J. Agr. Sci. Tech. (2023) Vol. 25(1): 185-198

Changes in Phenolic Acid Levels in Wheat Cultivars Inoculated with *Pyrenophora tritici-repentis* race 1

M. Ghorbi[1], H. Momeni[2*], V. Rashidi[1], A. Ahmadzadeh[3], and M. Yarnia[1]

ABSTRACT

Wheat is the main crop in the world. Tan spot caused by *Pyrenophora tritici-repentis* (*Ptr*) is a destructive disease in wheat-producing areas. Accumulation of phenolic acids at the onset of the fungal infection induces plant's resistance to tan spot. This study evaluated the effect of phenolic compound accumulation on the resistance to tan spot in wheat–pathogen interactions. Five different wheat cultivars including Glenlea, Salamouni, Moghan 3, Morvarid, and Bolani were studied at three different time points after inoculation with *Ptr*. The composition and concentration of phenolic acid including ferulic acid, *p*-coumaric acids, vanillic acid, chlorogenic acid, and rutin were detected using high-performance liquid chromatography and analyzed according to standard curves. Results showed considerable accumulation of ferulic acid, *p*-coumaric acids, vanillic acid, chlorogenic acid, and rutin in treatment with *Ptr* during 7 days post-inoculation in resistant and partially resistant cultivars compared with the susceptible ones. Ferulic acid was the most abundant phenolic compound in Salamouni (16.77±0.16 mg g^{-1} dw), Moghan 3 (17.76±0.00 mg g^{-1} dw), and Morvarid (23.11±0.00 mg g^{-1} dw) at 7 dpi. The obtained data indicated that the identified phenolic acids had enhanced and improved the wheat resistance to the fungal pathogen. Linear Pearson's coefficient analysis showed a positive correlation between some phenolic acids concentration and also between them and flavonoid rutin in wheat cultivars during infection. These findings highlighted the capacity of phenolic compounds as potential tools for the identification of resistance in wheat–pathogen interactions.

Keywords: Fungal infection, Resistance to tan spot, Tan spot disease, Wheat–pathogen interaction.

INTRODUCTION

Wheat is one of the main foods and major cereals consumed by people around the world (Van Hung *et al*., 2009). The importance of bran and germ in dietary fiber or phenolic acids are the health benefits of wheat. Phenolic acids play an important role in combating oxidative stress in the human body by maintaining a balance between oxidants and antioxidants (Temple, 2000).

Tan spot, caused by *Pyrenophora tritici-repentis* (Died.), is one of the major wheat diseases. This fungus, as a necrotrophic pathogen, is mainly known as a foliar pathogen that causes tan colored lesions often surrounded by a chlorotic halo, and associated as a seed-borne pathogen with infected grain, reported being a source of inoculum (Fernandez *et al*., 1994; Fernadez *et al*., 1997; Schilder and Bergstrom, 1994; Carmona *et al*., 2006). Based on the reaction of differential wheat hosts, eight races of this pathogen have been identified (Lamari

[1] Department of Agronomy and Plant Breeding, East Azarbaijan Science and Research Branch, Islamic Azad University, Tabriz, Islamic Republic of Iran.
[2]Department of Plant Disease, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization, Tehran, Islamic Republic of Iran.
* Corresponding author; e-mail: h.momeni@areeo.ac.ir
[3]Department of Agronomy and Plant Breeding, Islamic Azad University, Shabestar, Islamic Republic of Iran.

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

et al., 2003). According to Momeni *et al*. (2019) grouping of *Ptr* isolates was consistent with geographic origin regardless of race.

Phenolic compounds are secondary metabolites, synthesized during plant growth and in response to stress conditions (Brandolini *et al*., 2013). These compounds, found primarily in the wheat bran fraction, play an important role in plant growth and reproduction as they provide protection against pests and pathogens and contribute to the sensory properties of plant species. Phenolic compounds play a very effective role in the resistance of wheat cultivar Frontana to the fungal pathogen *Fusarium culmorum* (Siranidou *et al*., 2002). There is a relationship between increased resistance in plants and high content of phenolic compounds that play an important role in plant resistance to plant pathogens. The role of these compounds in plant resistance to fungal pathogens is prominent (Atak *et al*., 2021). Heim *et al*. (2002) reported that the beneficial effects of phenolic compounds have been attributed to their antioxidant activity. Antioxidants are molecules that can delay or prevent oxidative stress at low concentrations. The antioxidants of wheat are most concentrated in the bran fraction, and their amount depends on the type of grain. These compositions scavenge or neutralize free radicals and reduce oxidative damage to DNA, proteins and membrane lipids (Kim *et al*., 2006). Phenolic acids are both free and bound form in cereals (Li *et al*., 2008). Free phenolic compounds are found in the outer layers of the pericarp, but the bound ones are esterified at the cell wall and released from the cell matrix by acid or base hydrolysis (Mattila *et al*., 2005; Gani *et al*., 2012). The bound form is present mostly in wheat grains conjugated with other components such as saccharides starch, cellulose, β-glucan, and pentosane (Yu *et al*., 2001; Vaher *et al*., 2010). Phenolic acid is a derivative of benzoic acid and cinnamic acid and is found in all types of grains. They are divided into two main groups: hydroxycinnamic and hydroxybenzoic acids derivatives. Vanillic acid and salicylic acid are primarily hydroxybenzoic acid derivatives, while ferulic acid and coumaric acid are the most common derivatives of hydroxycinnamic acid (Abdel-Aal *et al*., 2001). Common phenolic acids in whole grains include ferulic acid, vanillic acid, caffeic acid, syringic acid, and coumaric acid (Sosulski *et al*., 1982; Liu, 2007). Ferulic acid is the main and most abundant phenolic acid found in wheat grains. Smaller concentrations of p-hydroxybenzoic, o-coumaric, *p*-coumaric, vanillic acid, syringic, salicylic and sinapic acids are also present in wheat (Moore *et al*., 2005; Liyana-Pathirana *et al*., 2006). Phenolic compounds have many functions. They act as stabilizers of the cell wall structure, but they may also be involved in the physical and chemical defense against various microorganisms, pests and insects. Furthermore, it is reported that they inhibit the biosynthesis of trichothecenes of *Fusarium* fungi, which are potent human toxins (Boutigny *et al*., 2009). In addition, phenolic acids formed in grain exhibit antifungal and antibacterial action (Cowan, 1999). According to the antioxidant activity of phenolic acids, the ability of these acids to bind to free radicals is determined as follows:

t-Cinnamic> Gallic> Caffeic> Benzoic> Sinapic> Syringic> Ferulic> *p*-Coumaric> Vanillic> Vanillin> Chlorogenic> 4-Hydroxybenzoic (Kinga Stuper-Szablewska and Perkowski, 2017). The presence of phenolic acids is of considerable importance in defense mechanisms during pathogenesis, through inhibition or activation of such enzymes as pectinases, plant amylase, phenoloxidase, succinate dehydrogenase, pancreatic RNase, and amino acid activating enzymes (Xu *et al*., 2014). Phenolic acids are potential alternatives to chemical pesticides used in agriculture. Natural origin substances that are effective fungicides used against *Fusarium oxysporum* include chlorogenic, ferulic and benzoic acids (Barkai-Golan, 2001). In the case of *Sclerotinia sclerotiorum* (causing sclerotinia rot), chlorogenic and ferulic acids effectively inhibit infection by this fungus (Martinez *et al*., 2011). Boutigny *et al*. (2008) observed the inhibitory effect of cinnamic, sinapic, caffeic, *p*-coumaric, chlorogenic and ferulic acids on the production

