

Determination of phenolic compounds in some almond hybrids varying in resistance to *Pseudomonas amygdali*

Misirli A., Gülcan R., Küden A., Demir G.

in

Ak B.E. (ed.).
XI GREMPA Seminar on Pistachios and Almonds

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 56

2001
pages 71-86

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=1600155>

To cite this article / Pour citer cet article

Misirli A., Gülcan R., Küden A., Demir G. **Determination of phenolic compounds in some almond hybrids varying in resistance to *Pseudomonas amygdali*.** In : Ak B.E. (ed.). *XI GREMPA Seminar on Pistachios and Almonds*. Zaragoza : CIHEAM, 2001. p. 71-86 (Cahiers Options Méditerranéennes; n. 56)



<http://www.ciheam.org/>
<http://om.ciheam.org/>

Determination of phenolic compounds in some almond hybrids varying in resistance to *Pseudomonas amygdali*¹

A. Mısırlı*, A. Küden**, G. Demir*** and R. Gülcan*

*Department of Horticulture, Faculty of Agriculture, Ege University, 35100 Bornova- İzmir, Turkey

**Department of Horticulture, Faculty of Agriculture, Cukurova University,
01330 Balcalı-Adana, Turkey

***Plant Protection Research Institute, 35100 Bornova- İzmir, Turkey

SUMMARY – Healthy young leaves were collected and analysed for their phenolic compound content in resistant and susceptible almond hybrids after artificial inoculation with *Pseudomonas amygdali*. Phenolic compounds were investigated by using spectrophotometry, thin layer and high-performance liquid chromatography. Consequently, average tannin content of medium resistant and resistant groups were higher than the others. When the phenolic compounds were investigated by using thin layer chromatography, some spots were determined only in resistant or in susceptible hybrids. In the analyses of phenolics by HPLC, chlorogenic acid content was found to be higher in the resistant hybrids.

Key words: Almond, *Pseudomonas amygdali*, disease resistance, phenolic compounds.

RESUME – "Détermination des composés phénoliques chez certains hybrides d'amandier ayant une résistance variable à *Pseudomonas amygdali*". De jeunes feuilles saines ont été prélevées et analysées pour connaître leur teneur en composés phénoliques chez des hybrides d'amandier résistants et susceptibles après inoculation artificielle avec *Pseudomonas amygdali*. Les composés phénoliques ont été étudiés par spectrophotométrie, chromatographie liquide à haute performance (HPLC) et en couche mince. Par conséquent, la teneur moyenne en tannins des groupes moyennement résistants et résistants était plus élevée que chez les autres. Lorsque l'on a examiné les composés phénoliques par chromatographie en couche mince (TLC), certains points ont été déterminés uniquement chez les hybrides résistants ou susceptibles. Dans les analyses de composés phénoliques par HPLC, la teneur en acide chlorogénique s'est révélée plus élevée chez les hybrides résistants.

Mots-clés : Amandier, *Pseudomonas amygdali*, résistance aux maladies, composés phénoliques.

Introduction

Plants are frequently exposed to numerous biotic and abiotic stressors and therefore have evolved efficient defence mechanisms (Eckey-Kaltenbach *et al.*, 1994). Plant cells respond to environmental stimuli by synthesising secondary metabolites which may protect them against the causal agents.

Phenolics belong to the secondary plant metabolites which are known to inhibit the feeding of many insects or have been shown to be toxic (Grayer *et al.*, 1992). The involvement of phenols in plant disease resistance is based on to large extent on their cytotoxicity. This is associated with their oxidation products (Aver'yanov and Lapikova, 1994).

Pathogen attack such as virus, bacteria or fungi induces a cascade of reactions which can lead to resistance being expressed at the site of infection or in other uninfected parts of the plant. This systemic resistance implies the existence of an endogenous signal translocated from the infection site to other parts of the plant (Kuc, 1983). It has been proposed that the first stage of the defence mechanism involves a rapid accumulation of phenols at the infection site, which function to slow down the growth of the pathogen (Matern *et al.*, 1988).

Phenolics consist of compounds like condensed tannin, flavonoid, phenylpropane compounds, etc. Flavonoids are fairly distributed in plant kingdom (Hermann, 1988). Besides, their many fold functions

¹ This study is a part of the project TOGTAG-1433.

in the plant, they possess insecticidal as well as antimicrobial effects (Tomas-Barberan *et al.*, 1988). Tannins are known to be toxic against a wide array of micro-organisms (Mila and Scalbert, 1994).

Phenolic compounds can be analysed by using spectrophotometry and, paper, gas and thin layer chromatography. Recently, High Performance Liquid Chromatography (HPLC) is used to analyse a wide spectrum of potential phenols in different materials. HPLC procedure allows a great separation and quantification of phenols (Treutter *et al.*, 1990).

The role of phenolic compounds in the disease resistance of plants has been dealt by many researchers. For example, tannin content of some resistant apricot hybrids to *S. laxa* was determined to be higher than the susceptible ones (Gülcan *et al.*, 1997). Similarly, there were some differences in relation to spots on the chromatograms (TLC) between resistant and susceptible hybrids (Gülcan *et al.*, 1997). Using TLC, an acid with R_f 0.62 was observed only in the case of Liberty variety resistant to *Venturia inaequalis* (Mikhailova and Vishanska, 1994). Thin layer chromatography showed that three compounds were extracted from the young leaves of the anthracnose resistant cv. Plimbite. They were not detected in extracts from the anthracnose susceptible cultivars (Plumley and Sweetmore, 1994). When phloem sap samples of resistant and susceptible rice plants were screened by means of HPLC, a higher level of phenolics was found in the resistant variety than in the susceptible ones (Grayer *et al.*, 1994).

