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ORIGINAL ARTICLE

Phenolic compounds of apple cultivars resistant or susceptible to *Venturia inaequalis*

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Abstract

Content: Phenolic compounds play an important role in the plant defense mechanism and are responsible for antioxidant capacity in fruits and vegetables. It is known that the phenolics can determine in the leaves of plants which are resistant/susceptible to fungal infections.
Objective: This study investigated the total phenolic compounds, content of shikimic acid from 33 different apple cultivars leaves infected with *Venturia inaequalis* [(Cke). Wint.] cultured in Fruit Research Station, in Egirdir, Isparta, Turkey.
Materials and methods: Leaves of apple cultivars were collected three times in an interval of 30 d from July to September in 2010, and analyzed using HPLC methods to detect changes in the amount of the phenolic compounds and shikimic acid.
Results: Total phenolic compounds and shikimic acid in resistant/moderate susceptible apple cultivars were higher than susceptible apple cultuvars, although not statistically different between resistant and susceptible apples. The content of shikimic acid was statistically higher only in the leaves of the domestic cultivar Ankara güzeli on all three dates.
Discussion and conclusion: Recently, there have been increased studies trying to explain the resistance mechanism in plants. Natural resistance genes are investigated in some apple cultivars and new resistance varieties which have resistant genes are identified daily. Our study hold to determine the relationship between the phenolic compounds and the expression of resistance seems to be promising.

Keywords

Apple scab, HPLC, *Malus domestica*, resistance, shikimic acid

History

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Introduction

Apple is a plant that can be grown in wide ranges and varied ecologies in the world. Many pests and diseases cause economic losses in apple production area. Apple scab [*Venturia inaequalis* (Cke).Wint.] is one of the most of devastating fungal diseases for apple trees and causes important economic losses. The disease is observed intensively in apple ochards in all regions in Turkey. Apple production decreases 20% by apple scab, as well as a negative impact on the market value caused by the depreciation of 30–60% in Turkey. Fungicides applied during spring and summer are used to control scab dieases caused *Vinaequalis* in apple orchards in Turkey (Boyraz et al., 2005). Most of the commercial apple cultivars are susceptible to the disease, and growers have to spray 20–30 times with fungicides in a season (Boyraz et al., 2005; Soriano et al., 2009). The use of pesticides adversely affect useful insects, microorganisms, plants, water, and even human health. For this reason, the development of scab-resistant varieties appears as the best means of control. It is known that many plants species are resistant to certain diseases naturally (Rühmann et al., 2002). The resistance genes of resistant apple genotypes possibly act as regulatory genes of phenol synthesis (Michalek et al., 1999). Phenolics play a role in defense against pathogens and are responsible for antioxidant capacity in fruits and vegetables. More than 8000 phenolic structures are currently known, the most numerous and widely distributed groups of substances in plants (Robbins, 2003). These compounds may react with and oxidize proteins. Thus, the enzyme causes a loss of movement and limits the movement of the pathogen or pathogen infection of the plant against an aggressive defense mechanism is stored in the cell wall (Agrios, 1997; Robbins, 2003).

Phenolic compounds are produced in plants via shikimic acid through the phenylpropanoid pathway, as by-products of the monolignol pathway and as breakdown products of lignin and cell wall polymers in vascular plants. Simple phenolic acid derivatives such as *p*-coumaroyl CoA is the shikimic acid derived starting unit in the biosynthesis of flavonoids (Dixon & Paiva, 1995; Winkel-Shirley, 2001).

The biosynthesis of a small phenolic compound (SA) occurs via the shikimic acid pathway, which is dependent on metabolites derived from glycolysis and pentose phosphate.

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Very little is known of the relationship between phenolic compounds and there are not many studies about shikimic acid and its relationship to apple scab resistance.

In this study, we investigated total phenolic compounds, the content of shikimic acid from 33 different apple cultivars leaves that were susceptible/moderate susceptible/resistant against *V. inaequalis* cultured in Fruit Research Station, in Egirdir, Isparta, Turkey. We studied the relationship between the levels of total phenolic content and shikimic acid that could be useful to screen-resistant (R), moderate susceptible (MS), and susceptible (S) apple cultivars.

