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ORIGINAL ARTICLE

Comparative evaluation of free and bound phenolic acid contents in early grains of durum wheat line for its resistance to fusarium head blight with some other sensitive varieties in Algeria

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Abstract

All plants contain varying levels of phenolic acids (metabolites) thus playing an important role in resistance mechanisms as constituents of cell walls, as constitutive antimicrobial compounds of plants or induced in response to infection against many diseases, in particular fusarium head blight caused by *Fusarium* species. To this end, the objective of this research was to study the variation in phenolic acid composition during the kinetics of filling wheat grains, in order to determine the best variety resistant to fusarium head blight. For this purpose, free and bound phenolic analyses were carried out by HPLC-DAD on five durum wheat varieties at the stage 5 to 8 days after the flowering stage (early grains). We showed that at the level of the samples analyzed, several phenolic acids were present at different concentrations, but others were absent [*cis*-ferulic acid (free phenolic acid), and sinapic acid (bound phenolic acid)]. The results also showed that the content of bound phenolic acids was much higher than that of free phenolic acids in all varieties. In addition, these phenolic acids existed in free soluble form or were mostly present in insoluble form bound to cell walls. For free acids, the results showed that significant amounts of *trans*-ferulic acid were detected in comparison to all free phenolic acids (56.72 μg · g^{-1} DM for G10). For bound acids, ferulic acid is the main bound phenolic acid which has much higher levels (4913.92 μg · g^{-1} DM for G1), followed by *p*-coumaric acid (3098.99 μg · g^{-1} DM for G1). Moreover, the sum of monomers (bound acids) was much higher than that of dehydrodiferulic acids (DiFA).

Keywords: bound phenolic acids, durum wheat varieties, flowering stage, free phenolic acids

Introduction

Worldwide, fusarium head blight (FHB) is a destructive cereal disease which primarly affects wheat and barley (Bai and Shaner 2004; Osborne and Stein 2007; Valverde-Bogantes *et al.* 2020). In Europe, this disease is considered to be one of the major diseases of cereals, and is mainly caused by several fungi of *Fusarium* genus. Particularly, *Fusarium* species pose a serious threat to food security due to their ability to produce a wide range of mycotoxins, including type B trichothecenes (Gauthier *et al.* 2015; Atanasova-Penichon *et al.* 2016; Gauthier *et al.* 2016). In Algeria, a preliminary study on the situation of fusarium head blight in wheat was carried out to identify the main species associated with this disease and also to show its consequences in the agro-ecological zones of durum wheat cultivation (Abdallah-Nekache *et al.* 2019). The severity of fusarium head blight in a given area is determined by environmental factors during critical phases of the disease's development (Kriss *et al.* 2010). In particular, ear infection develops e.g., when flowers open (flowering

stage), and can be aided by excessive humidity or humid conditions combined with warm temperatures (Shah *et al.* 2018; Lozowicka *et al.* 2022). Faced with this health situation, it is critical to implement effective and long-term management techniques to lower the disease's health risk (Atanasova-Penichon *et al.* 2016). The creation of varieties resistant to fusarium head blight continues to be the most efficient method of control (Gervais *et al.* 2003; Dhokane *et al.* 2016; Khaledi *et al.* 2017). As a result, plant phenolic compounds are known to have an important role in plant defense against pathogen infections (Lattanzio 2013). Specifically, phenolic compounds may have an important role in wheat cultivar resistance to fusarium head blight, mainly caused by *F. culmorum* (Siranidou *et al.* 2002; Jung *et al.* 2010). In particular, the phenolic acids of wheat are metabolites which actively participate in the defense system against *Fusarium* species and they are likely to affect resistance to fusarium head blight (FHB) (Chrpová *et al.* 2021).

