during infection by manipulating the immune response and the iron status. *Plant Physiol*. 150:1687-1696.
16. Dong, H., Delaney, T.P., Bauer, D. W. and Beer, S. V. 1999. Harpin induces disease resistance in Arabidopsis through the systemic acquired resistance pathway mediated by salicylic acid and the NIM1 gene. *Plant Journal* 2: 207–215.
17. Filek, M., Walas, S., Mrowiec, H., Rudolphy-Skorska, E., Sieprawska, A. and Biesaga-Koscielniak, J. 2012. Membrane permeability and micro and macro element accumulation in spring wheat cultivars during the short-term effect of salinity- and PEG-induced water stress. *Acta Physiol Plant* 34:985-995.
18. Foyer, C. H., Leadis, M. and Kunert, K. J. 1994. Photo oxidative stress in plants. Plant Physiology. 92:696-717.
19. Franza, T. and Expert, D. 2013. Role of iron homeostasis in the virulence of phytopathogenic bacteria: an 'à la carte' menu. *MOLECULAR PLANT PATHOLOGY* 14(4), 429–438.
20. Fuentes, G., Talavera., C., Oropeza, C., Desjardins, Y. and Santamaria, J. M. 2005. Exogenous sucrose can decrease *in vitro* photosynthesis but improve field survival and growth of coconut (Cocos nucifera L.) *in vitro* plantlets. In Vitro Cell Dev Biol Plant 41:69–76.
21. Gaudriault, S., Malandrin, L., Paulin, J. P. and Barny, M. A. 1997. DspA, an essential pathogenicity factor of *Erwinia amylovora* showing homology with AvrE of

Pseudomonas syringae, is secreted via the Hrp secretion pathway in a DspB dependent way. *Molecular Microbiology* 26:1057–1069.

22. Gusberti, M., Klemm, U., Meier, M. S., Maurhofer, M. and Hunger Glaser, I. 2015. Fire Blight Control: The Struggle Goes On. A Comparison of Different Fire Blight Control Methods in Switzerland with Respect to Biosafety, Efficacy and Durability. Int J Environ Res Public Health 12(9): 11422–11447.

23. Halliwell, B. and Gutteridge, J. M. 2015. Free radicals in biology and medicine: Oxford University Press, USA.

24. Hassani, M., Salami, SA., Nasiri, J., Abdollahi, H. and Ghahremani, Z. 2015. Phylogenetic analysis of PR genes in some pome fruit species with the emphasis on transcriptional analysis and ROS response under *Erwinia amylovora* inoculation in apple. Genetica, 1–14.

25. Hegeduse, A., Erde, S., Janda, T., Szalai, J., Dubits, D. and Horrath, G. 2002. Effects of low temperature stress on ferritin or aldose reductase overexpressing transgenic tobacco plants. Biochimica et Biophysica Acta Szeged 46:97-98.

26. Holtappels, M., Noben, G. P., Van Dijck, P. and Valcke, R. 2018. Fire blight host-pathogen interaction: proteome profiles of *Erwinia amylovora* infecting apple rootstocks. *Scientific Reports* 8: 11689.

27. Katyal, J. C. and Sharma, B. D. 1980. A new technique of plant analysis to resolve iron chlorosis. Plant and Soil 55: 105–119.

28. Kim, J. F. and Beer, S. V. 1998. HrpW of *Erwinia amylovora*, a new harpin that contains a domain homologous to pectate lyases of a distinct class. *J Bacteriol* 180: 5203-5210.

29. Krasuska, U. and Gniazdowska, A. 2012. Nitric oxide and hydrogen cyanide as regulating factors of enzymatic antioxidant system in germinating apple embryos. *Acta Physiologiae Plantarum* 34: 683–692.

