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Armut (*Pyrus communis* L.) Çeşit ve Genotiplerinde Ateş Yanıklığı (*Erwinia amylovora*) Hastalığına Dayanıklılığın Biyokimyasal Göstergeler Kullanılarak Belirlenmesi

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Armut (*Pyrus communis* L.) üretimi dünya genelinde 26 milyon tonun üzerindedir ve bu üretimin yaklaşık %70'den fazlası Çin'de gerçekleştirilmektedir. Armut verimini ciddi anlamda düşüren en önemli hastalık ise bakteriyel bir etmen olan *Erwinia amylovora*'nın neden olduğu ateş yanıklığıdır. Hastalığa dayanıklılık durumunda, çeşidin genetik özelliklerinin yanı sıra fenolik maddeler gibi biyokimyasal özelliklerin de önemli rol oynadığı bilinmektedir.
Bu çalışmada, ateş yanıklığı hastalığına dayanıklı genotipler ve duyarlı çeşitlerdeki dayanıklılık durumlarının bazı kimyasal analizlerle karşılaştırılması amaçlanmıştır. Elde edilen bulgulara göre; dayanıklı genotiplerde ortalama suda çözünebilir kuru madde (SÇKM) oranı ve pH değeri istatistiksel olarak anlamlı derecede yüksek bulunurken, toplam fenol değeri, kateşin ve arbutin miktarları da dayanıklı genotiplerde duyarlı çeşitlere göre yüksek bulunmuştur. Öte yandan, TA miktarı, toplam flavonoid ve klorogenik asit miktarı ise duyarlı çeşitlerde dayanıklılara göre daha yüksek sonuç vermesine rağmen sonuçlar istatistiksel olarak önemli bulunmamıştır. Tüm çeşit ve genotipler arasında, II-14-37 numaralı genotipin, toplam fenol, toplam flavonoid, klorogenik asit, kateşin ve arbutin miktarları bakımından diğerlerine göre en yüksek değerlere sahip olduğu belirlenmiştir. Bu sonuçlar, fenolik bileşiklerin armutta ateş yanıklığına karşı dayanıklılık mekanizmasında önemli rol oynadığını ve özellikle kateşin ve arbutin seviyelerinin potansiyel biyokimyasal göstergeler olarak kullanılabileceğini, ayrıca SÇKM ve pH değerlerinin de uygun biyokimyasal analizler olarak bu sonuçlara eklenebileceğini işaret etmektedir.

Determination of Fire Blight (*Erwinia amylovora*) Resistance in Pear (*Pyrus communis* L.) Cultivars and Genotypes Using Biochemical Indicators

Pear (*Pyrus communis* L.) production worldwide exceeds 26 million tons, with over 70% of this production occurring in China. The most significant disease that seriously reduces pear yield is fire blight, caused by the bacterial pathogen *Erwinia amylovora*. In terms of disease resistance, it is known that biochemical characteristics such as phenolic compounds play an important role in addition to the genetic characteristics of the variety.
This study aimed to compare the resistance levels of fire blight disease-resistant genotypes and susceptible cultivars using certain chemical analyses. According to the results, average the soluble solid content (SSC) ratio and pH value were found to be statistically significantly higher in resistant genotypes, while the total phenol value, catechin, and arbutin amounts were also found to be higher in resistant genotypes compared to susceptible cultivars. On the other hand, although titratable acidity (TA), total flavonoid and chlorogenic acid content were higher in susceptible varieties compared to resistant varieties, the results were not statistically significant. Among all cultivars and genotypes, genotype II-14-37 was found to have the highest values for total phenol, total flavonoid, chlorogenic acid, catechin, and arbutin content compared to the others. These results indicate that phenolic compounds play an important role in the resistance mechanism against fire blight in pears and that catechin and arbutin levels, in particular, can be used as potential biochemical indicators. Furthermore, SSC and pH values can also be added to these results as appropriate biochemical analyses.

INTRODUCTION

Pear (*P. communis* L.) is a pome fruit species belonging to the Rosaceae family and the genus *Pyrus*, cultivated almost everywhere in the world where apple cultivation is widespread (Sobiczewski et al., 1997). Türkiye has significant production potential thanks to its climate and soil conditions suitable for pear cultivation and ranks fourth in the world with a production of 534.513 tons of pears according to 2023 data (FAO, 2025). However, the most important factor limiting pear production is Fire Blight disease caused by the *E. amylovora* bacteria (Mirik, 2000).