Phytochemicals, such as phenolic compounds, are present in cereals (van Hung 2014). Thus, phenolic acids, which are significant phenylpropanoids, are the most frequent type of cereal phenolic compounds and are also found in many other plants (Atanasova-Penichon and Richard-Forget 2014; Boz 2015; Leváková and Lacko-Bartošová 2017). In cereals, they are derived directly from either hydroxycinnamic or hydroxybenzoic acids (El Gharras 2009; Nicoletti *et al.* 2013; Atanasova-Penichon and Richard-Forget 2014). There are two forms of phenolic acids, soluble (free) and insoluble (bound). The first form can be free or conjugated by ester or glycosidic bonds, whereas the second is linked to the cell wall by ester or ether bonds (Nicoletti *et al.* 2013; Atanasova-Penichon and Richard-Forget 2014). According to research carried out by Brandolini *et al.* (2013), more than 90% of phenolic acids were in bound form in three *Triticum* species. In addition, phenolic acids mainly exist in bound form in rice grains and wheat flour (Shao *et al.* 2021). In wheat flours, different extraction rates were carried out which showed significant variation in the composition of free and bound phenolic acids (Wang *et al.* 2013).

According to laboratory tests, the main phenolic acids found in triticale and wheat were ferulic, coumaric, and protocatechuic acids. Triticale was similarly high in gallic acid. Barley was high in ferulic, coumaric, hydroxybenzoic, and gallic acids. Ferulic, *p*-coumaric, gallic, and syringic acids were abundant in corn (Kandil *et al.* 2012). Thus, wheat (genus *Triticum*) is regarded as a significant source of polyphenols, however, there is little information about their compositions and concentrations in different species of *Triticum* genus (Leváková and Lacko-Bartošová 2017). Analysis of free, conjugated and bound phenolic acid content in the grains of some wheat varieties resulted in the determination of various phenolic acids namely ferulic, sinapic, *p*-coumaric, vanillic, 4-hydroxybenzoic, *cis*-isomers of ferulic acid and sinapic acid (Paznocht *et al.* 2020). In particular, ferulic acid is considered to be the most abundant and major phenolic acid contained in wheat grain at all stages of crop development (McKeehen *et al.* 1999; Anson *et al.* 2009; Leváková and Lacko-Bartošová 2017). In addition to ferulic acid, vanillic and syringic acids were the main phenolic acids in the wheat bran studied (Kim *et al.* 2006).

The objective of this research was to carry out biochemical analyses in the laboratory of phenolic acids having a key role in resistance to fusarium head blight. The work was focused on conducting a comparative analysis of the composition of free and cell wall-bound phenolic acids in early grains of two selected lines and three marketed parental varieties. The presence of these phenolic acids in early wheat grains, the variation of their composition during the filling kinetics of wheat grains and in response to *Fusarium* infection were studied. In our research, free soluble and insoluble wall-bound phenolic acids were examined in the flowering stage (5 to 8 days after flowering) (early grains).

Materials and Methods

The plant material used in our present work included a total of five durum wheat varieties, including three parental varieties with codes for each variety: G10 – Ardente, G11 – Waha, G9 – Simeto, and two genealogical lines carrying the symbols G1 and G4, composed of seeds resulting from diallel crosses between five parents: Ardente, Waha, Simeto, Vitron, and Saadi, obtained from the Laboratory of Crop Productions, ENSA, El-Harrach, Algiers. G9, G10 and G11 are the most susceptible varieties to fusarium head blight (Hadjout *et al.* 2017).

Extraction of free soluble phenolic acids

Phenolic extraction was carried out on ears harvested at the flowering stage (5 to 8 days after flowering) (BBCH: 61). These were frozen at –80°C and lyophilized for 48 h. All samples were ground with a centrifugal mill (Tissuelyser, Retsh, Germany) (0.50 mm grid).

For the extraction of free phenolic acids, 1 g of ground sample was put directly into a 50 ml tube. Lipid compounds were eliminated with a hexane extraction. Wheat powder was extracted twice with 5 ml of hexane, and stirred for 10 min at room temperature.

After centrifugation at 2800 rcf for 5 min, the organic phase was eliminated and the sample in the corning was dried under the hood for about 60 min. Then, the compounds of interest were extracted with a 10 ml mixture of methanol/water (80 : 20, v/v), purged with nitrogen for 10 s and agitated on a stirring wheel for 30 min. The samples were then centrifuged for 10 min at 2800 rcf.