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

of type B trichothecenes in *F. graminearum* and *F. culmorum*

Pandelova *et al*. (2012) compared the effect of ToxA and ToxB of *P. tritici-repentis* in the accumulation of the phenylpropanoid compounds in wheat. They showed that the transcriptional level of the second enzyme of Cinnamate Hydroxylase (C4H) in the phenylpropanoid pathway leading to the formation of phenolic compounds was increased by up to 60-fold with ToxA treatment, whereas lower fold changes were up-regulated in ToxB-treated leaves. Microarray data showed that toxin sensitive wheat treated with either ToxA or ToxB show an up-regulation in mRNA transcripts of enzyme PAL and other enzymes associated with the phenylpropanoid pathway. These data suggest that the production of phenolic compounds is elicited by ToxA or ToxB (Pandelova *et al*., 2009; Pandelova *et al*., 2012). Moreover, Dorneles *et al*. (2018) studied the effect of phenylpropanoid metabolism on the resistance to tan spot and identified some phenolic compounds that accumulated in response to the fungi attack. As a result, they reported that phenolic compounds accumulation reduced tan spot colonization of wheat leaf tissues.

In this study, we aimed to investigate the possible relationship between phenolic acid compounds and disease resistance in five different wheat cultivars against *P. tritici-repentis* at three different time points. Phenolic acid profiles of both free and bound phenolic were to be analyzed. In addition, we planned to map the phenolic compounds and compare the potential capacity of wheat cultivars in producing phenolic compounds.

MATERIALS AND METHODS

Plant Material

Genotypes of wheat cultivars used in this study were chosen based on a previous study around resistance of 40 Iranian wheat cultivars to the pathogen *P. tritici-repentis* (Ghorbi *et al*., 2021). The plants grew in a growth chamber in a cycle of 16 hours (h) light at 22°C and 8 hours in the dark at 18°C, after which they were inoculated with conidia of the pathogen. Disease intensity were measured based on a numerical rating system of 1 to 5 that reflected the size and type of the lesions and firstly was recognized by Lamari and Brenier (1989). Based on the results of that study, a number of disease resistant (Salamouni, Moghan3 and Morvarid) and susceptible (Glenlea and Bolani) cultivars were selected for evaluations related to phenolic compounds in the present study.

Inoculation of P. tritici-repentis

Three sampling times were chosen in the first, third, and seventh days after inoculation. One- day post inoculation was considered to see plant reaction right after inoculation in term of phenolic acids concentrations, three-days post inoculation was to evaluate phenolic compound amounts in the mid-term of the incubation period, and seven-days post inoculation was the time needed for the appearance of disease symptoms in the inoculated plants. After each time point, plants were collected and dried for phenolic acids analysis. Seeds of each genotype were sown at a rate of six seeds per pot in 10-cm diameter plastic pots filled with soil. The seedlings were maintained in a growth cabinet at 20/18°C (day/night) with a 16 h photoperiod until they were inoculated at the three-leaf stage. IR 46 as an isolate of dominant race1 of *P. tritici-repentis* (Momeni *et al*., 2014), was used in the experiments to inoculate the wheat germplasms. Fungal growth and inoculum production were performed according to Momeni *et al*. (2014). Plants were inoculated by spraying a conidial suspension (3,000 conidia mL^{-1} and one drop of 100 mL Tween 20 suspension) onto leaves of 3-week old wheat plants as a fine mist using a hand-held manual sprayer until runoff. Immediately after inoculation, plants were transferred to a humid chamber

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

maintained at 25±2ºC, 90±5% relative humidity, and a 12 hour photoperiod. After 24 hours in the humid chamber, plants were transferred to the growth cabinet and monitored daily for symptom development. Tan spot reaction was assessed one, 3- and 7-days post inoculation.

Extraction of Free Phenolic Acids

Extraction of free phenolic acids was according to the method of Kim *et al*. (2006) with some modification. One gram powdered wheat sample was extracted twice with 80% methanol at a 10:1 ratio (v/w) for 50 minutes at room temperature and, then, centrifuged at 4,000 rpm for 15 minutes. The supernatants were transferred to pear shape flasks and rotary evaporated to dryness at 40ºC. The extracts were redissolved in 4 mL of HCl solution (pH 2.0) and extracted with 4 mL of ethyl acetate/ethyl ether (1:1, v/v) three times. The organic layers were combined and rotary evaporated to dryness at 40ºC. The solid extracts were reconstituted to 10 ml with methanol and frozen at −20ºC before further analysis.

Extraction of Bound Phenolic Acids

The residues (one gram) after methanol extraction were dispersed with 40 mL NaOH solution (2.0 mol L^{-1}) in a 150 mL conical flask. The flasks were purged with nitrogen to reduce the oxidation of phenolic acids. The mixture was hydrolyzed in a mechanical shaker for 4 hours at room temperature. The suspension was adjusted to pH 7.0 with 4.0 mol L^{-1} HCl and centrifuged at 4,000 rpm for 15 minutes. The supernatant was adjusted to pH 2.0 and re-extracted with ethyl acetate/ethyl ether (1:1, v/v). After evaporating ethyl acetate and ethyl ether, the extraction of phenolic compounds was re-constituted in 10 mL with methanol (Zhang *et al*., 2018).

HPLC Analysis

Waters liquid chromatography devices consisting of separation modules: Waters 2695 (USA) and PDA detector Waters 996 (USA) were used for HPLC analysis. Data acquisition and integration were done using Millennium32 software. The injection was Autosampler injector equipped. The chromatographic assay was performed on a 15 cm×4.6 mm with pre-column, Eurospher 100-5 C18 analytical column provided by waters (Sunfire) reversed phase matrix (3.5 µm) (Water) and elution was carried out in a gradient system with methanol as the organic phase (solvent A) and distilled water (solvent B) with the flow-rate of 1 mL min^{-1}. Peaks were monitored at 195-400 nm wavelength. The injection volume was 20 µL and the temperature was kept at 25°C. Phenolic acid standards of Ferulic, Vanillic, Coumaric, Chlorogenic and Flavonoid Rutin were purchased from Sigma-Aldrich®.