The objective of the present work is to compare the difference in phenolic content among almond hybrids different resistant and susceptible to *Pseudomonas amygdali* after artificial inoculation.

Material and method

Young leaf samples were taken for analyses of phenolic compounds from resistant, medium resistant and susceptible almond hybrids after the inoculation with *Pseudomonas amygdali*. Genotypes with different level of resistance to *P. amygdali* are seen in Table 1.

Table 1. Hybrids in different resistance group

Resistance level	Hybrid no.
Susceptible	1/7,2/1,3/8,4/3,5/18,6/12,7/5,8/4,9/5,10/19,11/8,12/12,13/1,14/9,16/4,16/12,17/28
Medium resistant	2/5,3/12,4/2,5/15,6/21,7/2,8/11,9/18,10/14,11/3,12/9,13/12,14/6,15/3,16/15,17/11
Resistant	1/6,2/7,3/9,4/8,5/11,6/1,7/10,8/6,9/10,10/12,11/2,12/4,13/8,16/4,17/29

In the experiment, healthy leaves of resistant and susceptible hybrids were taken. In quantitative determination of tannin, the young leaf samples were extracted two times with ethanol 80%. The content of tannin was measured by using spectrophotometry (Mısırlı *et al.*, 1994). p-DMASA was used as a reagent. In thin layer and high performance liquid chromatography, young leaf samples were dried at 65°C and extracted with ethanol 96%. For TLC; the silica gel plates (Merck 5577) were used and 50 µl of samples were applied on the right corner of each plate and developed in butanol: acetic acid: water (4:1:5) and acetic acid: water (5:95). In order to identify the classes of compounds, Naturstoff (diphenyl boric acid-2 amino ethylester) was used. Chromatograms were examined under UV light (366 nm). R_f value and colour intensity of each spot were determined. For HPLC, 250/4 nucleosil 120-5 phenyl column was used. Solvent A was 1% aqueous acetic acid, solvent B was methanol/butanol (5:1). Flow rate was 0.05 ml/min in A, 0.45 ml/min in B. Detection was carried out 290 nm wave length. 10 µl of this solution were injected into the HPLC. Peak identification was done according to the standards. Quercetin, catechin and chlorogenic acid were used as standards.

Results and discussion

The obtained data reveal that the tannin content of investigated almond hybrids display significant differences according to the types in different resistance levels Figs 1, 2 and 3. Tannin content of

susceptible, medium resistant and resistant hybrids ranged between 0.100-0.210% (Fig. 1), 0.095-0.210% (Fig. 2) and 0.075-0.210% (Fig. 3), respectively. The highest tannin content in different resistance groups was the same. Similarly, there was no correlation between tannin content and resistance to *Erwinia amylovora* (Evrenoso_lu *et al.*, 1999). On the other hand, a comparison among different groups based on the mean values showed that resistant (0.158%) and medium resistant (0.159%) hybrids contained more tannin than the others (0.150%). Confirming this, it was stated that tannin content of resistant apricot hybrids were higher than the others (Gülcan *et al.*, 1997).

Tannin content (%)

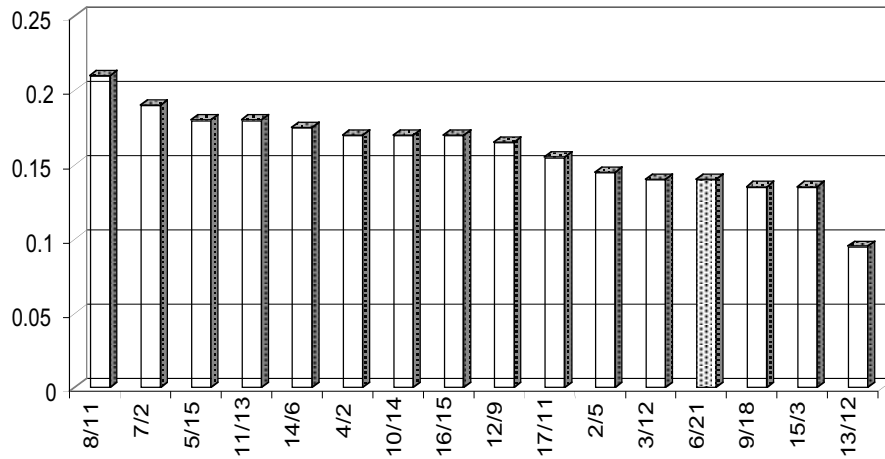


Fig. 1. Tannin content of susceptible hybrids.

Tannin content (%)

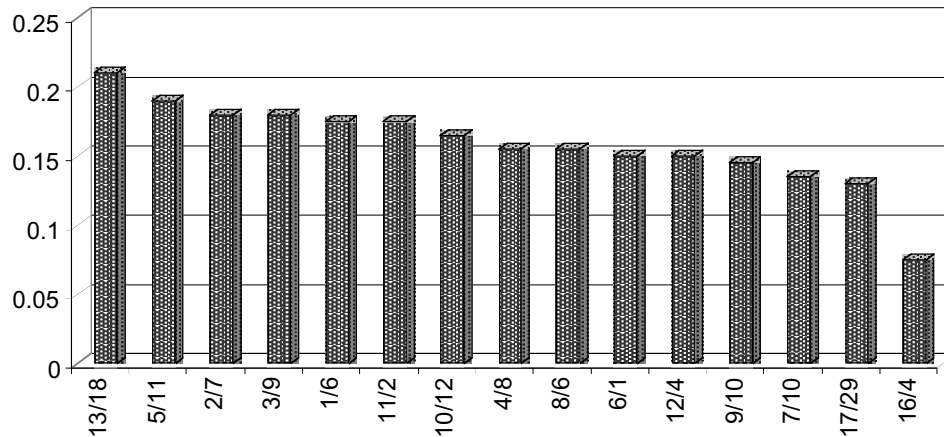


Fig. 2. Tannin content of medium resistant hybrids.