Fire blight is one of the most destructive bacterial diseases affecting many fruits in the Rosaceae family, such as pears, apples, and quinces, causing the death of flower buds, shoots, and even entire trees. Due to the environmental impacts of chemical methods and the risk of resistance development in combating the disease, the breeding of resistant cultivars is of great importance (Peil et al., 2009).

Biochemical compounds involved in the plant defense system play a critical role in the resistance of plants to diseases. Phenolic compounds, in particular, form the basis of natural defense mechanisms against plant pathogens and pests. The main phenolic compounds in pears include epicatechin, arbutin, and chlorogenic acid, and these compounds are also known to contribute to antioxidant capacity. Previous studies have found that the amounts of phenolic compounds differ between resistant and susceptible pear cultivars. In particular, substances such as chlorogenic acid and arbutin have been found to play important roles in disease resistance (Evrenosoğlu, 2002; Peil vd., 2009; Bernoville vd., 2011; Karacif, 2012; Pu vd., 2018; Skłodowska vd., 2018).

This study aims to compare the biochemical profiles (SSC, pH, TA, antioxidant capacity, total phenols, total flavonoids, and chlorogenic acid, catechin, and arbutin determined by HPLC) of pear cultivars and genotypes that are resistant and susceptible to *E. amylovora*. The data obtained will contribute to the identification of potential biochemical markers associated with resistance to fire blight.

MATERIAL AND METHOD

Material

The study material consisted one year old saplings of four commercial pear cultivars grafted on BA29 quincee rootstock (Santa Maria, Akça, Williams, Ekşi) determined to be susceptible to fire blight through inoculation studies, and four pear genotypes again cultivars grafted on BA29 quincee rootstock (I-16-18, I-16-17, I-16-28, II-14-37) determined to be resistant. Prior to identifying these cultivars and genotypes forming the two groups, inoculations were also performed on the Magness, Moonglow, Kieffer, Taş, Güz, and Ankara cultivars; however, these cultivars were not included in the biochemical analyses due to inconsistent results. In the artificial inoculation of hybrid plants, seven *E. amylovora* isolates with very high virulence levels isolated from different locations (Adana, Amasya, Bursa, Eskişehir, Karaman, and Konya) on the studies of Aysan et al. (2004), Saygılı et al. (2004), and Yılmaz and Aysan (2009) were used.

Method

The susceptibility levels of cultivars and genotypes to fire blight were determined using the artificial inoculation method (Thibault and Lezec, 1990). During inoculation, the shoots of the varieties and genotypes used were inoculated with an equal amount (0.1 ml) of bacterial suspension using a syringe at the top part. After inoculation with the pathogenic bacteria, the plants were kept in a greenhouse under conditions of 80-90% relative humidity and 27°C (Quamme, Van Der Zwet and Dirks., 1976) for 8 weeks, and routine fertilization and watering procedures were continued. The evaluation was performed using the ratio of the length of the necrotic portion to the total shoot length as a percentage. The susceptibility level was grouped according to the criteria in Table 1 based on the determined percentage blight rate (Layne ve Quamme, 1975).

$$\text{Cultivar Susceptibility Value (VS \%)} = \frac{\text{Lenght of Infected Shoot (cm)} \times 100}{\text{Total Shoot Lenght (cm)}}$$

After eight weeks, the infected portion of the marked branches on each plant was measured and recorded, and the VS values were calculated for each plant. The values were adapted according to the table shown below, as applied by Thibault et al. (1987), and the susceptibility characteristics and classes of the individuals were determined (Tablo 1) (Thibault, Lecomp, Hermann and Belouin 1987)

Table 1. Susceptibility assessment based on artificial inoculation [19]

Cultivar Susceptibility Value (VS)	0–10%	11–20%	21–40%	41–60%	61–100%
Susceptibility Class	A	B	C	D	E
Susceptibility Characteristic	Very Low	Low	Medium	High	Very High

For biochemical analyses, fruit juice samples (SSC, pH, TA, vitamin C, antioxidant capacity, total phenols) and fresh leaf samples (total flavonoids and specific phenolic compounds) were used.