30. Smirnoff, N. and Arnaud, D. 2018. Hydrogen peroxide metabolism and functionsin plants. New Phytologist 221:1197–1214

31. Lukac, R. J., Aluru, M. R. and Reddy, M. B. 2009. Quantification of ferritin from staple food crops. Journal of Agriculture and Food Chemistry 57:2155-2161.

32. Maleki, R., Abdollahi, H. and Piri, S. 2021. Variation of active iron and ferritin content in pear cultivars with different levels of pathogen resistance following inoculation with

Erwinia amylovora. *Journal of Plant Pathology*. https://doi.org/10.1007/s42161-021-00998-9.

33. Malnoy, M., Venisse, J. S., Brisset, M. N. and Chevreau, E. 2003. Expression of bovine lactoferrin cDNA confers resistance to *Erwinia amylovora* in transgenic pear. *Mol Breed* 12:231–244.
34. Narayanasamy, P. 2008**.** Molecular Biology in Plant Pathogenesis and Disease Management: Disease Management. Springer.
35. Norliza, A. B., Mohd, Z. S., Nor, M. J., Rafidah, B. and Johari, S. 2018. Induction of Systemic Acquired Resistance in Papaya by Foliar Application of HrpN Recombinant Protein for Increased Resistance against Papaya Dieback Pathogen. *Curr Inves Agri Curr Res* 2(3)- CIACR. MS.ID.000136. DOI: 10.32474/CIACR.2018.02.000136.
36. Ong, S. T., Ho, J. Z. S., Ho, B. and Ding, J. L. 2006. Iron-withholding strategy in innate immunity. *Immunobiology* 211:295–314**.**
37. Oswald, O., Martin, T., Dominy, P. J. and Graham, I. A. 2001. Plastid redox state and sugars: interactive regulators of nuclear-encoded photosynthetic gene expression. Proc Natl Acad SciUSA 98:2047–2052.
38. Pandey, S. S. 2023. The Role of Iron in Phytopathogenic Microbe–Plant Interactions: Insights into Virulence and Host Immune Response. Plants 12, 3173.
39. Qiu, D., Wei, Z.-W., Bauer, D.W. and Beer, S.V. 1997. Treatment of tomato seed with harpin enhances germination and growth and induces resistance to *Ralstonia solanacearum*. *Phytopathology* 87, S80.
40. Quoirin, M., Lepoivre, P. 1977. Etude de milieux adaptes aux cultures *in vitro* de Prunus. Acta Horticulturae.
41. Sahin, M., Misirli, A., Gokkur, S., Aksoy, D. and Ozaktan, H. 2020. Application of hybridization Breeding Technique for Fire Blight Resistance on Cydonia Oblonga: A Base Study on Susceptibility, Heterosis, and Heterobeltiosis Parameters. Int. J. Fruit Sci., **20:** 1458–S1469
42. Sairam, R. K. and Srivastava, G. C. 2001. Water stress tolerance of wheat *Triticum aestivum* L.: Variation in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotype. J. Agron. Crop Sci., **186:** 63-70.