Materials and methods

In this study, 33 domestic and commercial apple varieties with different levels of resistance grown in the experimental field of the Fruit Research Station in Egirdir, Isparta, Turkeys were used (Tables 1 and 2). The experiment was carried out during the growing period 2010. Leaf samples were taken from 13-year-old apple trees grafted on MM 106 rootstock.

Table 1. The domestic cultivars in Fruit Research Station in Egirdir, Isparta, Turkey.

Cultivars	Host reaction
Yaz elması (2563)	R
2438[a]	R
200888 (1–2) [a]	MS
Amasya 9	R
33[a]	MS
E 42[a]	MS
4[a]	R
Sarıgöbek	R
Ankara güzeli	R
Aksu 4	R
13[a]	MS
E 473	R
62-2[a]	R
Beyaz elma (2575)	MS
E 40	S
Harım	S
E 496[a]	R
Amasya	R

[a]Cultivar has not yet been named.

Table 2. Apple cultivars in Fruit Research Station in Egirdir, Isparta, Turkey.

Cultivars	Host reaction
Granny Smith Spur	MS
Auwil Spur	S
Crabapples	R
Yellow Spur	S
Red Rome 262	MS
Elite	S
Stark	MS
Calville Rouge Datan	MS
Scarlet Staymaret	MS
Summerred	S
Hi–Early	S
Wayne Spur	S
Cooper 7 SB-2	S
Red Stayman	S
Golden Sel B	S

The infected leaves harvested three times in an interval of 30 d, immediately treated with liquid nitrogen and stored at −80 °C until extraction. Phenolic compounds were consisted according Kosar et al. (2004).

Extraction of phenolic compounds

Frozen samples were powdered with liquid nitrogen in a mortar. This powder (3 g), 10 mL of acetone, water (1:4, v/v) mixture, and 0.1 mL of trifluoroacetic acid were added into the flask and refluxed for 1 h. After it was cooled, the mixture was filtered and made up to 10 mL with distilled water. These samples were directly used for HPLC analyses.

High-performance liquid chromatography (HPLC)

In this study, liquid chromatographic apparatus (Hewlett-Packard HP-1100, Palo Alto, CA) was used. Samples were detected at 200–600 nm. Three replicates from each sample were used for HPLC analyses. All samples and standards were injected three times, and mean values were used (Kosar et al., 2004).

Statistical analyses

All statistical analyses were carried out using SPSS 16 (SPSS Inc., Chicago, IL). Data were subjected to analysis of variance (ANOVA) and were separated using Duncan's Multiple Range Test at a level of $p < 0.05$.

Results and discussion

In this investigation, we compared the content of total phenolic compounds and shikimic acid in the leaves of 33 domestic and commercial apple varieties (12 scab-resistant, 10 scab susceptible, and 11 scab susceptible apple cultivars) cultured in Fruit Research Station, in Egirdir, Isparta, Turkey. We identified that total phenolic content and shikimic acid in resistant/moderate susceptible apple cultivars were higher than and susceptible apple cultuvars (Tables 3 and 4).

In July, the amount of phenolic compounds in the leaves of the domestic cultivar E 473 (R) was almost 2–6 times as high as in the remaining cultivars. The total phenolic content ranged from 63.67 μg/g [Summerred (S)] to 664.00 μg/g (E 473 domestic cultivar/R) in the leaves. In August and September, total phenolic compounds in all cultivars decreased and were found lower than in July. The highest phenolic compounds was determined 381.33 μg/g in leaves of domestic cultivar Amasya (R) in August, whereas the lowest value was 79 μg/g from cultivar Scarlet Staymaret (MS). In September, the leaves of resistant cultivars contained more phenolic compounds than those of susceptible cultivars. The highest total phenolic content was contained in domestic cultivar Amasya (R) and Summerred (S).

We found that the amount of the total phenolic compounds in many resistant/moderate susceptible cultures was higher than and susceptible apple cultuvars. Even then, the results of our experiment in which we compared the total phenolic compounds in the leaves of scab-resistant apple cultivars and susceptible cultivars did not show any differences. Similar results were reported by Veberic et al. (2005) which

Table 3. Amount of total phenolic compounds (μg/g) in the leaves of apple cultivars measured at different dates.