Leaf extracts of almond hybrids were investigated by thin layer chromatography. Some differences appeared in relation to distribution of spots in susceptible hybrids (Table 2). Totally, 24 different spots were determined. Spot 1 was common in all hybrids except 17/28. Spots 2 and 4 occurred in most of the hybrids. Spots 3, 5, 6, 7, 9, 12, 14, 19, 20, 23, 30 and 31 were observed in some hybrids. Spots 15, 16, 17, 18, 21, 25, 27, 28, 29, 32 and 33 which were found in the other groups were not identified in extracts from susceptible hybrids. On the contrary, spots 34 and 35 were peculiar only in two hybrids.

Tannin content (%)

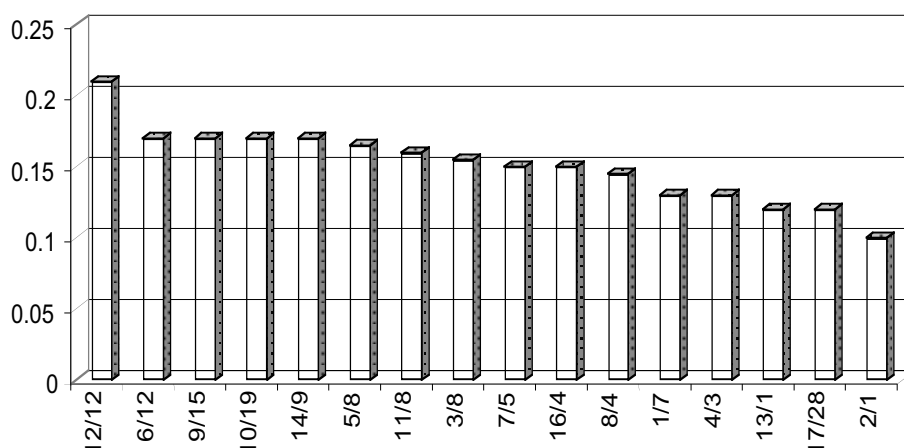


Fig. 3. Tannin content of resistant hybrids.

In the quantitative evaluation of the spots at applied concentrations, spots 4 and 9 were found to be high in some hybrids. Some spots were observed either at high or low densities according to the hybrids. The other spots were seen as faint.

The distribution of spots in medium resistant hybrids is given in Table 3. Spot number of this group was 24. Spot 1 was present in all hybrids. Spots 2, 3 and 7 were detected in many hybrids. Spots 11, 15, 16, 17, 18, 21, 25, 27, 33, 34 and 35 were absent in these hybrids, but spots 28 and 29 occurred in some hybrids.

In relative evaluation according to the size and colour of spots, spot 1 was found the highest concentration in 5/15 and 6/21. The density of the same spot differed in other hybrids. Spots 2, 7 and 9 were observed to be high in some hybrids.

25 different spots were counted on the chromatograms regarding resistant hybrids (Table 4). Spot 1 was common in all hybrids. Also, spot 2 appeared in all hybrids except 10/2. Spots 10, 12, 14, 15, 16, 17, 18, 20, 21, 25, 27, 30 and 33 were specific for some hybrids. Spots 8, 9, 13, 23, 24, 26, 28, 29, 34 and 35 were not detected in the extracts of resistant hybrids. On the other hand, spots 15, 16, 17, 18, 21, 27 and 33 were identified in extracts of resistant hybrids, however they were not found in the other groups.

When the spots were evaluated quantitatively, spot 7 had the highest concentration in 11/2. The other spots were determined to be in different concentrations.

R_f value, colour reaction and identification of spots are given in Table 5. Orange coloured spots were accepted to represent flavonoids and blue coloured spots to belong phenylpropane compounds (Tanner, 1982).

When all hybrids were considered, 35 different spots were determined (Fig. 4). Some spots (8, 9, 13, 23, 25, 26 and 34) were present only in susceptible and medium resistant hybrids. On the contrary, spots 15, 16, 17, 18, 21, 27 and 33 were characteristic for resistant hybrids. Similarly, spots 28 and 29 were characteristic for some medium resistant hybrids. Spots 34 and 35 occurred in some susceptible ones. Thus, some spots can be evaluated for distinguishing different resistance levels. The results of this experiment were found parallel to data obtained by Evrenosoğlu *et al.* (1999) in pear, Gülcan *et al.* (1997) in apricot hybrids, Mikhailova and Vishanska (1994) in apple varieties, Salle *et al.* (1994) in poplar and Plumbley and Sweetmore (1994) in yam.

Table 2. The distribution of phenolic compounds in susceptible hybrids[†][illegible]

Table 2 (cont.). The distribution of phenolic compounds in susceptible hybrids†

Spot no.	Hybrid no.															
	1/7	2/1	3/8	4/3	5/18	6/12	7/5	8/4	9/15	10/19	11/8	12/12	13/1	14/9	16/12	17/28
30				++										++		
31				++++												
32																
33																
34			++													
35														++		

†+: Little dense; +++++: Very dense.

Table 3. The distribution of phenolic compounds in medium resistant hybrids†

[illegible]

Table 3 (cont.). The distribution of phenolic compounds in medium resistant hybrids[†]

Spot no.	Hybrid no.														
	2/5	4/2	5/15	6/21	7/2	8/11	9/18	10/14	11/13	12/9	13/12	14/6	15/3	16/15	17/11
16															
17															
18															
19	+			+	+	+	+	+	++						++
20				++	+	+									
21															
22			+++								++				
23			++				++				+				
24			++	++			+++								
25															
26					+	+									
27															
28				+											
29					+	+									
30								+	++						
31		++		+			+				+++				
32											++				
33															
34															
35															

[†]+: Little dense; +++++: Very dense.