Supernatants contained free phenolic compounds, and the pellets (solid part) contained phenolic compounds bound to the cell wall. An 8 ml volume of the supernatant was taken from the solid part and dried on a Petri dish overnight to allow the extraction of the bound phenolic acids. After methanol/water (80 : 20, v/v) extraction of phenolic compounds, free phenolic acids were extracted by liquid-liquid extraction with ethyl acetate. They were concentrated twice by evaporation under nitrogen flow at 40°C, and 6 ml of water were added. Aqueous solutions were acidified with 1 M HCl at a pH of 2 then 10 ml of ethyl acetate were added. At this pH, phenolic acids are more soluble in the organic phase than in the aqueous phase. The sample was stirred for 5 min and centrifuged for 5 min at 2800 rcf. Then, 8 ml of the organic phase (upper) was taken from a 10 ml glass tube, and evaporated to dryness under nitrogen flow. Before the HPLC-DAD assay, the extract was taken up with 200 µl of MeOH/water (50 : 50, v/v) and filtered through 0.22 µm porosity filter paper.

Extraction of insoluble phenolic acids bound to cell walls

Bound phenolic acids were extracted from about 100 mg of the solid part (finely ground grains). Four milliliters of sodium hydroxide (2 M) were added to the tube. After purging with nitrogen for 10 s, the tube was shaken for 2 h. Then, the filtrates were acidified to pH 2 using hydrochloric acid (12 M). The samples were then extracted with 5 ml of ethyl acetate. After centrifugation at 2800 rcf for 5 min, 4 ml of the ethyl acetate phase were placed in a 10 ml glass tube. The extraction was repeated a second time and 8 ml of the organic phase were evaporated to dryness at 40°C under nitrogen flow. Finally, the dry samples were taken up in 500 µl of a methanol/water mixture (50 : 50, v/v) and filtered through a 0.22 µm porosity filter.

HPLC-DAD phenolic acids determination

Phenolic acids (free and bound) were analyzed by 1100 Series High Performance Liquid Chromatography (HPLC) system (Agilent, Massy, France) coupled to an Agilent photodiode array detector (DAD) according to a modified protocol described by Atanasova-Penichon *et al.* 2014. This method has been developed to separate many phenolic acid compounds: chlorogenic acid, *p*-coumaric acid, *trans*-ferulic acid, sinapic acid, syringic acid, protocatechuic acid, vanillic acid, ferulic acid, 8-5′-dehydrodiferulic acid (8-5'-DiFA), 5-5′-dehydrodiferulic acid (5-5′-DiFA), 8-O-4′-dehydrodiferulic acid (8-O-4′-DiFA) and 8-5′-benzofurandehydrodiferulic acid (8-5′-benzofuran-DiFA). These compounds and some of their characteristics are reported in Table 1. The separation of the phenolic acids was carried out on a Kinetex XB-C18 100 Å (150 by 4.6 mm, 2.6 µm) (Phenomenex, Le Pecq, France) maintained at 45°C. The mobile phase consisted of water acidified with 0.20% formic acid (v/v) (solvent A) and acetonitrile acidified with 0.20% formic acid (v/v) (solvent B). Phenolic acids were separated by an elution gradient as follows: from 5 to 15% of B in 30 min, from 15 to 50% of B in 10 min, from 50 to 90% of B in 5 min, 90% B for 3 min, 90 to 5% B in 1 min, and 5% B for 10 min. The injection volume was 5 µl and the flow rate was maintained at 1 ml/min for a total duration of 68 min. UV-VIS spectra were recorded from 200 to 550 nm and chromatographic peaks were measured at 260 nm, 280 nm and 320 nm, according to the phenolic acids studied. Monomeric phenolic acids' quantification and ferulic acid dimers were carried out using an external calibration performed with standard solutions of phenolic acids prepared from pure commercial powders purchased from Sigma-Aldrich (France).