	Total phenolic compounds (μg/g)		
	Dates		
Cultivars	July	August	September
Granny Smith Spur	349.00 ± 1.15 j	190.00 ± 4.04 h	71.00 ± 0,57 b
Auwil Spur	328.00 ± 1.15 h	167.67 ± 0.33 e	101.00 ± 1.73 de
Yaz elması (2563)	132.00 ± 0.57 c	145.00 ± 5.77 e	166.00 ± 1.73 n
Crabapples	511.00 ± 0.57 v	238.67 ± 0.33 l	168.00 ± 5.77 n
Yellow Spur	230.00 ± 2.85 d	210.67 ± 0.33 i	113.00 ± 1.73 gh
Red Rome 262	495.67 ± 0.33 u	171.00 ± 5.77 f	170.33 ± 0.88 no
2438	385.00 ± 1.15 n	213.00 ± 2.30 i	158.67 ± 0.33 m
Elite	326.00 ± 3.46 h	119.67 ± 0.33 d	234.00 ± 0.57 s
Stark	381.00 ± 2.30 n	191.67 ± 0.33 h	175.00 ± 0 op
200888 (1–2)	346.00 ± 1.15 j	108.00 ± 5.77 c	98.00 ± 2.30 d
Calville Rouge Datan	295.00 ± 1.15 f	194.67 ± 0.33 h	69.67 ± 0.33 b
Scarlet Staymaret	442.00 ± 1.73 r	79.00 ± 3.46 a	139.00 ± 4.61 l
Amasya 9	333.00 ± 1.15 i	234.67 ± 0.33 kl	158.00 ± 2.88 m
33	347.00 ± 1.15 j	179.00 ± 1.15 g	138.00 ± 1.73 l
E 42	110.67 ± 3.17 b	109.67 ± 0.33 c	112.67 ± 0.33 gh
4	443.00 ± 2.30 r	111.67 ± 0.33 c	118.67 ± 0.33 hi
Sarıgöbek	337.00 ± 0.57 i	120.00 ± 0.57 d	100.67 ± 0.33 de
Ankara güzeli	403.00 ± 1.15 o	195.00 ± 2.30 h	186.67 ± 0.33 r
Summerred	63.67 ± 0.33 a	123.67 ± 0.33 d	241.33 ± 1.45 t
Hi–Early	296.00 ± 1.15 f	125.00 ± 1.15 d	230.00 ± 3.46 s
Aksu 4	267.00 ± 0.57 e	171.67 ± 0.33 f	166.00 ± 1.15 n
13	376.00 ± 1.15 m	88.67 ± 0.33 b	122.00 ± 1.73 ij
E 473	664.00 ± 1.15 y	173.00 ± 2.88 fg	126.00 ± 0.57 jk
Wayne Spur	370.00 ± 01	126.67 ± 0.33 de	130.00 ± 1.73 k
62-2	334.00 ± 1.73 i	226.00 ± 1.15 j	177.00 ± 0.57 p
Beyaz elma (2575)	356.00 ± 0 k	111.00 ± 3.46 c	19.33 ± 0.33 a
E 40	309.67 ± 0.33 g	120.67 ± 2.60 d	110.00 ± 0.57fg
Harım	402.00 ± 1.73 o	228.00 ± 0 jk	117.67 ± 0.33 hi
E 496	475.00 ± 0 s	151.00 ± 1.15 e	85.00 ± 0.57 c
Cooper 7 SB-2	484.00 ± 0.57 t	265.00 ± 1.73 n	101.00 ± 2.30 de
Red Stayman	381.33 ± 4.05 n	241.00 ± 1.73 l	105.00 ± 1.15 ef
Golden Sel B	229.33 ± 0.33 d	119.00 ± 1.15 d	166.00 ± 1.73 n
Amasya	412.00 ± 1.15 p	398.00 ± 2.30 n	333.00 ± 5.77 u

Total phenolic compounds in columns designated with the same letter do not differ significantly (Duncan's test, $p < 0.05$). Average total phenolic compounds were compared between individual apple cultivars for each date separately.

compared the total phenolic compounds between resistant and susceptible apple cultivars. The highest content of total phenolic compounds was measured in the cultivars ''Majda'' (S) and ''Remura'' (R).