Table 4. The distribution of phenolic compounds in resistant hybrids[†][illegible]

Table 4 (cont.). The distribution of phenolic compounds in resistant hybrids[†]

Spot no.	Hybrid no.														
	1/6	2/7	3/9	4/8	5/11	6/1	7/10	8/6	9/10	10/12	11/2	12/4	13/8	16/4	17/29
30										+++					
31	++									++		+	++	++	
32													+	+	
33													+		
34															
35															

[†]+: Little dense; +++++: Very dense.

Table 5. Rf value, colour reaction and identification of spots

Spot no.	BAW [†]	AA ^{††}	Colour	Identification
1	0.54	0.32	Orange	Flavonoid
2	0.48	0.46	Orange	Flavonoid
3	0.68	0.34	Orange	Flavonoid
4	0.55	0.77	Blue	Phenylpropane
5	0.47	0.56	Orange	Flavonoid
6	0.76	0.48	Orange	Flavonoid
7	0.55	0.66	Blue	Phenylpropane
8	0.61	0.58	Blue	Phenylpropane
9	0.58	0.71	Blue	Phenylpropane
10	0.42	0.69	Blue	Phenylpropane
11	0.55	0.58	Blue	Phenylpropane
12	0.55	0.38	Orange	Flavonoid
13	0.89	0.35	Orange	Flavonoid
14	0.64	0.55	Orange	Flavonoid
15	0.66	0.32	Blue	Phenylpropane
16	0.90	0.61	Orange	Flavonoid
17	0.42	0.64	Blue	Phenylpropane
18	0.78	0.56	Blue	Phenylpropane
19	0.50	0.71	Blue	Phenylpropane
20	0.25	0.51	Orange	Flavonoid
21	0.84	0.17	Blue	Phenylpropane
22	0.37	0.80	Blue	Phenylpropane
23	0.54	0.67	Blue	Phenylpropane
24	0.50	0.45	Orange	Flavonoid
25	0.57	0.12	Orange	Flavonoid
26	0.15	0.78	Orange	Flavonoid
27	0.87	0.46	Orange	Flavonoid
28	0.42	0.43	Orange	Flavonoid
29	0.24	0.67	Orange	Flavonoid
30	0.47	0.79	Blue	Phenylpropane
31	0.49	0.61	Orange	Flavonoid
32	0.42	0.68	Orange	Flavonoid
33	0.93	0.87	Orange	Flavonoid
34	0.95	0.38	Blue	Phenylpropane
35	0.69	0.22	Blue	Phenylpropane

[†]BAW: Butan-1-ol: acetic acid: water (4:1:5).

^{††}AA: Acetic acid: water (0.95: 0.05).

Leaf phenolic compounds were determined by using high performance liquid chromatography. Figs 5-13 show the amount of phenolic compounds in HPLC procedure.

Quercetin content was found to range between 0.342-24.388 mg/g in susceptible hybrids (Fig. 5); 1.628-17.104 mg/g in medium resistant hybrids (Fig. 6) and 2.091-25.232 mg/g in resistant hybrids (Fig. 7). Mean values in relation to this compound for susceptible, medium resistant and resistant hybrids were 8.329 mg/g; 6.737 mg/g and 8.472 mg/g, respectively.

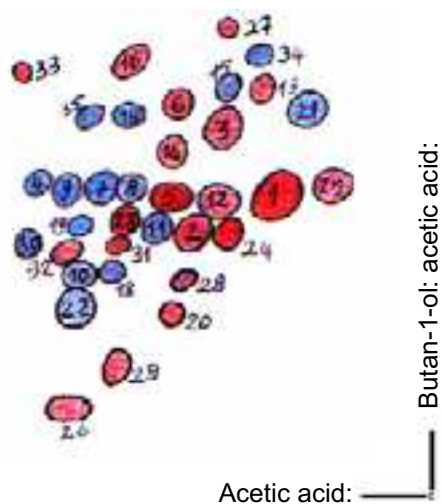


Fig. 4. The main chromatogram of spots.

Quercetin

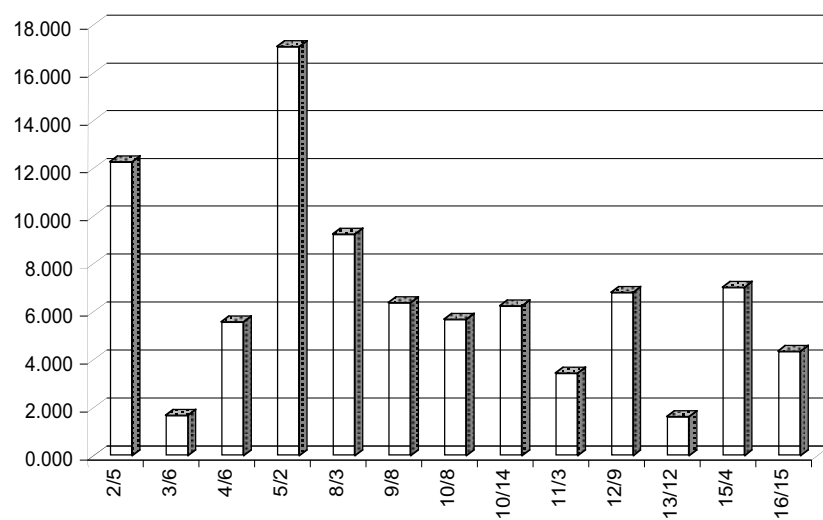


Fig. 5. Quercetin content of susceptible hybrids.