538 **43.** Shetty, N. P., Jorgensen, H. J. L., Jensen, J. D., Collinge, D. B. and Shetty, H. S. 2008.
539 Roles of reactive oxygen species in interactions between plants and pathogens. *Eur. J.*
540 *Plant. Pathol*., **121:** 267–280.
541 44. Taheri Shahrestani, A., Abdollahi, H., Yakhchali, B., Mehrabi, R. and EiniGandomani,
542 O. 2020. Determination of the role of HrpN effector protein, as a key factor in course of
543 interaction between *Erwinia amylovora* with chloroplasts of pear (Pyrus communis L.).
544 *J. Plant. Pathol*., **102:** 1041–1050.
545 45. Taheri Shahrestani, A., Abdollahi, H., Yakhchali, B., Mehrabi, R. and EiniGandomani,
546 O. 2017. Comparison of the effects of *Erwinia amylovora* effector proteins on pear
547 cultivars in active and inactive chloroplastic electron transport chain conditions. *New.*
548 *Genetic*., **3:** 333-345.
549 46. Vanneste, J. L. (2000). Fire Blight: The Disease and its Causative Agent, *Erwinia*
550 *amylovora*. CABI Publishing, Wallingford, UK.
551 47. Venisse, J. S., Barny, M. A., Paulin, J. P. and Brisset, M. N. 2003. Involvement of three
552 pathogenicity factors of *Erwinia amylovora* in the oxidative stress associated with
553 compatible interaction in pear. *FEBS. Letters*., **537:** 198–202.
554 48. Venisse, J. S., Gullner, G. and Brisset, M. N. 2001. Evidence for the involvement of an
555 oxidative stress in the initiation of infection of pear by *Erwinia amylovora*. *Plant.*
556 *Physiology*., **125:** 2164–2172.
557 49. Wang, Y., Gi, D., Chen, T., Li, B., Zhang, Z., Qin, G. and Tian, S. 2019. Production,
558 Signaling, and Scavenging Mechanisms of Reactive Oxygen Species in Fruit–Pathogen
559 Interactions. Int. J. Mol. Sci., **20(12):** 2994.
560 50. Wei, Z. M., Laby, R.J., Zumoff, C. H., Bauer, D. W., He, S. Y., Collmer, A. and Beer,
561 SV. 1992. Harpin, elicitor of the hypersensitive response produced by the plant pathogen
562 *Erwinia amylovora*. *Science*., **257:** 85-88.
563 51. Xie, Z. and Chen, Z. 2000. Harpin-induced hypersensitive cell death is associated with
564 altered mitochondrial functions in tobacco cells. *Molecular. Plant-Microbe. Interactions*.,
565 **13:** 183–190.
566 52. Xi, L., Xu, K., Qiao, Y., Qu, S., Zhang, Z. and Dai, W. 2011. Differential expression of
567 ferritin genes in response to abiotic stresses and hormones in pear (Pyrus pyrifolia). *Mol.*
568 *Biol. Rep.*, **38 (7):** 4405-13.

53. Yabuta, Y., Mieda, T., Rapolu, M., Nakamura, A. and Motoki, T. 2007. Light regulation of ascorbate biosynthesis is dependent on the photosynthetic electron transport chain but independent of sugars in Arabidopsis. J. Exp. Bot., **58:** 2661–2671.
54. Yadav, K., Patel, P., Srivastava, A. K. and Ganapathi, TR. 2017. Overexpression of native ferritin gene *MusaFer1* enhances iron content and oxidative stress tolerance in transgenic banana plants. *PLoS. ONE*., **12(11):** e0188933.
55. Zang, X., Geng, X., Wang, F., Liu, Z., Zhang, L., Zhao, Y., Tian, X., Ni, Z., Yao, Y., Xin, M., Hu, Z., Sun, Q. and Peng, H. (2017). Overexpression of wheat ferritin gene TaFER-5B enhances tolerance to heat stress and other abiotic stresses associated with the ROS scavenging. *BMC Plant Biol.*, **17:** 1-13.
56. Zhao, Y., Blumer, S.E. and Sundin, G.W. 2005. Identification of *Erwinia amylovora* genes induced during infection of immature pear tissue. *J. acteriol.* **187:** 8088– 8103.

Table 1 Strains used in this work**.**

Designation	Mutated gene	Relevant characteristics
CFBP[a]7956	*hrp*N$^-$	Tn3-gus-km^R
CFBP7980	*hrp*W$^-$	Mvd 11734-km^R
CFBP7981	*dsp*A/E$^-$	*dsp*A/E 605: vidA-kan- Expressed a b,glucoruronidase fusion
ATCC[b] 49,946	Wild-type	Wild

[a] CIRM-CFBP: International Centre for Microbial Resources-French**.**
[b] American type culture collection.