We studied scab-resistant, susceptible, moderate susceptible apple cultivars with respect to their defence-related secondary compounds with inoculation by the fungus *V. inaequalis*. In the group of resistant cultivars, the July leaves contained the highest quantities of shikimic acid; its content decreased in August and September. Total shikimic acid content varied from 10 μg/g (E 42/MS) to 72 μg/g (domestic cultivar Ankara güzeli/R). The leaves of scab-resistant cultivars contained up to 10 times as high shikimic acid content as the leaves of scab susceptible/moderate susceptible cultivars.

The content of shikimic acid was statistically higher only in the leaves of the domestic cultivar Ankara güzeli on all three dates. In the remaining, 11 scab-resistant cultivars, Yaz Elması (2563), Crabapples, 2438, Amasya 9, 4, Sarıgöbek, Aksu 4, E 473, E 496, 62-2, domestic cultivar Amasya, the content of shikimic acid did not differ significantly from that in the leaves of scab susceptible cultivars. The leaves of resistant cultivars contained more shikimic acid than those of susceptible cultivars. In August, shikimic acid was present at the highest concentration in the apple leaves, ranging from 6.0 μg/g in the domestic cultivar E 42 (moderate susceptible) to 44.66 μg/g in the domestic cultivar Ankara güzeli (R).

Nevertheless, the results of our experiment in which we compared the shikimic acid in the leaves of scab-resistant apple cultivars and susceptible cultivars did not show any differences. In general, research investigated chlorogenic acid in the apples to apple scab (Mayr et al., 1997; Mikulic Petrovsek et al., 2003, 2009; Veberic et al., 2005). The results agree partly with the findings of Mikulic Petrovsek et al. (2003) which reported that in the leaves of three scab-resistant apple cultivars and two scab susceptible ones did not show any differences in the content of chlorogenic acid.

In our study, the amount of phenolic compounds was lower in August and September compared to July. It was concluded from these results that the accumulation of phenolic compounds in apple leaves is also influenced by the growing season. Environmental conditions influence the synthesis of phenolic compounds (Hamauzu, 2006; Treutter, 2001), and therefore, the individual year had a significant influence on the amount of total phenolic compounds. Similar results

Table 4. The content of shikimic acid (μg/g) in the leaves of apple cultivars measured at different dates.