Quercetin

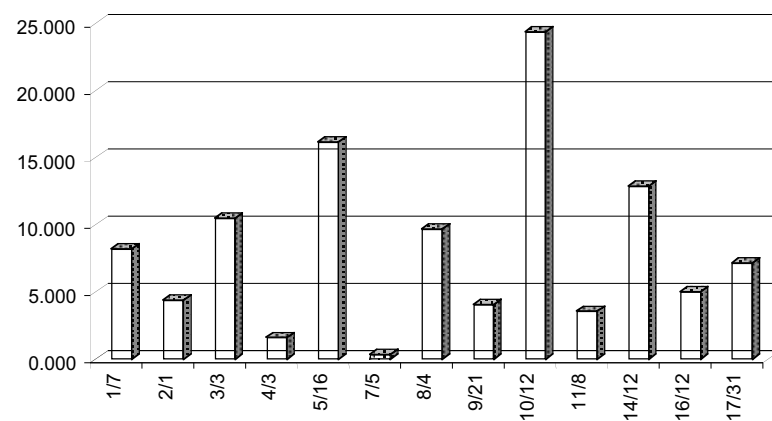


Fig. 6. Quercetin content of medium resistant hybrids.

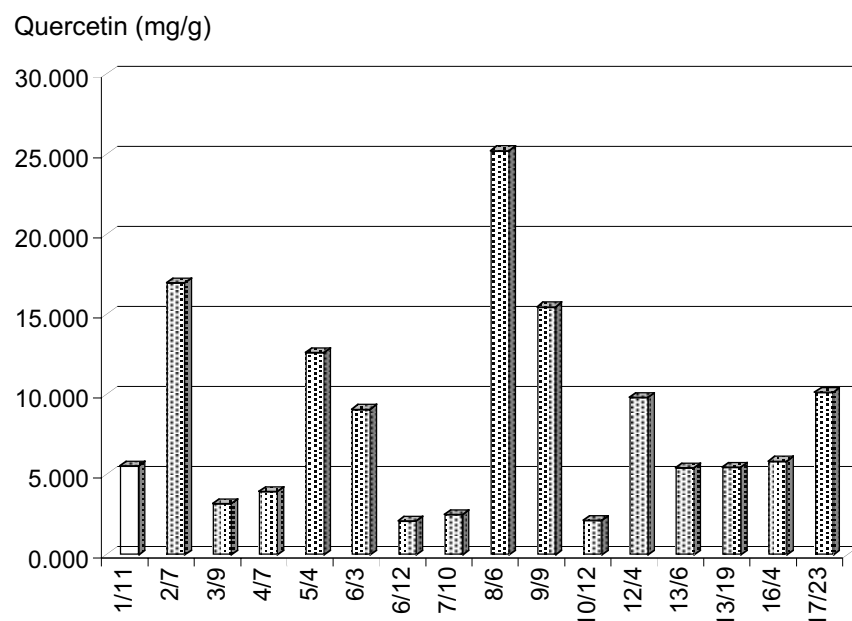


Fig. 7. Quercetin content of resistant hybrids.

The range of variation in catechin content was 0.108-1.109 mg/g for susceptible (Fig. 8), 0.047-0.528 mg/g for medium resistant (Fig. 9) and 0.093-0.704 mg/g for resistant (Fig. 10). Average values were found 0.448 mg/g, 0.337 mg/g and 0.417 mg/g, respectively. It was seen that the catechin content of resistant hybrids was lower than the susceptible ones. When cherry leaves were infected by *Blumeriella jaapii*, catechin content of susceptible ones were determined to be higher in the HPLC analysis of the leaf extracts (Niederleitner *et al.*, 1994).

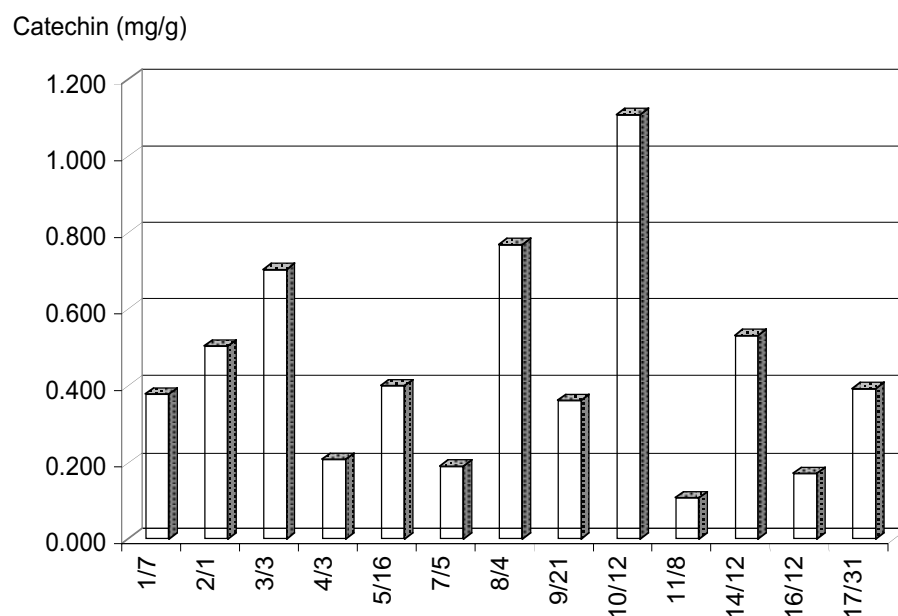


Fig. 8. Catechin content of susceptible hybrids.