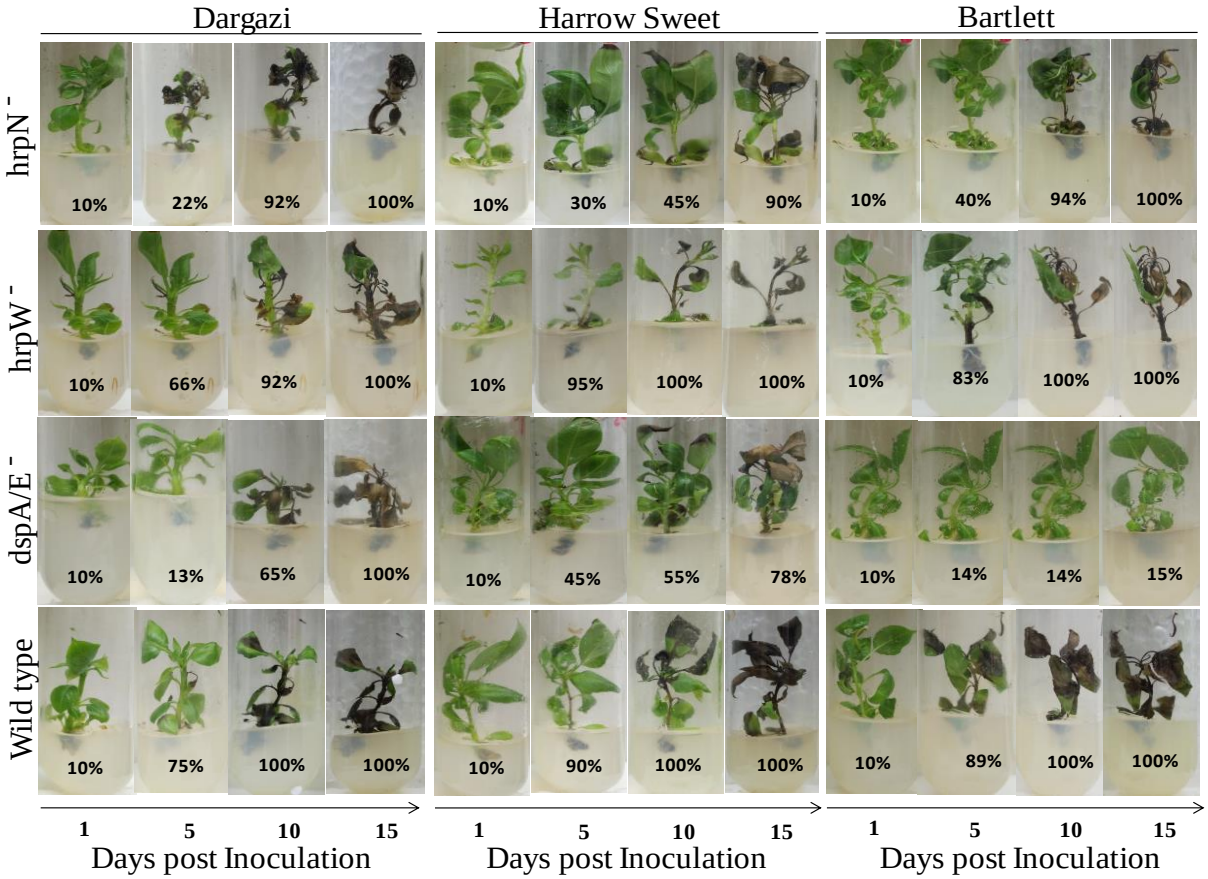

Fig. 1 Comparison of *in vitro* necrosis progression in Dargazi (resistant), Harrow sweet (tolerant), and Bartlett (susceptible) pear cultivars after inoculation with wild-type strain (a) and three mutants of *Erwinia amylovora* (*hrp*N$^-$, *hrp*W$^-$ and *dsp*A/E$^-$). The percentages expressed in the lower part of each cell represent the mean electrolyte leakage of the pear cultivars after inoculation with the wild-type strain of *Erwinia amylovora*.