Statistical Analysis

All analyses consisted of three independent repeats. The analysis of variance and the Duncan test of mean comparison was done using the SAS software ver. 9.4. A value of $P \leq 0.05$ was considered significant. Moreover, correlation coefficients were computed among all phenolic compounds using SPSS ver. 22. To access the Principal Component Analysis (PCA) and Heatmap Graphpad Prism 9.0 software (GraphPad Software Inc., San Diego, USA) was used. The results of the experiments are expressed as mean±Standard Deviation (SD).

RESULTS

Five cultivars out of forty previously studied ones (Ghorbi *et al*., 2021) including Glenlea (as a susceptible standard), Salamouni (as a resistant standard), Bolani (susceptible), Morvarid 3 (semi-resistant)

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

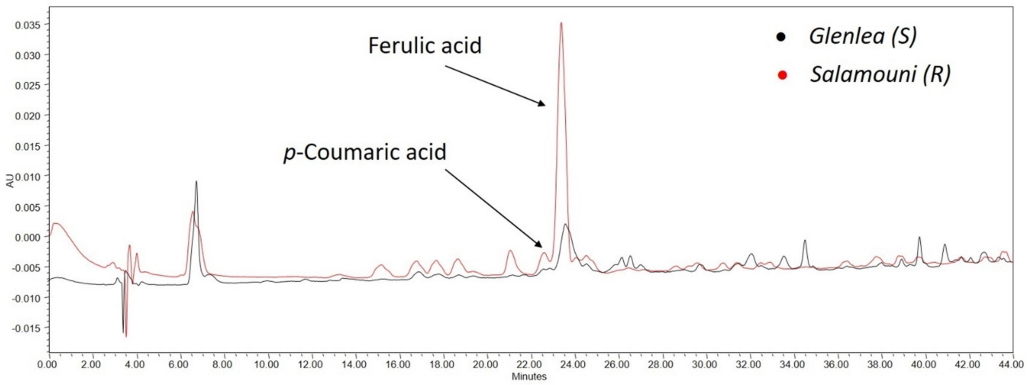
and Moghan (semi-resistant) were used in this study to evaluate the changes in phenolic compounds by HPLC.

HPLC Analysis of Extracts

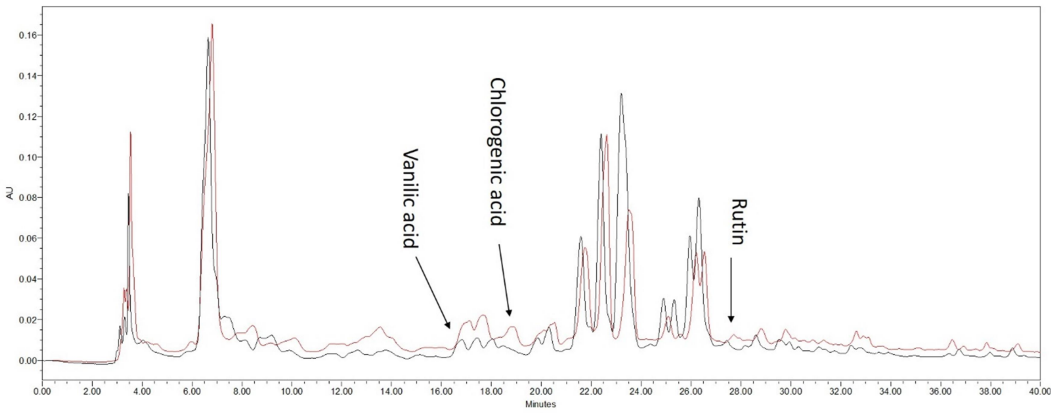
The phenolic profile of the free and bound phenolic was determined by HPLC (Figure 1). Both the free and bound fractions had different phenolic acid profiles, with the most common phenolic acids being found in bound form. (Figure 1-A). Phenolic acids such as chlorogenic and vanillic acid were detected in the free fraction (Figure 1-B). In addition, *p*-coumaric acid and ferulic acid were also found in the bound fraction. Phenolic acids in the bound fraction were hydroxycinnamic acids such as *p*-coumaric acid and ferulic acid. The highest bound ferulic acid content was 23.11±0.00 mg g^{-1} dry basis meanwhile p-coumaric acid level was 3.18±0.00 mg g^{-1} dry basis (Table 1).

Phenolic Composition and Concentration

Using HPLC analysis, Ferulic acid, Coumaric acid, Vanillic acid, and Chlorogenic acid were the four phenolic acids and Rutin as flavonoids were



(a)



(b)

Figure 1. High-performance liquid chromatography chromatogram of Phenolic acids in treated wheat varieties [Glenlea (S) and Salamouni (R)) by *P. tritici-repentis*. (A) Bound Phenolic Acids and (B) Free Phenolic Acids.

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

identified in five different wheat cultivars against *P. tritici-repentis* in three different time points (Table 1).

Although the accumulation of coumaric acid was observed in all cultivars one day post inoculation, but the highest values 0.93±0.00, 0.77±0.00, and 0.55±0.00 mg g^{-1} dw were detected at 7 days post inoculation (dpi) in Salamouni, Moghan 3 and Bolani, respectively. However, it decreased in Glenlea (0.20±0.00 mg g^{-1} dw) and Morvarid (1.41±0.00 mg g^{-1} dw). A strikingly strong increase occurred for Ferulic acid during 7 dpi in Salamouni (16.77±0.16 mg g^{-1} dw), Moghan 3 (17.76±0.00 mg g^{-1} dw) and Morvarid (23.11±0.00 mg g^{-1} dw), but in Glenlea (1.76±0.00 mg g^{-1} dw) and Bolani (3.31±0.00 mg g^{-1} dw), it was lower in comparison. Vanillic acid content showed a decline until 3 dpi, then after, it increased at 7 dpi in all cultivars except for Glenlea, which showed increasing patterns during time-courses. A significant increase for Chlorogenic acid occurred at 1, 3, and 7 dpi in Moghan 3 (1.22±0.00 mg g^{-1} dw), whereas, this compound showed decreasing patterns in all other cultivars, except for Salamouni at 3 dpi. Rutin showed a linear increment in content during the treatment period in Moghan 3, while its level was reduced at 3dpi in Salamouni and Morvarid, then increased at 7dpi.

Correlation

The relationship between phenolic acids and flavonoids accumulated in different wheat cultivars infected with *P. tritici-repentis* was investigated (Figure 2).