Chlorogenic acid content varied between 0.007-0.077 mg/g in susceptible (Fig. 11), 0.009-0.055 mg/g in medium resistant (Fig. 12) and 0.012-0.155 mg/g in resistant groups (Fig. 13). Mean values of this compound were calculated to be 0.029 mg/g for susceptible, 0.022 mg/g for medium resistant and

0.039 mg/g for resistant. The chlorogenic acid content of resistant hybrids were higher than the susceptible group. Mondolot-Cosson and Andary (1994), showed the accumulation of caffeoylquinic derivatives in healthy zone and reported the high level of this compound in resistant variety and hybrids.

Catechin (mg/g)

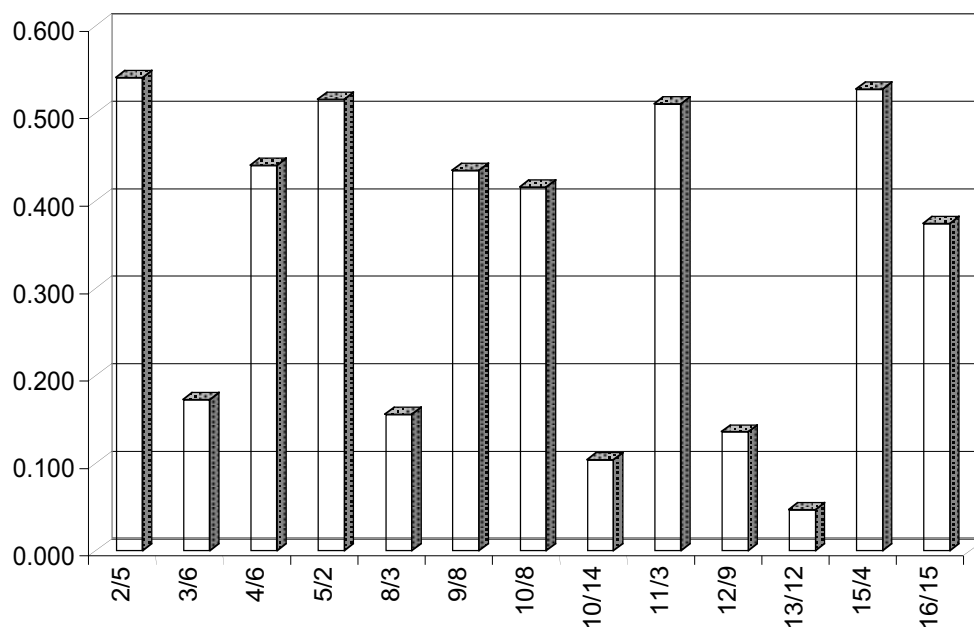


Fig. 9. Catechin content of medium-resistant hybrids.

Catechin (mg/g)

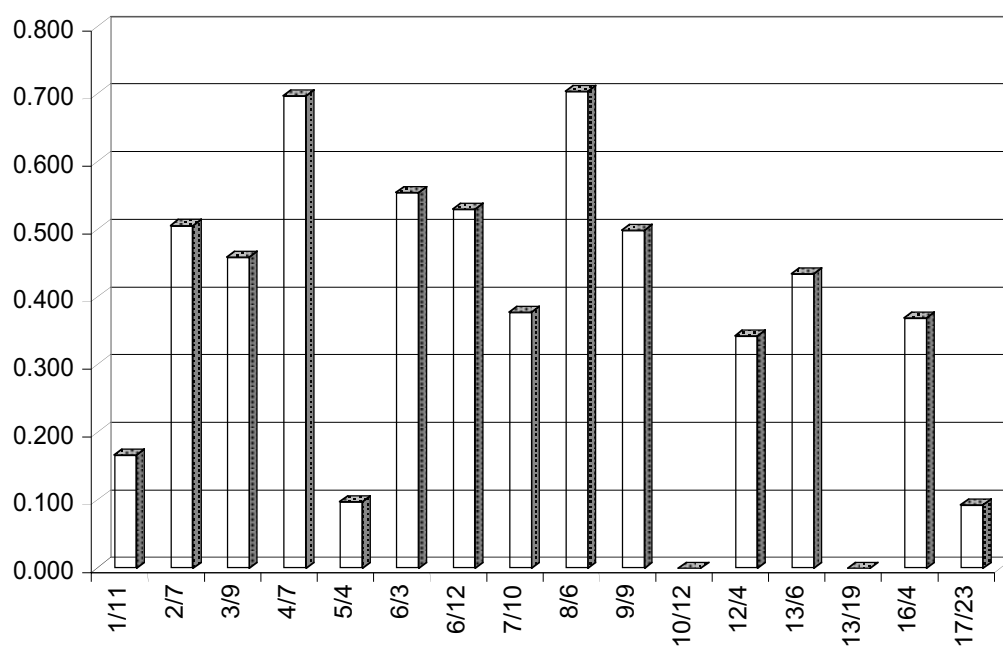


Fig. 10. Catechin content of resistant hybrids.