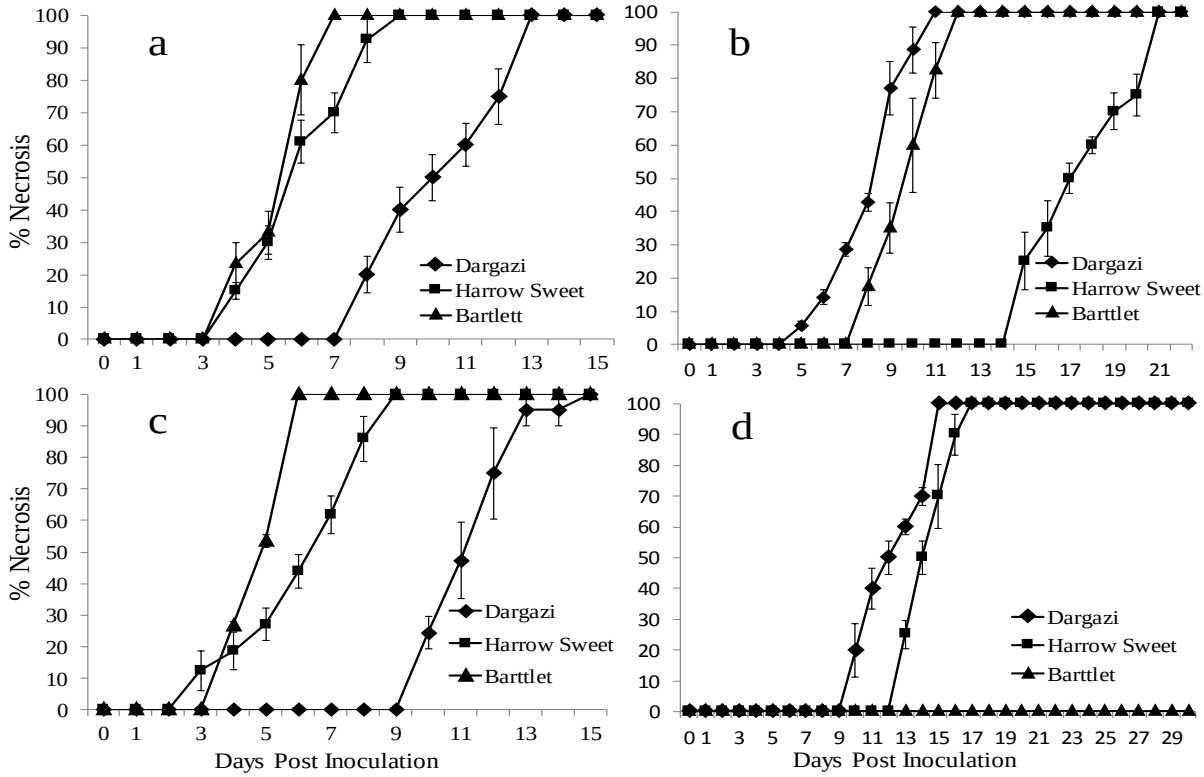

Fig.2 Comparison of necrosis development in Dargazi (resistant), Harrow sweet (tolerant), and Bartlett (susceptible) pear cultivars after inoculation with the wild-type strain (a) and three mutants of *Erwinia amylovora* including *hrp*N$^-$ (b), *hrp*W$^-$ (c), and *dsp*A/E$^-$ (d). The values are the mean of five replications and the bars are means ± standard errors.