Table 1. Characteristics of phenolic compounds separated by the HPLC analysis method developed during the study

Phenolic compounds	R_T [min]	λ_{max} [nm]
Phenolic acids (derived from benzoic acids)		
Protocatechuic acid	8.86	260
Vanillic acid	18.90	260
Syringic acid	21.70	280
Phenolic acids (derived from cinnamic acids)		
Ferulic acid	33.60	320
p-coumaric acid	28.10	320
Sinapic acid	33.80	320
Chlorogenic acid	16.60	320
Dehydrodiferulic acids (DiFA)		
8-5′-DiFA (open form)	38.80	320
5-5′-DiFA	42.80	320
8-O-4′-DiFA	43.90	320
8-5′-benzofuran-DiFA (benzofuran form)	44.10	320

R_T – retention time; λ_{max} – lambda max; *p*-coumaric acid – *para*-coumaric acid; 8-5′-DiFA – 8-5′-dehydrodiferulic acid; 5-5′-DiFA – 5-5′-dehydrodiferulic acid; 8-O-4′-DiFA – 8-O-4′-dehydrodiferulic acid; 8-5′-benzofuran-DiFA – 8-5′-benzofurandehydrodiferulic acid

Statistical analysis

For variance analysis, statistical analysis of all results was performed using statgraphics software version 15.1.0. Then, means multiple comparison was carried out using LSD test (Least Significant Difference) to determine homogeneous groups at the 5% significance level.

Results

At 5 to 8 days after flowering (early grains), free and cell wall-bound phenolic acids content of the varieties studied was analyzed by HPLC-DAD and presented in Table 2.

Phenolic acid analysis results in the early grains showed that the contents of bound phenolic acids were much higher than that of free phenolic acids at the flowering stage.

In early grains, analytical results for free phenolic acids revealed the absence of *cis*-ferulic acid. However, chlorogenic, *p*-coumaric, *trans*-ferulic, sinapic, syringic, protocatechuic and vanillic acids were detected in a variety of ears. Indeed, chlorogenic and protocatechuic acid contents were very low, while slightly higher contents were detected in *p*-coumaric, syringic, vanillic acids and sinapic acid with slightly higher amounts than in the last three acids. Finally, *trans*-ferulic acid was detected with significant amounts compared to all free phenolic acids in early grains. In all cases, the values were relatively very close for all varieties.

Statistically, the G1 line did not show a significant difference with the other varieties for the content of chlorogenic, *p*-coumaric, *trans*-ferulic, syringic and vanillic acids. However, it showed a significant difference with the G11 variety for sinapic ($F = 3.37$; $p < 0.05$) and protocatechuic ($F = 3.48$, $p < 0.05$) acids.

For bound phenolic acids at the level of the early grains, the absence of sinapic acid was noted and the presence of *p*-coumaric, ferulic, syringic, vanillic acids and dehydrodiferulic acids (8-5'-DiFA, 5-5'-DiFA, 8-O-4'-DiFA and 8-5'-benzofuran-DiFA) was seen. In particular, ferulic acid was the main bound phenolic acid which had much higher levels.

Statistical analysis revealed that the G1 line showed significant differences with G4, G9 and G11 for *p*-coumaric acid, with G9 and G11 for ferulic acid, with G4, G9 and G10 for syringic acid and with G9 and G11 for dehydrodiferulic acids (8-5'-DiFA, 5-5'-DiFA, 8-O-4'-DiFA and 8-5'-benzofuran-DiFA), sum of cell-wall-bound monomers and sum of cell-wall-bound DiFA. No significant differences were observed between the G1 line and other varieties for vanillic acid.