Chlorogenic acid

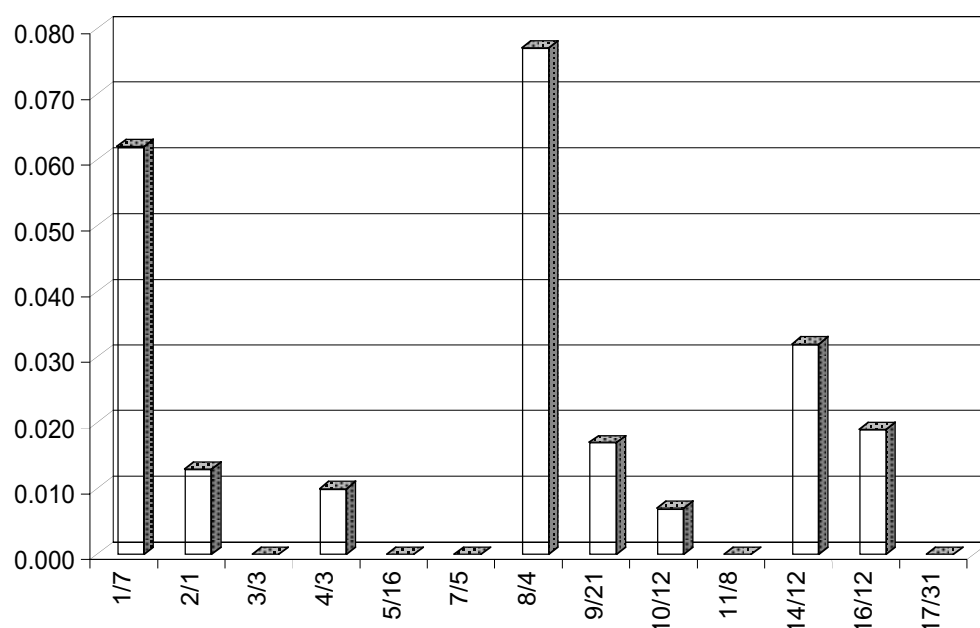


Fig. 11. Chlorogenic acid of susceptible hybrids.

Chlorogenic acid

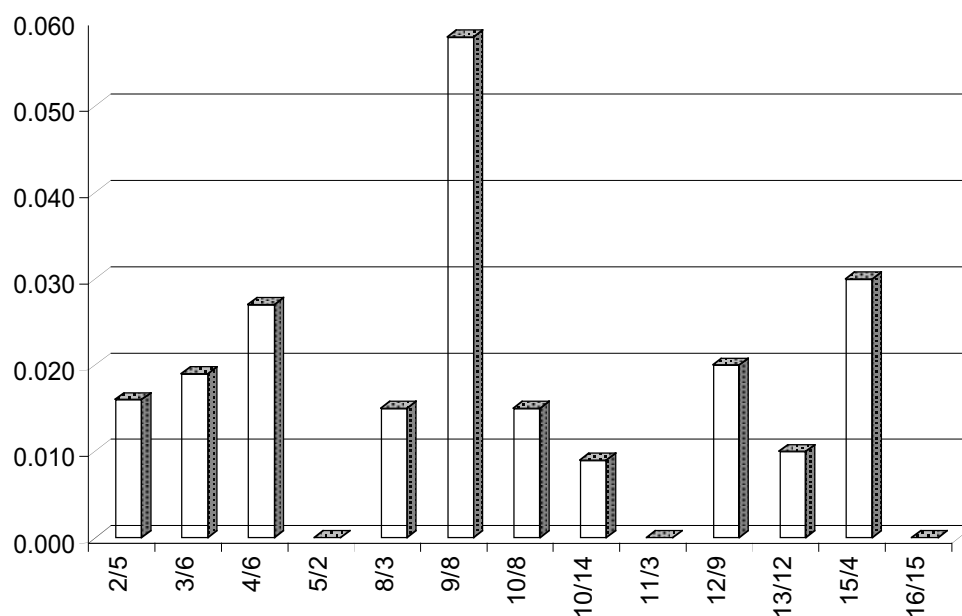


Fig. 12. Chlorogenic acid of medium resistant hybrids.

Unknown peaks were determined in HPLC separation of the leaf extract of hybrids. Their retention time and area differed from each other. Different peaks which were not found in the other hybrids occurred on the HPLC chromatograms of the medium resistant hybrids (hybrid no.: 2/5, 3/6, 5/2, 8/3 and 15/4) and resistant hybrids (hybrid no. 4/7, 6/12, 7/10, 8/6, 9/9 and 11/6). The analysis of HPLC profiles from the *M. promise* (susceptible) healthy extracts showed that two peaks were not found in Latham (moderately resistant to *D. applanata*) extracts (Kozłowska and Krzywanski, 1994).

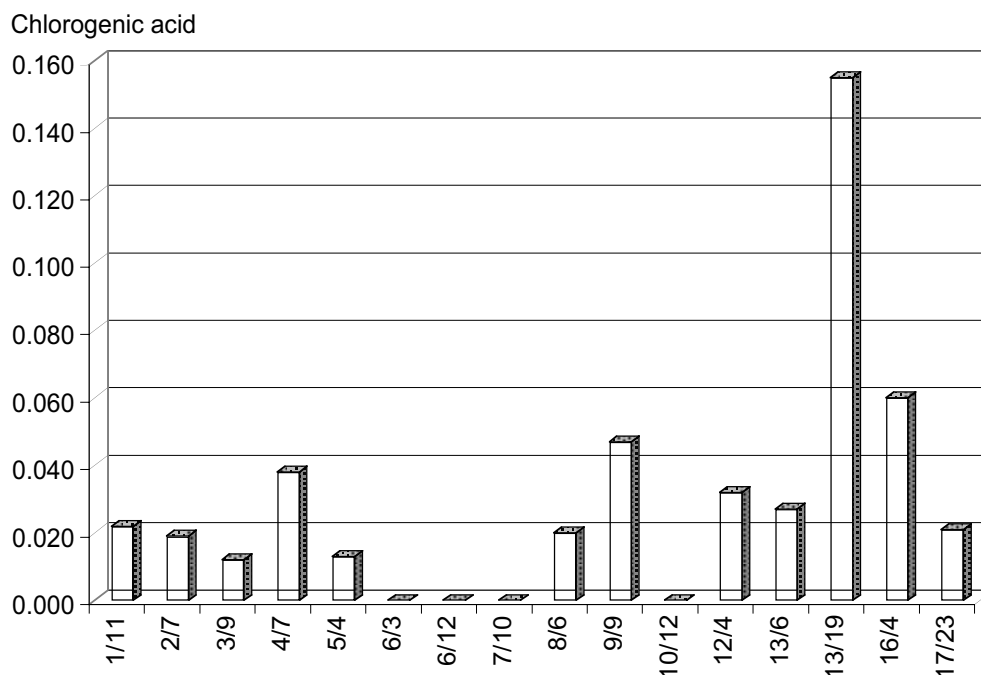


Fig. 13. Chlorogenic acid of resistant hybrids.