	The content of shikimic acid (μg/g)		
	Dates		
Cultivars	July	August	September
Granny Smith Spur	41,00 ± 2.89 g–i	9.00 ± 1.73 a	8.00 ± 0.58 ab
Auwil Spur	30.00 ± 0 de	27.00 ± 1.73ef	18.00 ± 2.31 e
Yaz elması (2563)	44.00 ± 1.15 i–l	32.00 ± 0.58 hi	22.00 ± 1.73 f
Crabapples	67.00 ± 0.58 no	44.00 ± 1.15 k	38.67 ± 0.33 j
Yellow Spur	29.00 ± 2.31 de	28.00 ± 1.15 e–g	19.00 ± 1.15 ef
Red Rome 262	52.00 ± 1.151	38.00 ± 1.15 j	32.00 ± 1.15 hi
2438	46.00 ± 1.73 g	39.00 ± 3.46 j	29.00 ± 0 gh
Elite	29.00 ± 2.31 de	14.00 ± 1.73 b	10.00 ± 1.1 bc
Stark	48.00 ± 4.04 kl	31.67 ± 0.33 g–i	30.00 ± 1.73 gh
200888 (1–2)	37.00 ± 2.89 f–h	24.00 ± 1.53 de	19.33 ± 0.88 ef
Calville Rouge Datan	11.00 ± 2.89 g	8.00 ± 0.58 a	6.00 ± 1.15 a
Scarlet Staymaret	23.00 ± 1.15 c	17.00 ± 0 b	14.67 ± 0.33 d
Amasya 9	17.00 ± 2.31 b	15.67 ± 0.3 b	12.00 ± 1.15 cd
33	42.00 ± 0.58 h–j	18.00 ± 1.73 bc	8.67 ± 0.33 ab
E 42	10.00 ± 0 a	6.00 ± 1.73 a	6.67 ± 0.33 a
4	32.00 ± 0.58 ef	24.00 ± 0.58 de	21.00 ± 1.15 ef
Sarıgöbek	57.00 ± 1.15 m	55.00 ± 1.151	49.00 ± 1.15 k
Ankara güzeli	72.00 ± 058 o	69.00 ± 1.15 m	49.00 ± 1.15 k
Summerred	62.00 ± 0.58 mn	44.00 ± 1.73 k	38.00 ± 1.15 j
Hi–Early	32.00 ± 1.15 ef	29.00 ± 1.15 f–h	20.00 ± 1.15 ef
Aksu 4	59.00 ± 1.15 m	38.00 ± 1.156 j	33.00 ± 0.58 i
13	32.00 ± 1.15 ef	18.00 ± 1.15 bc	14.00 ± 0 d
E 473	62.00 ± 0.58 mn	52.00 ± 0.581	49.67 ± 0.3 k
Wayne Spur	23.33 ± 0.88 c	14.00 ± 0.58 b	12.00 ± 0 cd
62-2	46.00 ± 2.31 i–l	38.67 ± 0.33 j	28.67 ± 0.33 g
Beyaz elma (2575)	29.00 ± 058 de	24.00 ± 0.58 de	21.6667 ± 0.33 f
E 40	27.66 ± 0.33 c–e	16.66 ± 0.33 b	12.00 ± 0.58 cd
Harım	48.00 ± 1.15kl	33.33 ± 0.88 i	29.00 ± 1.15 gh
E 496	47.00 ± 1.15 j–m	21.00 ± 1.15 cd	18.00 ± 1.15 e
Cooper 7 SB-2	46.00 ± 0.58 i–l	29.66 ± 0.33 fi	15.00 ± 0.58 d
Red Stayman	25.00 ± 1.15 cd	18.00 ± 1.15 bc	14.00 ± 1.15 d
Golden Sel B	36.00 ± 1.73 fg	25.00 ± 2.31 e	21.0000 ± 1.15 ef
Amasya	57.66 ± 0.33 m	47.00 ± 0.58 k	39.00 ± 1.15 j

The content of shikimic acid in columns designated with the same letter do not differ statistically (Duncan's test, $p < 0.05$). Average contents of shikimic acid were compared between individual apple cultivars for each date separately.

were reported by Veberic et al. (2007) and Mikulic Petrovsek et al. (2011).

Conclusion

In present study, the total phenolic compounds and shikimic acid in resistant/moderate susceptible apple cultivars were higher than susceptible apple cultuvars, although not statistically different between resistant and susceptible apples. The accumulation of different phenolic compounds as well as the possible activation of the shikimic acid patway and phenylpropanoid pathway may explain the broad and unspecific prevention of plant diseases such as apple scab (Croteau et al., 2000; Harkin et al., 1973; Mikulic Petrovsek et al., 2011; Treutter, 2001; Treutter & Feucht, 1990a,b; Winkel-Shirley, 2001). However, there are still many questions as to whether the constitutive levels of some phenolic compounds is really involved in resistance mechanisms, or if it represents only the capacity of the tissue to produce large amount of these compounds (Treutter, 2001). Further research would be required about shikimic acid to apple scab disease.

Recently, there has been an increased number of studies trying to explain the resistance mechanism in plants. Natural resistance genes are investigated in some apple cultivars and new resistance varieties which have resistant genes are identified daily. In our study performed to determinate the relationship between the phenolic compounds and the expression of resistance seems to be promising.

As a result, this and similar studies using more apple cultivars should be considered. In this way, it will contribute to the elucidation of mechanisms of resistance and it will reduce the existing problems to control plant diseases. The use of resistant cultivars could reduce the cost to growers and may also contribute to a cleaner environment and to a reduction of fungicide residuals on apples used by consumers.

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Declaration of interest

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