As a result, the G1 line was still in first position with the highest content of bound phenolic acids compared to the other varieties. It showed 3098.99 $\mu g \cdot g^{-1}$ of DM in *p*-coumaric acid, 4913.92 $\mu g \cdot g^{-1}$ DM in ferulic acid, 27.06 $\mu g \cdot g^{-1}$ DM in syringic acid, 24.42 $\mu g \cdot g^{-1}$ DM in vanillic acid, 90.35 $\mu g \cdot g^{-1}$ DM in 8-5'-DiFA, 273.38 $\mu g \cdot g^{-1}$ DM in 5-5'-DiFA, 522.71 $\mu g \cdot g^{-1}$ DM in 8-O-4'-DiFA, 413.06 $\mu g \cdot g^{-1}$ DM in 8-5'-benzofuran-DiFA, 8064.39 $\mu g \cdot g^{-1}$ DM in monomers and 1299.48 in dehydrodiferulic acids.

According to these results, syringic and vanillic acids were present in a variety of ears but in low quantities and a difference between varieties which was not important. For dehydrodiferulic acids, the results showed that their composition was less important than that of ferulic and *p*-coumaric acids but it was more important than that of syringic and vanillic acids. Indeed, the 8-5'-benzofuran-DiFA and the 8-O-4'-DiFA are the two main acids of dehydrodiferulic acid, thus representing quantities substantially similar to each other. The 5-5'-DiFA was the third dehydrodiferulic acid and finally came the 8-5'-DiFA with lower levels than the other dehydrodiferulic acids. Moreover, the sum of the monomers was much higher than dehydrodiferulic acids at the level of all varieties.

Comparing varieties, the content of all bound phenolic acids (monomeric form and dehydrodiferulic acids) in the G1 line was much higher than in other varieties, especially with susceptible varieties (G9 and G11), except for vanilic acid from which no significant differences were observed. For this last acid, the G1 line presented a lower content than G4, G10 and G11 varieties. It can be assumed that this line had not yet formed its cell walls.

Discussion

We focused our discussion on the variation of phenolic acid levels at the flowering stage of durum wheat. Indeed, phenolic acids are major phenylpropanoids and are derived directly from either cinnamic acid or benzoic acid (Atanasova-Penichon *et al.* 2016). In cereals, benzoic acid derivatives include gallic, *p*-hydroxybenzoic, vanillic, syringic and protocatechuic acids while cinnamic acid derivatives include caffeic, chlorogenic, *p*-coumaric, sinapic and ferulic acids (Das and Singh 2015; Atanasova-Penichon *et al.* 2016). Indeed, phenolic acids found in cereals exist in a soluble form (free form), either conjugated esterified in sugar or in an insoluble form (form bound to cell walls), specifically, bound to various polysaccharides and lignin by ester and ether bounds (Atanasova-Penichon *et al.* 2016; Žilić 2016).

Table 2. Mean contents of the main free and cell wall bound phenolic acids contained in the ears of the five durum wheat varieties harvested at the flowering stage (μg · g^{-1} DM)