Data analysis of Glenlea showed a positive correlation between phenolic acids including ferulic acid, coumaric acid, and chlorogenic. A significant negative correlation was shown between ferulic acid, vanillic acid, and coumaric acid with the content of the flavonoid rutin, also between chlorogenic acid with vanillic acid, but no significant correlation was observed between vanillic and chlorogenic acids with rutin content. In Salamouni, our result indicated a positive correlation between ferulic acid, coumaric acid, vanillic acid and rutin, while a significant negative correlation was shown between ferulic acid, chlorogenic acid, vanillic acid, and coumaric acid. Moreover, data analysis of Moghan 3

Table 1. Phenolic acid compositions in wheat varieties at 1, 3 and 7 days after treated by *P. tritici-repentis*.[a]

Wheat cultivars	Time after treatment (Day)	Coumaric acid (mg g^{-1}dw)	Ferulic acid (mg g^{-1} dw)	Vanilic acid (mg g^{-1} dw)	Chlorogenic acid (mg g^{-1} dw)	Rutin (mg g^{-1} dw)
Glenlea	1	0.40 ±0.00 ^{efg}	3.90±0.00 ^{g}	0.15±0.00 ^{J}	0.05±0.00 ^{i}	0.023±0.00 ^{fg}
	3	0.15±0.00 ^{ghi}	1.50±0.00 ^{l}	0.27±0.00 ^{h}	0.00±0.00 ^{j}	0.026±0.00 ^{e}
	7	0.20±0.00 ^{fghi}	1.76±0.00 ^{k}	0.35±0.00 ^{f}	0.00±0.00 ^{j}	0.024±0.00 ^{f}
Salamouni	1	0.00±0.00 ^{i}	0.38±0.00 ^{n}	2.34±0.00 ^{b}	0.98±0.00 ^{c}	0.060±0.00 ^{b}
	3	0.06±0.00 ^{hi}	1.09±0.00 ^{m}	2.18±0.00 ^{c}	1.03±0.00 ^{b}	0.045±0.00 ^{c}
	7	0.93±0.00 ^{c}	16.77±0.16 ^{c}	2.76±0.02 ^{a}	0.87±0.00 ^{d}	0.075±0.00 ^{a}
Moghan 3	1	0.00±0.00 ^{i}	1.15±0.00 ^{m}	0.25±0.00 ^{i}	0.09±0.00 ^{g}	0.024±0.00 ^{f}
	3	0.01±0.00 ^{hi}	1.98±0.00 ^{j}	0.15±0.00 ^{j}	0.12±0.00 ^{f}	0.028±0.00 ^{e}
	7	0.77±0.00 ^{cd}	17.76±0.00 ^{b}	0.24±0.00 ^{i}	1.22±0.00 ^{a}	0.046±0.00 ^{c}
Morvarid	1	3.18±0.00 ^{a}	9.48±0.00 ^{d}	0.67±0.00 ^{d}	0.54±0.00 ^{e}	0.030±0.00 ^{d}
	3	0.52±0.58 ^{de}	7.47±0.00 ^{e}	0.00±0.00 ^{I}	0.00±0.00 ^{j}	0.021±0.00 ^{gh}
	7	1.41±0.00 ^{b}	23.11±0.00 ^{a}	0.08±0.00 ^{k}	0.00±0.00 ^{j}	0.024±0.00 ^{f}
Bolani	1	0.45±0.00 ^{ef}	4.72±0.00 ^{f}	0.40±0.00 ^{e}	0.06±0.00 ^{h}	0.018±0.00 ^{j}
	3	0.29±0.00^{efgh}	2.99±0.00 ^{i}	0.40±0.00 ^{e}	0.07±0.00 ^{h}	0.019±0.00 ^{ij}
	7	0.55±0.00 ^{de}	3.31±0.00^{h}	0.31±0.00 ^{g}	0.05±0.00 ^{i}	0.020±0.00 ^{hi}

[a] All data are presented as the mean±SD with n= 3. Means with different letters are significant at P≤ 0.05 as determined by the Duncan test.

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

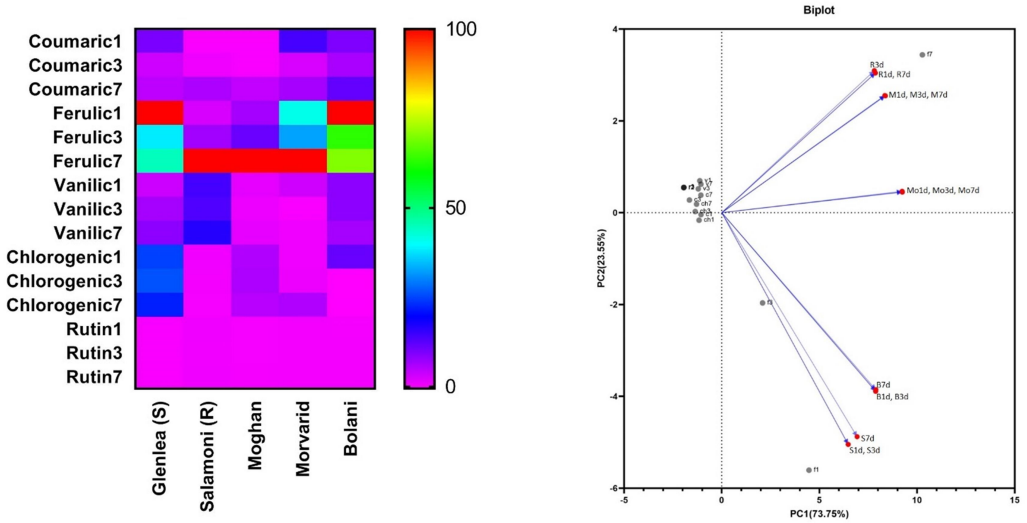


Figure 2. Dynamic changes of phenolic metabolites in wheat varieties (Glenlea, Salamouni, Moghan 3, Morvarid and, Bolani) during 1, 3, and 7 days post inoculation with *P.tritici-repentis* measured by high-performance liquid chromatography. (A) Analysis of five metabolites involved in a metabolic pathway is shown as a heatmap. The colors in boxes show the direction and magnitude of the correlations: Pale pink and red indicate strong negative and positive correlations, respectively. Each row of heatmap indicates an average of three biological replications for each time. (B) Biplots of principal component analysis using data during the 7 days of treated wheat varieties (f: Ferulic, v: Vanillic, ch: Chlorogenic, c: Coumaric and r: Rutin) and (S: Glenlea, R: Salamouni, M: Moghan 3, Mo: Morvarid and, B: Bolani).

declared a positive correlation between ferulic, coumaric and chlorogenic acids with the production of rutin, but no significant correlation was observed between ferulic acid, vanillic acid, coumaric acid, and also between rutin with the contents of chlorogenic acid and vanillic acids. About phenolic compound analysis of Morvarid, the result showed a positive correlation between coumaric, vanillic and chlorogenic acids with rutin content, but no significant correlation was observed between ferulic acid, coumaric acid, vanillic acid, chlorogenic acid and rutin production. In addition, about Bolani, our data indicated a positive correlation between vanillic and chlorogenic acids. In contrast, a significantly negative correlation was shown between coumaric acid and chlorogenic acid, also between vanillic and ferulic acids with rutin content, but no significant correlation was observed between ferulic, coumaric, vanillic, and chlorogenic acids. Moreover, a significant negative correlation ($p \leq 0.05$), between coumaric acid with vanillic acid and chlorogenic acid with rutin was observed (Table 2).

A subset of metabolites with PCA was created that accounted for 73.75 and 23.55% of the total variables within the data set (Figure 2-B). The heat-map analysis showed dynamic changes of phenolic compounds in pathogen-treated wheat cultivars. The results also showed a correlation between disease intensity (Ghorbi *et al.*, 2021) and production of phenolic compounds.