Conclusion

There was some qualitative and quantitative differences between the phenolic fingerprints of the sensitive and the resistant hybrids. This could constitute a quick selection criterion. In order to elucidate the role of these compounds in defence mechanisms, it is necessary to know the exact time schedule of the biosynthesis and their localisation in the tissues. To clarify differences between sensitive and less sensitive cultigens, future studies should be carried out before and after inoculation.

References

- Aver'yanov, A.A. and Lapikova, V.P. (1994). Participation of active forms of oxygen in the mechanism of ferulic acid toxicity. *Biology Bulletin* (Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya), N4: 352-357.
- Eckey-Kaltenbach, H., Ernst, D., Heller, W. and Sandermann, H.J. (1994). Cross-induction of defensive phenylpropanoid pathways in parsley plants by ozone. *Acta Horticulturae*, 381: 192-198.
- Evrenoso_lu, Y., Mısırlı, A. and Gülcan, R. (1999). Determination of phenolic compounds in pear cultivars resistant and susceptible to *Erwinia amylovora*. In: 8th International Workshop on Fire Blight, Ku_adası (Turkey), 12-15 October 1998. *Acta Horticulturae*, 489: 327-333.
- Grayer, R.J., Harborne, J.B., Kimmins, F.M., Stevenson, P.C. and Wijayagunasekera, H.N.P. (1994). Phenolics in rice phloem sap as sucking deterrents to the brown planthopper, *Nilaparvata lugens*. *Acta Horticulturae*, 381(2): 691-694.
- Grayer, R.J., Kimmins, F.M., Padgham, D.E., Harborne, J.B. and Ranga Rao, D.V. (1992). Condensed tannin levels and resistance of groundnuts (*Arachis hypogea*) against *Aphis craccivora*. *Phytochemistry*, 31: 3795-3800.
- Gülcan, R., Mısırlı, A., Özeker, E., Tengiz, F., _lbi, H., Saatçi, N. and Asma, B. (1997). Melez kayısılarda erken seleksiyon parametreleri üzerinde ara_tırmalar. TOGTAG-1432 no'lu proje, sonuç raporu.
- Hermann, K. (1988). On the occurrence of flavanol and flavone glycosides in vegetables. *Z. Lebensmittel Untersuch. Fors.*, 186: 1-5.
- Kozłowska, M. and Krzywanski, Z. (1994). The possible role of phenolic compounds in red raspberry resistance to *Didymella applanata* (Niessl) Sacc. *Acta Horticulturae*, 381: 671-674.

- Kuc, J. (1983). Induced systemic resistance in plants to diseases caused by fungi and bacteria. In: *The Dynamics of Host Defense*, Bailey, J.A. and Deverall, B.J. (eds). Academic Press, New York, pp. 192-221.
- Matern, U., Strasser, H., Wendorf, H. and Hamerski, D. (1988). Coumarins and furanocoumarins. In: *Cell Culture and Somatic Cell Genetics of Plants*, Vol. 5, *Phytochemicals in Plant Cell Cultures*, Constabel, F. and Vasil, I.K. (eds). Academic Press, New York, pp. 3-21.
- Mikhailova, N.P. and Vishanska, J.V. (1994). Phenolic acids in leaf and bark tissues of *Venturia inaequalis* susceptible and resistant apple varieties. *Acta Horticulturae*, 381: 646-649.
- Mila, I. and Scalbert, A. (1994). Tannin antimicrobial properties through iron deprivation: A new hypothesis. *Acta Horticulturae*, 381(2): 749-755.
- Mısırlı, A., Gülcan, R. and Tanrıseven, A. (1994). Determination of phenolic compounds of some almond cultivars. *Acta Horticulturae*, 373: 185-189.
- Mondolot-Cosson, L. and Andary, C. (1994). Resistance factors of a wild species of sunflower, *Helianthus resinosus* to *Sclerotinia sclerotiorum*. *Acta Horticulturae*, 381(2): 642-645.
- Niederleitner, S., Zinkernagel, V., Treutter, D. and Feucht, W. (1994). Accumulation of flavanols in cherry leaves after infection by the fungus *Blumeriella jaapii*. *Acta Horticulturae*, 381: 767-770.
- Plumbley, R.A. and Sweetmore, A. (1994). Phenolic compounds and resistance of yam (*Dioscorea alata*) to anthracnose caused by *Colletotrichum gloeosporioides*. *Acta Horticulturae*, 381: 667-670.
- Salle, G.C., Hariri, E.B. and Andary, C. (1994). Polyphenols and resistance of poplar (*Populus* spp.) to mistletoe (*Viscum album* L.). *Acta Horticulturae*, 381: 756-762.
- Tanrıseven, A. (1982). Kiraz grubu prunus türlerinde flavan içeriği ile büyüme gücü arasındaki ilişki üzerine araştırmalar. *E.Ü.Z.F. Derg.*, 19(2): 39-49.
- Tomas-Barberan, F.A., Msonthi, J.D. and Hostettmann, K. (1988). Antifungal epicuticular methylated flavonoids from *Helichrysum nitens*. *Phytochemistry*, 27: 753-755.
- Treutter, D., Schmid, P.P. and Feucht, W. (1990). Wall-bound phenols and peroxidase activity in shoots of *Prunus*. I. Isolation and identification of phenolic acids. *Gartenbauwissenschaft*, 55(2): 69-72.