Phenolic acids		Varieties					*F* value and significance
		G1	G4	G9	G10	G11	
Free phenolic acids	Chlorogenic acid	0.57 ± 0.20 a	0.79 ± 0.40 a	0.69 ± 0.34 a	0.37 ± 0.16 a	0.85 ± 0.58 a	*F* = 0.95 ns
	p-coumaric acid	6.55 ± 1.82 a	5.71 ± 2.06 a	6.48 ± 1.07 a	5.42 ± 2.15 a	5.07 ± 2.03 a	*F* = 0.47 ns
	*tra*ns-ferulic acid	43.29 ± 9.61 a	49.97 ± 17.09 a	53.90 ± 8.91 a	56.72 ± 22.27 a	50.62 ± 21.14 a	*F* = 0.32 ns
	Sinapic acid	15.95 ± 2.25 bc	6.44 ± 4.51 c	21.48 ± 5.31 ab	20.69 ± 3.87 ab	28.04 ± 13.53 a	*F* = 5.99**
	Syringic acid	4.19 ± 1.08 a	4.45 ± 1.28 a	3.74 ± 1.02 a	3.19 ± 1.13 a	4.48 ± 0.89 a	*F* = 0.99 ns
	Protocatechic acid	1.07 ± 0.16 b	1.30 ± 0.19 ab	1.34 ± 0.23 ab	1.20 ± 0.43 ab	1.53 ± 0.22 a	*F* = 1.65 ns
	Vanillic acid	4.59 ± 1.08 a	3.99 ± 0.93 a	3.64 ± 1.01 a	3.48 ± 0.96 a	4.14 ± 0.68 a	*F* = 0.91 ns
Bound phenolic acids	*p*-Coumaric acid	3,098.99 ± 1,046.51 a	2,047.35 ± 566.07 b	1,443.49 ± 205.97 b	2,120 .61 ± 54.85 ab	1601.71 ± 912.32 b	*F* = 3.65*
	Ferulic acid	4,913.92 ± 1,209.31 a	3,839.93 ± 881.70 ab	2,916.39 ± 492.68 b	3,848.99 ± 1,031.73 ab	3,228.05 ± 1,060.84 b	*F* = 3.40*
	Syringic acid	27.06 ± 9.29 a	16.53 ± 4.44 b	19.98 ± 3.33 b	18.14 ± 6.20 b	18.89 ± 7.89 ab	*F* = 2.90 ns
	Vanilic acid	24.42 ± 8.70 a	27.27 ± 19.46 a	16.20 ± 9.24 a	28.23 ± 13.41 a	32.24 ± 17.42 a	*F* = 0.62 ns
	8-5′-DiFA	90.35 ± 26.42 a	74.20 ± 18.59 ab	52.36 ± 9.09 bc	66.92 ± 23.73 abc	44.54 ± 17.78 c	*F* = 4.09*
	5-5′-DiFA	273.38 ± 74.52 a	246.56 ± 75.10 ab	174.60 ± 44.11 b	206.11 ± 76.46 ab	156.11 ± 56.35 b	*F* = 2.54 ns
	8-O-4′-DiFA	522.71 ± 162.99 a	440.31 ± 133.71 ab	302.48 ± 71.56 b	380.74 ± 153.37 ab	265.32 ± 111.32 b	*F* = 3.01 ns
	8-5′-benzofuran-DiFA	413.06 ± 127.66 a	347.69 ± 107.29 ab	231.95 ± 47.12 b	302.85 ± 114.67 ab	223.45 ± 95.58 b	*F* = 2.98 ns
Sum of cell-wall-bound monomers		8,064.39 ± 2,264.77 a	5,931.06 ± 1457.26 ab	4,390.06 ± 697.01 b	6,015.97 ± 1767.28 ab	4,880.89 ± 1,975.33 b	*F* = 3.46*
Sum of cell-wall-bound DiFA		1,299.48 ± 390.98 a	1,108.77 ± 334.13 ab	761.38 ± 170.83 b	956.61 ± 367.97 ab	689.41 ± 281.43 b	*F* = 3.00 ns

This table records the average values; the *p* value of independent test is presented with its threshold of significance; *$p < 0.05$; **$p < 0.01$; ns – not significant)

Values with the same letters in a column are not statistically different at the 5% significance level according to Fisher's Least Significance Difference (LSD) Test (*p*-value > 0.05); mean values ±SD of means; DiFA – dehydrodiferulic acid; 8-5′-benzofuran-DiFA – 8-5′- benzofurandehydrodiferulic acid (benzofuran form)

In our study, the following free phenolic acids: chlorogenic, *p*-coumaric, *trans*-ferulic, sinapic, syringic, protocatechuic and vanillic acids were detected in wheat ears at the level of all varieties. Several studies have reported the presence of these different phenolic acids in wheat (Onyeneho and Hettiarachchy 1992; Zhou *et al.* 2004; Kim *et al.* 2006). Mccallum and Walker (1991) studied changes in soluble hydroxycinnamic acids during wheat grain development. The HPLC analysis revealed that the *trans*-ferulic acid level decreased in the soluble fraction, but the bound fraction increased steadily during grain development. Moreover, comparison of the studied cultivars revealed significant differences in ferulic acid content per grain 20 days after emergence of the ear but little difference at maturity. Sinapic acid is the second major acid.