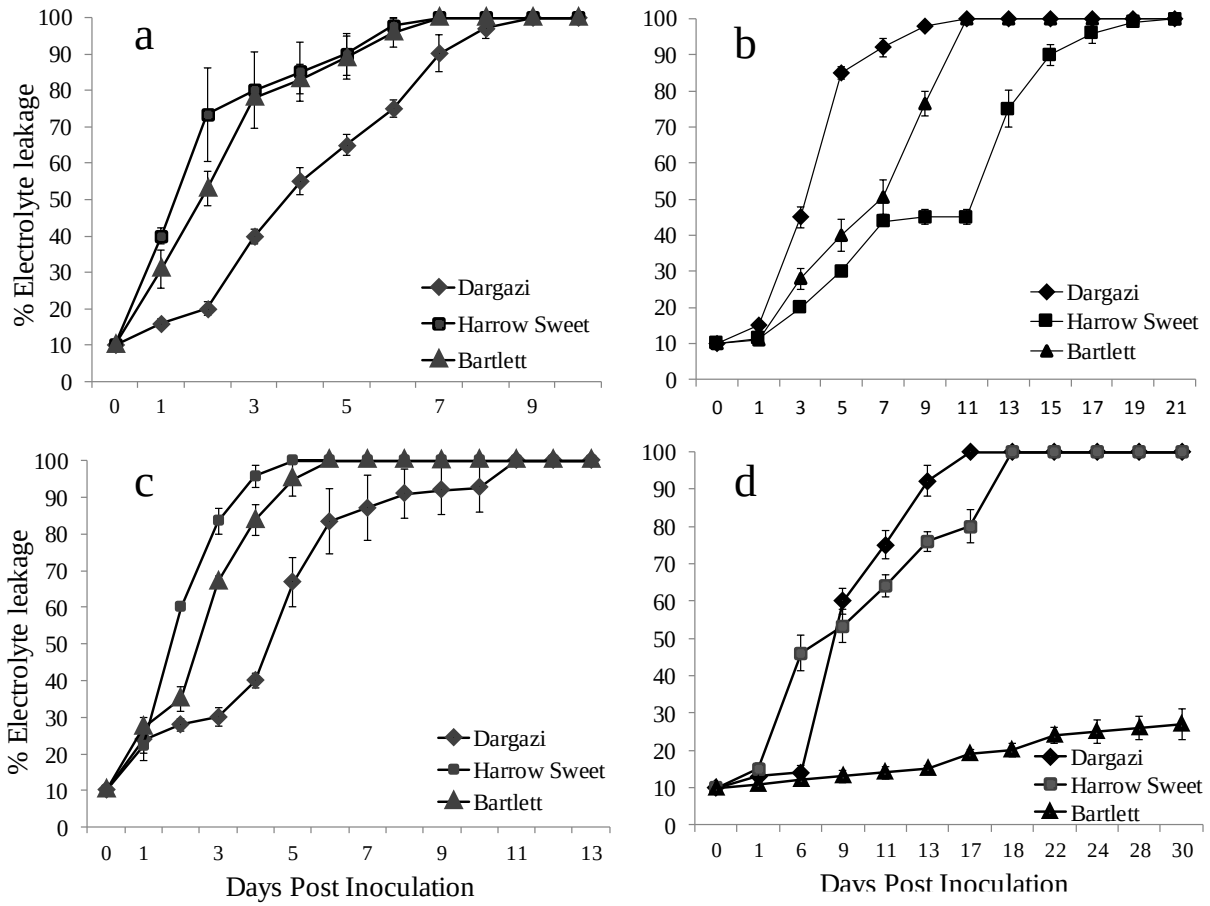

Fig. 3 Comparison of electrolyte leakage changes during post-inoculation of Dargazi (resistant), Harrow sweet (tolerant), and Bartlett (susceptible) pear cultivars with the wild-type strain (a) and three mutants of *Erwinia amylovora* including *hrp*N$^-$ (b), *hrp*W$^-$ (c), and *dsp*A/E$^-$ (d). The values are the mean of five replications and the bars are means ± standard errors.