The overall results could indicate that the resistance of wheat to *P. tritici-repentis* is related to the production and accumulation of phenolic compounds (Figure 2-A).

DISCUSSION

This study was performed to investigate the changes in phenolic compounds in some wheat cultivars resistant or susceptible to *P.*

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

Table 2. Linear Pearson's coefficient correlations measured between phenolic acids and flavonoid production among five wheat cultivars in response to *P. tritici-repentis* infection during the 7 dpi.

	Ferulic acid	*p*-Coumaric acid	Vanillic acid	Chlorogenic acid	Rutin
Glenlea					
Ferulic acid	1				
p-coumaric acid	.995**	1			
Vanillic acid	-.872**	-.818**	1		
Chlorogenic acid	.862**	.806**	-1.000**	1	
Rutin	-.872**	-.917**	.520	-.503	1
Salamouni					
Ferulic acid	1				
p-coumaric acid	.998**	1			
Vanillic acid	.952**	.930**	1		
Chlorogenic acid	-.931**	-.906**	-.996**	1	
Rutin	.841**	.806**	.963**	-.975**	1
Moghan 3					
Ferulic acid	1				
p-coumaric acid	1.000**	1			
Vanillic acid	.373	.398	1		
Chlorogenic acid	1.000**	1.000**	.390	1	
Rutin	.993**	.989**	.262	.991**	1
Morvarid					
Ferulic acid	1				
p-coumaric acid	-.067	1			
Vanillic acid	-.207	.961**	1		
Chlorogenic acid	-.393	.917**	.981**	1	
Rutin	-.091	.970**	.993**	.951**	1
Bolani					
Ferulic acid	1				
p-coumaric acid	.300	1			
Vanillic acid	.346	-.792*	1		
Chlorogenic acid	.189	-.880**	.987**	1	
Rutin	-.834**	.277	-.806**	-.699*	1

** Correlation is significant at the 0.01 level and, * Correlation is significant at the 0.05 level.

tritici-repentis as a pathogenic fungus that is prevalent in the North and North-West of Iran. After inoculation with the pathogen, the trend of changes in phenolic compounds was evaluated at three time points to determine differences in the production of phenolic compounds in the plant.

Several factors are involved in resistance, among which phenolic acid compounds could play an important role. According to the results of this study, increase in phenolic compounds concentration after inoculation with *P. tritici-repentis* can provide the basis for plant resistance to the pathogen.

In addition to determining the phenolic compounds concentration, some correlations were also investigated to see the possible relationship between increases or decreases of compounds in different cultivars after inoculation with pathogen.

This study showed that accumulation of phenolic acids including ferulic acid, *p*-coumaric acids, vanillic acid, chlorogenic acid, and rutin changed in all wheat cultivars

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

(Glenlea, Salamouni, Moghan 3, Morvarid and Bolani) used in reaction against *P. tritici-repentis.* This accumulation was more in Salamouni (resistant), Moghan 3 (semi-resistant), and Morvarid (semi-resistant) compared with Glenlea and Bolani (susceptible). A considerable accumulation of ferulic, *p*-coumaric, vanillic, chlorogenic acids and rutin was observed in treatment with *P. tritici-repentis* during 7dpi in resistant and semi-resistant wheat cultivars compared with the susceptible ones. Phenolic compounds are strongly involved in plant-pathogen interactions, both contributing to physiological roles in the plant and being naturally toxic to pathogenic microorganisms (Mierziak *et al*., 2014). In consistence with the findings of the present study, wheat resistance to *P. tritici-repentis* was also associated with an increase in phenolic compounds, which promote cell wall strengthening, affecting both the infection and colonization processes (Mierziak *et al*., 2014), especially when compound accumulation occurs at the onset of the fungal infection (Fortunato *et al*., 2014). In the present study, a high level of ferulic acids and *p*-coumaric acids accumulation occurred at 7dpi in Salamouni (resistant), Moghan 3 (semi-resistant) and Morvarid (semi-resistant). It was previously reported that *p*-coumaric acids are used in lignin biosynthesis and cell wall strengthening (Miedes *et al*., 2014), which inhibited the growth of *P. tritici-repentis* (Lattanzio *et al*., 2006; Shalaby and Horwitz, 2015). In addition, ferulic acid showed fungal toxic activity against *P. triticirepentis* in in vitro studies, affecting conidia germination and/or hyphal development. The observed fungal toxic effects included swelling of the fungal tip, hyphal and hyphal granulation, and hyphal hyper-branching (Dorneles *et al*., 2018). These changes are potentially a result of the delayed penetration of *P. tritici-repentis* into the epidermal plant cell, which is its main route of infection (Larez *et al*., 1986). In agreement with these studies, the result of the present findings showed that cultivars with a high amount of ferulic acids and *p*-coumaric acids are more resistant than others against *P. tritici-repentis*.

It was demonstrated that rutin has mediated priming in plants in response to pathogen attacks (Yang *et al*., 2016; Nazari *et al*., 2017). In the present study, the amount of rutin showed a significantly increasing pattern in Moghan 3. Furthermore, Dorneles *et al*. (2018) showed that this compound induced hyper-branching, fungal tip swelling, and increased granulation of germ tubes and hyphae in *P. tritici-repentis* (Dorneles *et al*., 2018), which can delay penetration (Larez *et al*., 1986). On the basis of this knowledge, a high accumulation of rutin occurred at 7 dpi in the resistant and semi-resistant cultivars, but was not observed in the susceptible ones (Figure 3).

Moreover, our findings in the present study i.e. the amount of vanillic acid in Salamouni, Moghan 3 and Morvarid at 7 dpi showed a significant increase, is in agreement with Dorneles *et al.* (2018), who reported Hydroxybenzoic acid such as vanillic acid completely inhibited mycelial growth and conidia germination of *P. tritici-repentis*. The present result was fluctuated among susceptible ones. (Figure 3). Barkai-Golan *et al*. (2001) showed that natural substances such as chlorogenic had fungicidal effects. Moreover, chlorogenic effectively inhibits infection of *Sclerotinia sclerotiorum* fungus (Martinez *et al*., 2011). According to this knowledge, the result of the present study showed high accumulation of chlorogenic in Moghan3 only and did not occur in the other cultivars (Figure 3). The increase of this compound in Moghan 3 can be one of the reasons for its semi resistant reaction against *P. tritici-repentis.*

Kinga Stuper-Szablewska and Perkowski (2017) reported that the antioxidant activity capacity of phenolic acids to bind free radicals was found to be as Ferulic> *p*-Coumaric> Vanillic> Chlorogenic. Furthermore, ferulic acid is the primary and the most abundant phenolic acid in wheat grains while smaller concentrations of p-