For the five varieties, ferulic acid is an insoluble phenolic acid bound to the walls, largely predominant with higher contents. This result is comparable with other research that has reported it to be the most abundant in wheat bran (Zhou *et al.* 2004; Parker *et al.* 2005; Kim *et al.* 2006; Moore *et al.* 2006; Mpofu *et al.* 2006). It is also known that the ferulic acid content varies between wheat varieties (Moore *et al.* 2006; Mpofu *et al.* 2006). In plants, ferulic acid is largely predominant in grains and it clearly has an inhibitory effect on the biosynthesis of toxins by all *Fusarium* isolates tested, including different chemotypes and species cultivated *in vitro* (Boutigny *et al.* 2009). Furthermore, ferulic acid is the most abundant primary phenolic acid in wheat grain, and is mainly responsible for the antioxidant activity of wheat, especially the bran fraction (Leváková and Lacko-Bartošová 2017). The second major bound acid is *p*-coumaric acid. Also, Boutigny (2007) reported that a few days after the flowering stage (10–12 and 20 days after flowering), *p*-coumaric acid was the second major phenolic acid. This same researcher also found that at the mature stage, *p*-coumaric acid represents the main phenolic acid after ferulic acid. Qualitative variations in phenolic acid contents have already been observed during wheat grain maturation (McKeehen *et al.* 1999). Research by Atanasova-Penichon *et al.* (2012) reported that the accumulation of phenolic compounds varies considerably during grain filling and the level is generally highest at the beginning of grain filling, as reported in corn. In addition, several previous studies have shown that the nature of grain phenolic acids varies between wheat varieties (Régnier and Macheix 1996; Lempereur *et al.* 1997; Lempereur *et al.* 1998; Peyron *et al.* 2002; Moore *et al.* 2006; Mpofu *et al.* 2006). These compounds could be good candidates for mycotoxin biosynthesis reduction characteristics in grains (Boutigny *et al.* 2008).

At 5 to 8 days after flowering, bound phenolic acid contents are much higher than that of free phenolic acids in all varieties. Work by Mccallum and Walker (1991), reported that bound insoluble phenolic acids are predominant. For the monomeric form, ferulic acid is the main acid, followed by *p*-coumaric acid. The content for these two acids is very important for all varieties. These results corroborate those obtained by McKeehen *et al.* (1999) who noted that the resistant cultivar (cv. Sumai) synthesized higher concentrations of bound ferulic acid than the susceptible cultivar (cv. Roblin) in the first 25 days after anthesis. Indeed, ferulic acid content in the Sumai cultivar was twice that of Roblin at 7 days after anthesis (1400 against 700 $\mu g \cdot 100\ grains^{-1}$). Together with ferulic acid, *p*-coumaric acid can contribute significantly to plant resistance mechanisms through cell wall enrichment and lignification (Atanasova-Penichon *et al.* 2016). Since anthesis occurs when cultivars are most susceptible to infection, results of McKeehen *et al.* (1999) suggest that ferulic and *p*-coumaric acids may potentially contribute to disease resistance.

Conclusions

Phenolic acids can be a very interesting lead to identifying varieties accumulating selected toxins. To better identify potential biochemical characters that could be related to the lower susceptibility to fusarium head blight of the selected G1 line, we compared the phenolic acid compositions in early grains of the five varieties considered in the present study. Overall phenolic acid composition results indicated that there was a predominance of monomeric forms and dehydrodiferulic acids (DiFA) in the ears of the five durum wheat varieties harvested at the flowering stage, as well as higher concentrations of free and cell wall-bound phenolic acids in the G1 line ears. Furthermore, our data probably represent a new argument for the role of cell wall composition in *Fusarium* resistance. We suggest that cell-wall traits could potentially be used as molecular markers for breeding durum wheat cultivars with increased resistance to fusarium head blight. Moreover, it would certainly be very relevant to consider, in addition to phenylpropanoids, other candidate compounds involved in cell-wall composition.

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