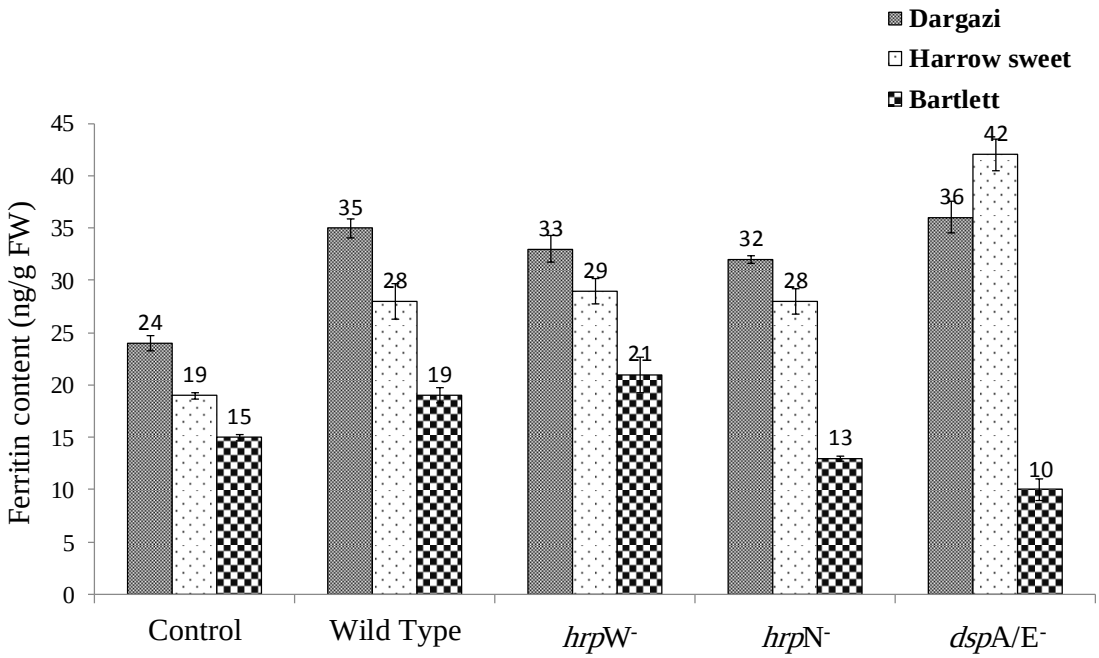

Fig. 4 Changes in ferritin contents in the Dargazi (resistant), Harrow sweet (tolerant), and Bartlett (susceptible) pear cultivars before inoculation and after 2 days post-inoculation with wild type and mutant strains of *Erwinia amylovora*. The values are mean of 3 replications and the bars are mean ± standard errors.