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

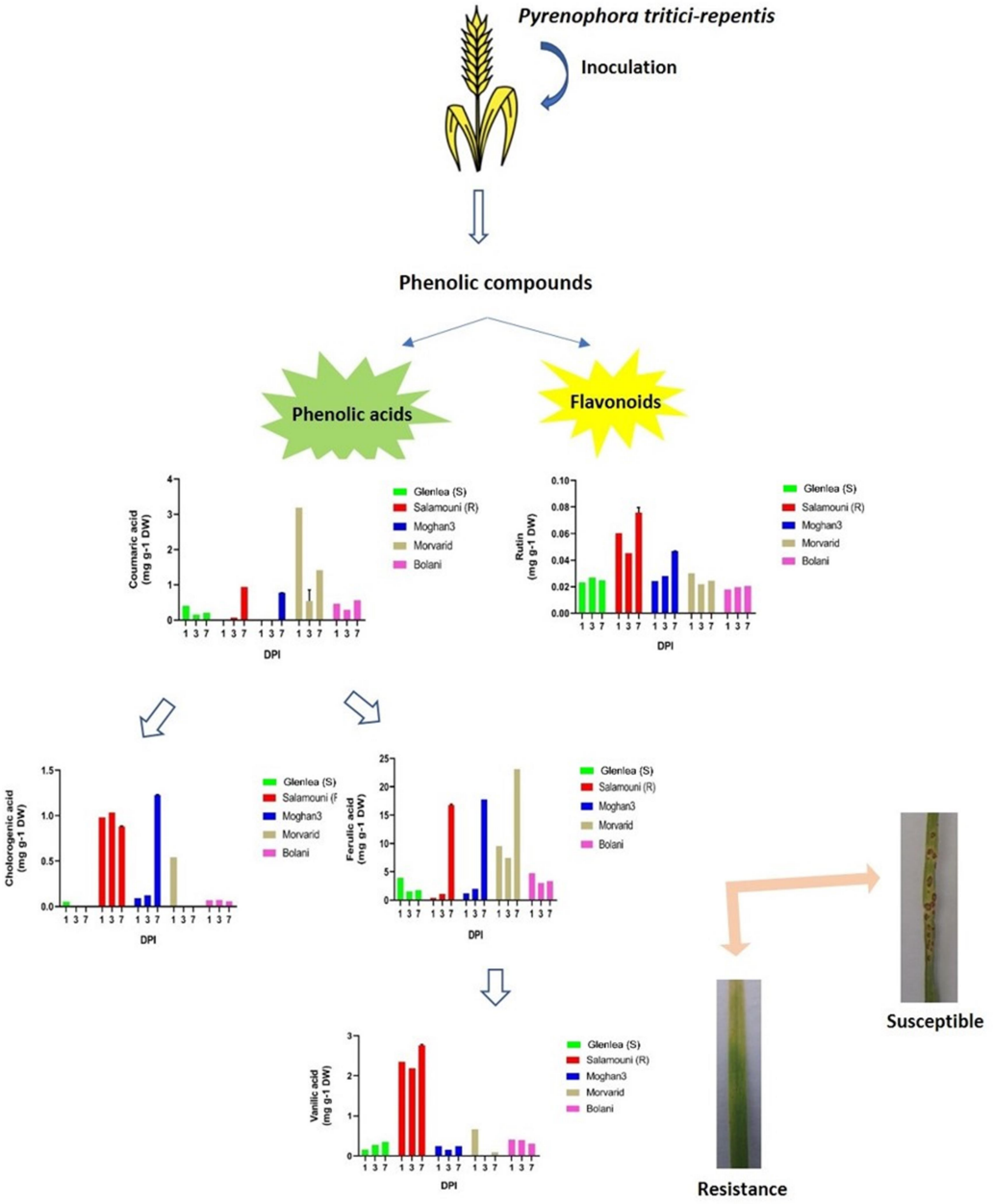


Figure 3. Phenolic biosynthetic pathway and cross-talk among various kinds of phenolics. The presented work suggested a correlation among the co-induction of phenolic acids (ferulic, vanillic, coumaric, and chlorogenic acids), and flavonoid (rutin), in five wheat cultivars used during the time courses after inoculation with *P. tritici-repentis* which have affected in resistance.

hydroxybenzoic, *p*-coumaric, vanillic and chlorogenic acids are present in wheat (Moore *et al*., 2005; Liyana-Pathirana *et al*., 2006). All of this knowledge is in agreement with the present data highlighted that ferulic acid content increased in all wheat cultivars compared to other phenolic acids. (Table 1). This finding can be useful in the identification of resistant wheat cultivars.

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

A direct correlation between plant resistance and phenolic compounds accumulation was previously reported (Miedes *et al*., 2014). Moreover, Dorneles *et al*. (2018) reported that phenolic compounds individually and/or in combination contributed to the reduction of tan spot colonization of wheat leaf tissues. The present work suggested a correlation among the co-induction of phenolic acids (ferulic, vanillic, coumaric, and chlorogenic acids), and flavonoid (rutin) in five wheat cultivars used during the time courses after inoculation with *P. tritici-repentis*, resistance (Figure 3).

Thus, taking all this data together showed that phenolic acids could be used as biomarkers to understand the interaction of wheat and *P. tritici-repentis* model system in different wheat cultivars including Glenlea, Salamouni, Moghan 3, Morvarid and Bolani. Although so much is known about the roles of phenolic acids in plant immunity interaction with a fungal infection, the knowledge is still incomplete and needs to be expanded. Therefore, similar pattern in the accumulation of phenolic acids among resistant plants might be the reason for the existence of some possible relations between them; but this needs more investigations. The results of our investigation could provide the fields to use phenolic acids as potential tools for identification of resistance in wheat–pathogen interactions.

ACKNOWLEDGEMENTS

The authors would like to thank Shirin Goudarzi (Instrumental Analysis Laboratory, Plants and Medicinal Materials Research Institute, Shahid Beheshti University, Iran) who helped us to establish the HPLC methods.

REFERENCES

1. Abdel-Aal, E. S. M., Hucl, P., Sosulski, F. W., Graf, R., Gillott, C. and Pietrzak, L. 2001. Screening Spring Wheat for Midge Resistance in Relation to Ferulic Acid Content. *J. Agric. Food Chem*., **49:** 3559–3566.
2. Atak, A., Göksel, Z. and Yılmaz, Y. 2021. Changes in Major Phenolic Compounds of Seeds, Skins, and Pulps from Various *Vitis* spp. and the Effect of Powdery and Downy Mildew Diseases on Their Levels in Grape Leaves. *Plants*, **10:** 2554.
3. Barkai-Golan, R. 2001. Postharvest Diseases of Fruits and Vegetables. *Dev. Control. Elsevier*, **6:** 418-423.
4. Boutigny, A. L., Richard-Forget, F. and Barreau, C., 2008. Natural Mechanisms for Cereal Resistance to the Accumulation of *Fusarium trichothecenes*. *Eur. J. Plant Pathol.*, **121**: 411–423.
5. Boutigny, A. L., Barreau, C., Atanasova-Penichon, V., Verdal-Bonnin, M. N., Pinson-Gadais, L. and Richard-Forget, F. 2009. Ferulic Acid, an Efficient Inhibitor of Type B Trichothecene Biosynthesis and Tri Gene Expression in Fusarium Liquid Cultures. *Mycol. Res.,* **113:** 746–753.
6. Brandolini, A., Castoldi, P., Plizzari, L. and Hidalgo, A. 2013. Phenolic Acids Composition, Total Polyphenols Content and Antioxidant Activity of *Triticum monococcum*, *Triticum turgidum* and *Triticum aestivum*: A Two-Years Evaluation. *J. Cereal Sci.,* **58:** 123–131.
7. Carmona, M., Ferrazini, M. and Barreto, D. E. 2006. Tan Spot of Wheat Caused by *Drechslera tritici-repentis*: Detection, Transmission, and Control in Wheat Seed. *Cereal Res. Commun.,* **34:** 1043–1049.
8. Cowan, M. M. 1999. Plant Products as Antimicrobial Agents. *Clin. Microbiol. Rev.,* **12:** 564–582.
9. Dorneles, K. R., Dallagnol, L. J., Pazdiora, P. C., Hoffmann, J. F., Chaves, F. C., Monte, L. G. and Rodrigues, F. A. 2018. Wheat Leaf Resistance to *Pyrenophora tritici-repentis* Induced by Silicon Activation of Phenylpropanoid Metabolism. *Plant Patho.,* **67:** 1713-1724.

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

10. Fernandez, M. R., Clarke, J. M., Depauw, R., Irvine, R. B. and Knox, R. 1994. Black Point and Red Smudge in Irrigated Durum Wheat in Southern Saskatchewan in 1990–1992. *Can. J. Plant Pathol.,* **16:** 221–227.
11. Fernadez, M. R., Clarke, J. M., de Pauw, R. M., Lefkovitch, L. P., 1997. Emergence and Growth of Durum Wheat Derived from Red Smudge-Infected Seed. *Crop Sci.,* **37**: 510–514.
12. Fortunato, A. A., Da Silva, W. L., Rodrigues, F. A., 2014. Phenylpropanoid Pathway is potentiated by Silicon in the Roots of Banana Plants during the Infection Process of *Fusarium oxysporum* f. sp *cubense*. *Phytopathol.,* **104:** 597-603.
13. Gani, A., Wani, S. M., Masoodi, F. A., Hameed, G., 2012. Whole-Grain Cereal Bioactive Compounds and Their Health Benefits: A Review. *Int. J. Food Process. Technol.,* **3:** 1–10.
14. Ghorbi, M., Momeni, H., Rashidi, V., Ahmadzadeh, A. and Yarnia, M. 2021. Resistance of Some Wheat Cultivars to the Main Race of Tan Spot Disease in Ardabil Province. *J. Appl. Res. Plant Prot.,* **11(2):** 1-16. (in Persian)
15. Heim, K. E., Tagliaferro, A. R. and Bobilya, D. J. 2002. Flavonoid Antioxidants: Chemistry, Metabolism and Structure–Activity Relationships. *J. Nutr. Biochem.,* **13:** 572–584.
16. Kim, K. H., Tsao, R., Yang, R. and Cui, S. W. 2006. Phenolic Acid Profiles and Antioxidant Activities of Wheat Bran Extracts and the Effect of Hydrolysis Conditions. *Food Chem.,* **95:** 466–473.
17. Lamari, L. and Bernier, C. C., 1989. Evaluation of Wheat Lines and Cultivars to Tan Spot [*Pyrenophora tritici-repentis*] Based on Lesion Type. *Can. J. Plant Pathol.,* **11:** 49- 56.
18. Lamari, L., Strelkov, S. E., Yahyaoui, A., Orabi, J. and Smith, R. B. 2003. The Identification of Two New Races of *Pyrenophora tritici-repentis* from the Host Centre of Diversity Confirms a One to One Relationship in Tan Spot of Wheat. *Phytopathol.,* **93:** 391-396.
19. Larez, C. R., Hosford, R. M. and Freeman, T. P. 1986. Infection of Wheat and Oats by *Pyrenophora tritici-repentis* and Initial Characterization of Resistance. *Phytopathol.,* **76:** 931-938.
20. Lattanzio, V., Lattanzio, V. M. T. and Cardinali, A. 2006. Role of Phenolics in the Resistance Mechanisms of Plants against Fungal Pathogens and Insects. *Phytochem.,* **37:** 23-67.
21. Li, L., Shewry, R. and Ward, J. L. 2008. Phenolic Acids in Wheat Varieties in the Health Grain Diversity Screen. *J. Agric. Food Chem.,* **56:** 9732–9739.
22. Liu, R. H. 2007. Whole Grain Phytochemicals and Health. *J. Cereal Sci.,* **46:** 207–219.
23. Liyana-Pathirana, C., Dexter, J. and Shahidi, F. 2006. Antioxidant Properties of Wheat as Affected by Pearling. *J. Agric. Food Chem.,* **54:** 6177–6184.
24. Martínez, J. A., Valdés, R., Gómez-Bellot, M. J. and Bañón, S. 2011. Effects of Indole- 3-Acetic Acid on *Botrytis cinerea* Isolates Obtained from Potted Plants. *63rd Inter. Sym. Crop Prot.,* (ISCP 2011), Ghent, Belgium.
25. Mattila, P., Pihlava, J. M. and Hellstrom, J. 2005. Contents of Phenolic Acids, Alkyl- and Alkenylresorcinols, and Avenanthramides in Commercial Grain Products. *J. Agric. Food Chem.,* **53:** 8290–8295.
26. Mierziak, J., Kostyn, K. and Kulma, A. 2014. Flavonoids as Important Molecules of Plant Interactions with the Environment. *Molecules*, **19**: 16240-16265.
27. Miedes, E., Vanholme, R., Boerjan, W. and Molina, A. 2014. The Role of the Secondary Cell Way in Plant Resistance to Pathogens. *Front. Plant Sci.,* **5:** 1-12.
28. Momeni, H., Aboukhaddour, R., Javan-Nikkhah, M., Razavi, M., Naghavi, M. R., Akhavan, A. and Strelkov, S. E. 2014. Race Identification of *Pyrenophora tritici-repentis* in Iran. *J. Plant Pathol.,* **96:** 287-294.
29. Momeni, H., Akhavan, A., Aboukhaddour, R., Javan-Nikkhah, M., Razavi, M., Naghavi, M. R. and Strelkov,

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

S. E. 2019. Simple Sequence Repeat Marker Analysis Reveals Grouping of *Pyrenophora tritici-repentis* Isolates Based on Geographic Origin, *Can. J. Plant Pathol.,* **41:** 218-227.