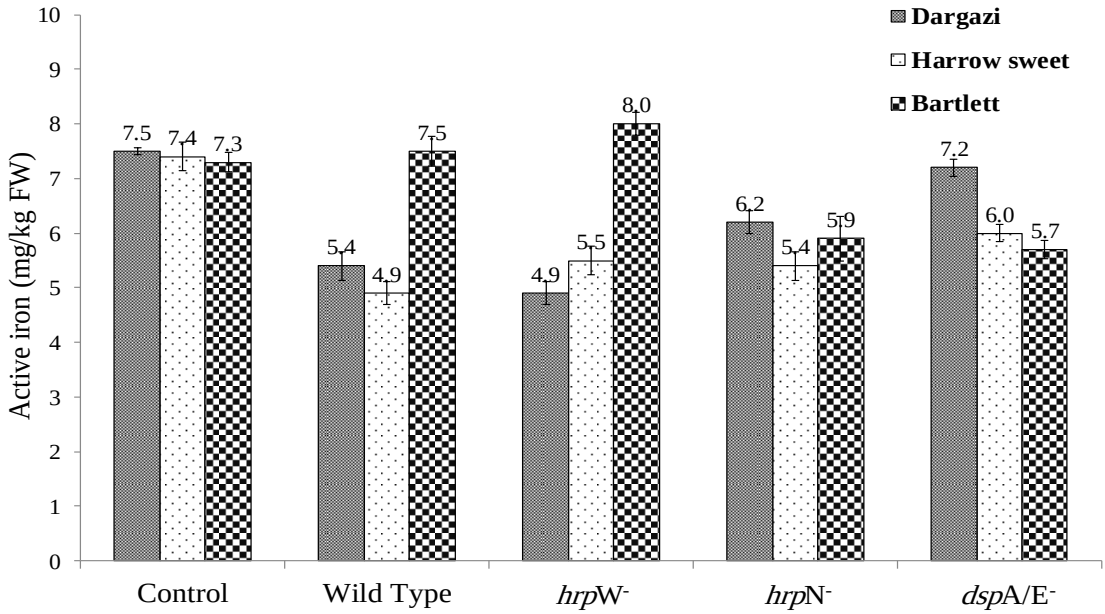

Fig. 5 Changes in Fe^{2+} contents in Dargazi (resistant), Harrow sweet (tolerant), and Bartlett (susceptible) pear cultivars before inoculation and after 2 days post-inoculation with wild type and mutant strains of *Erwinia amylovora*. The values are mean of 3 replications and the bars are means ± standard errors.

پروتئین های موثره باکتری Erwinia amylovora شامل-HrpN ، -HrpWو -DspA/E قبل از ایجاد ضایعه نکروز باعث تغییر محتوای آهن و فریتین در گلابی شدند

ر، مالکی. ح، عبداللهی. س، پیری و ک، پهلوان افشاری

چکیده

Erwinia amylovora دارای پروتئین‌های موثره HrpN،DspA/E و HrpW است که طی مراحل بیماری‌زایی از طریق مسیر ترشحی نوع 3 به داخل سلول های گیاهان میزبان ترشح می‌شوند. به منظور بررسی اثر متقابل این پروتئین های موثره با گیاهان میزبان، ارقام گلابی مقاوم (درگزی)، متحمل (هارو سویت) و حساس (بارتلت)، در شرایط درون شیشه ای با سویه های نوع وحشی و جهش یافته (hrpN- E. amylovora، dspA/E- و hrpW-) تلقیح شدند. بر اساس نتایج، احتمال تاثیر پروتئین HrpW در بیماریزایی رقم درگزی وجود دارد. سطوح مختلف بیماری زایی توسط پروتئین موثره DspA/E در ارقام گلابی مشاهده شد. نتایج نشان داد پروتئین موثره HrpN در سیستم دفاعی اکتسابی رقم مقاوم درگزی نقش کلیدی و در رقم هاروسوئیت نقش بیماریزایی دارد. علیرغم افزایش فریتین در تمامی ارقام گلابی پس از تلقیح با سویه نوع وحشی، ارقام مقاوم و متحمل گلابی سطوح فریتین بالاتری را نسبت به رقم حساس نشان دادند. همچنین کاهش Fe2+ تنها در رقم مقاوم و متحمل مشاهده شد داده های به دست آمده نشان می دهد که پروتئین HrpW تاثیری در تغییرات میزان آهن ندارد. تلقیح رقم درگزی و هاروسوئیت با همه سویه ها باعث افزایش فریتین و کاهش Fe2+ همراه بود. بر اساس نتایج، امکان ارتباط جداگانه هر یک از پروتئین های موثره با تغییرات فریتین و Fe2+ وجود ندارد. به طور کلی می توان نتیجه¬گیری کرد، توانایی رقم گلابی مقاوم در افزایش میزان فریتین و کنترل آهن می تواند یکی از دلایل مقاومت آن به بیماری آتشک باشد. با توجه به این یافته ها، مسیرهای مختلفی توسط ارقام گلابی برای پاسخ به عامل بیماری آتشک استفاده می شود.