30. Moore, J., Hao, Z., Zhou, K., Luther, M., Costa, J. and Yu, L. L. 2005. Carotenoid, Tocopherol, Phenolic Acid, and Antioxidant Properties of Maryland-Grown Soft Wheat. *J. Agric. Food Chem.,* **53**: 6649–6657.
31. Nazari, F., Safaie, N., Soltani, B. M., Shams–Bakhsh, M. and Sharifi, M. 2017. *Bacillus subtilis* Affects miRNAs and Flavanoids Production in *Agrobacterium*–Tobacco Interaction. *Plant Physiol. Biochem.,* **118:** 98–106.
32. Pandelova, I, Betts, M. F., Manning, V. A., Wilhelm, L. J., Mockler, T. C., Ciuffetti, L. M., 2009. Analysis of Transcriptome Changes Induced by Ptr ToxA in Wheat Provides Insights into the Mechanisms of Plant Susceptibility. *Mol. Plant.,* **2**: 1067–1083.
33. Pandelova, I., Figueroa, M., Wilhelm, L. J., Manning, V. A., Mankaney, A. N., Mockler, T. C., and Ciuffetti, L. M. 2012. Host-Selective Toxins of *Pyrenophora tritici-repentis* Induce Common Responses Associated with Host Susceptibility. *PLoS One*. 7: e40240.
34. Schilder, A. M. C. and Bergstrom, G. C. 1994. *Pyrenophora-tritici-repentis* as a Component of the Fungal Flora of Winter-Wheat Seed in New-York. *Seed Sci. Technol.,* **22**: 285–297.
35. Shalaby, S. and Horwitz, B. A. 2015. Plant Phenolic Compounds and Oxidative Stress: Integrated Signals in Fungal–Plant Interactions. *Curr. Genet.,* **61:** 347-357.
36. Siranidou, E., Kang, Z. and Buchenauer, H. 2002. Studies on Symptom Development, Phenolic Compounds and Morphological Defense Responses in Wheat Cultivars Differing in Resistance to Fusarium Head Blight. *J. Phytopathol.,* **150:** 200-208.
37. Sosulski, F., Krygier, K. and Hogge, L. 1982. Free, Esterified, and Insoluble-Bound Phenolic Acids. Composition of Phenolic Acids in Cereal and Potato Flours. *J. Agric. Food Chem*., **30:** 337–340.
38. Stuper-Szablewska, K. and Perkowski, J., 2017. Phenolic Acids in Cereal Grain: Occurrence, Biosynthesis, Metabolism and Role in Living Organisms, *Crit. Rev. Food Sci. Nutr.,* **59(4):** 664-675.
1. 35. Temple, N. J. 2000. Antioxidants and Disease: More Questions than Answers. *Nutr. Res. Rev.,* **20:** 449-459.
39. Vaher, M., Matso, K., Levandi, T., Helmja, K. and Kaljurand, M. 2010. Phenolic Compounds and the Antioxidant Activity of the Bran, Flour and Whole Grain of Different Wheat Varieties. *Procedia Chem.,* **2:** 76–82.
40. Van Hung, P., Maeda, T., Miyatake, K. and Morita, N. 2009. Total Phenolic Compounds and Antioxidant Capacity of Wheat Graded Flours by Polishing Method. *Int. Food Res.,* **42:** 185–190.
2. 38. Xu, T. F., Zhao, X. C., Jiao, Y. T., Wei, J. Y., Wang, L. and Xu, Y. 2014. A Pathogenesis Related Protein, VpPR-10.1, from *Vitis pseudoreticulata*: An Insight of Its Mode of Antifungal Activity. *PLoS One.* 9: 95102.
41. Yang, W., Xu, X., Li, Y., Wang, Y., Li, M., Wang, Y., Ding, X., and Chu, Z. 2016. Rutin-Mediated Priming of Plant Resistance to Three Bacterial Pathogens Initiating the Early SA Signal Pathway. *PloS One*, **11:** 1-15.
42. Yu, V., Vasanthan, T. and Temelli, F. 2001. Analysis of Phenolic Acids in Barley by High-Performance Liquid Chromatography. *J. Agric. Food Chem.,* **49**: 4352–4358.
43. Zhang, J., Ding, Y., Dong, H., Hou, H. and Zhang, X. 2018. Distribution of Phenolic Acids and Antioxidant Activities of Different Bran Fractions from Three Pigmented Wheat Varieties. *J. Chem.,* 9 pages.

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]

تغییرات سطح اسید فنولیک در ارقام گندم تلقیح شده توسط نژاد یک قارچ *Pyrenophora tritici-repentis*

م. قربی، ح. مومنی، و. رشیدی، ع. احمدزاده، و م. یارنیا

چکیده

گندم یکی از محصولات مهم زراعی در دنیا می باشد. لکه خرمایی ناشی از قارچ بیماریزای *Pyrenophora tritici-repentis*یک بیماری مخرب در مناطق تولید گندم است. تجمع اسیدهای فنولیک در شروع عفونت قارچی باعث ایجاد مقاومت گیاه در برابر لکه خرمایی می شود. این مطالعه اثر تجمع ترکیبات فنلی را بر مقاومت به بیماری لکه خرمایی در برهمکنش‌های گندم -عامل بیماری‌زا مورد ارزیابی قرار داد. پنج رقم مختلف گندم شامل Glenlea، Salamouni، مغان ۳، مروارید و بولانی در سه فاصله زمانی مختلف پس از مایه زنی با قارچ مورد مطالعه قرار گرفتند. ترکیب و غلظت اسید فنولیک شامل اسید فرولیک، اسیدهای p-کوماریک، اسید وانیلیک، اسید کلروژنیک و روتین با استفاده از کروماتوگرافی مایع با کارایی بالا شناسایی و بر اساس منحنی‌های استاندارد آنالیز شد. نتایج نشان دهنده تجمع قابل توجهی از اسید فرولیک، اسیدهای p-کوماریک، اسید وانیلیک، اسید کلروژنیک و روتین در تیمار با قارچ طی ۷ روز پس از مایه زنی در ارقام مقاوم و نیمه مقاوم در مقایسه با ارقام حساس بود. اسید فرولیک فراوان ترین ترکیب فنلی در Salamouni (16.77±0.16 mg g−1dw)، مغان ۳ (17.76±0.00 mg g−1dw) و مروارید (23.11±0.00 mg g−1dw) در ۷ روز پس از مایه زنی بود. داده‌های به‌دست‌آمده نشان می‌دهد که اسیدهای فنولیک شناسایی شده، مقاومت گندم را در برابر پاتوژن قارچی افزایش داده و بهبود بخشیده است. تجزیه و تحلیل ضریب پیرسون خطی نشان داد که بین غلظت برخی از اسیدهای فنولیک و همچنین بین آنها و روتین فلاونوئیدی در ارقام گندم در طی آلودگی، همبستگی مثبت وجود دارد. این یافته‌ها ظرفیت ترکیبات فنلی را به عنوان ابزار بالقوه برای شناسایی مقاومت در تعاملات گندم- بیمارگر نشان می دهد.

[Downloaded from jast.modares.ac.ir on 2024-04-18]

[DOI: 10.52547/jast.25.1.185]