The SSC and pH determined in fruit juice samples were measured using a refractometer and pH meter according to standard methods. TA was determined by titration, and the results were expressed as malic acid equivalent (mg/100 mL) (Karaçalı, 2002). Vitamin C was calculated using the iodine titration method (0.005 NI_2 solution) and the results were expressed as mg/100 mL (Spinola et al., 2013). Antioxidant Capacity was determined using the DPPH method and the results were expressed as % inhibition (Polat et al., 2018). To determine the total phenolic content, a colorimetric reaction method was used with the folin ciocalteu reagent, and readings were taken at 765 nm using a spectrophotometer. Results were given as gallic acid equivalent (mg gallic acid/L) (Selçuk and Erkan, 2016).

Total flavonoid content in fresh leaf samples was determined by measuring absorbance values at 510 nm using a catechin standard, and the results were calculated as catechin equivalents (mg catechin/L) (İçli, 2017). The determination of phenolic compounds (chlorogenic acid, catechin, arbutin) was performed using High Performance Liquid Chromatography (HPLC, Agilent 1100 series). Optimal separation was achieved by testing different mobile phases, gradient systems, and columns Ölmez, 2022).

The data were analyzed using the one-way ANOVA procedure in the Minitab-17 software package. Differences between groups were identified using the Tukey (HSD) multiple comparison test at a 5% significance level.

RESULTS AND DISCUSSION

The blight rates for parents and hybrids in the post-inoculation period, along with the susceptibility group and average, are shown in Table 2. Accordingly, Moonglow, Magness, Kieffer, Taş, Güz, and Ankara cultivars, which showed inconsistencies between artificial inoculations, were not included in the biochemical analyses.

Accordingly, Santa Maria, Akça, Williams, and Ekşi cultivars were classified in group E, the most susceptible group, with high blight rates (83.34-100.00%), while four genotypes (I-16-18, I-16-17, I-16-28, II-14-37) were classified in the most resistant group (A) with blight rates of 1.82-8.33%.

When examining the SSC levels in fruit juice samples from fire blight-resistant and susceptible cultivars and genotypes, the average SSC value of resistant genotypes (17.70%) was found to be statistically significantly higher than the average value of susceptible cultivars (11.92%) (Table 3). The significantly higher average SSC value of resistant genotypes compared to susceptible cultivars suggests that these genotypes may have a more advantageous physiological structure in terms of carbohydrate accumulation and sugar metabolism. It is known that SSC varies depending on the genotype and is directly related to the storage of photosynthetic products. The high SSC values observed in resistant genotypes suggest that these genotypes manage carbon metabolism more efficiently under stress conditions (Núñez-Lillo et al., 2024; Yin et al., 2024; Kırca and Kırca, 2025).

In fruit juice samples from fire blight-resistant genotypes and susceptible cultivars, the average pH value (4.755) in resistant genotypes was statistically higher than that in susceptible cultivars (3.875) (Table 3). Although no statistically significant difference was observed between fire blight disease-resistant genotypes and susceptible cultivars, this situation was reversed in TA values; the average value in susceptible cultivars (0.34 mg/100 mL) was higher than in resistant genotypes (0.20 mg/100 mL) (Table 3). The significantly higher pH values in resistant genotypes indicate that organic acid accumulation is more limited in these genotypes and that the acid-sugar balance in the fruit is shaped in a different direction. However, TA values did not differ statistically between the two groups ($P = 0.057$). This suggests that the relationship between pH and TA may depend not only on the total acid content but also on the type of acid composition and buffering capacity (Paulson and Stevens, 1974; Etienne et al., 2013).

No statistically significant difference was found in vitamin C content between resistant genotypes and susceptible cultivars (Table 3). Antioxidant capacity values were found to be higher in susceptible cultivars (average 58.25%) compared to resistant genotypes (average 44.25%), but this difference was again not statistically significant (Table 3). The relatively higher antioxidant capacity in susceptible cultivars indicates that defense mechanisms cannot be explained solely by antioxidant capacity levels, but this difference was again not statistically significant. This situation reveals that resistance mechanisms are more complex and must be evaluated in conjunction with the type of phenolic compounds, lignification level, cell wall structure, and signaling molecules (Yadav et al., 2020; Ninkuu et al., 2022; Wang et al., 2023).

Table 2. Percentage of blight and susceptibility groups in all cultivars and genotypes

VARIETY/ GENOTYPE	Shoot Lenght	Blight Lenght	Blight Percentage (%)	Susceptibility Class	Mean of Blight	Mean of Class
Akça	15	15	100.00	E	100.00	E
Akça	31	31	100.00	E		
Ekşi	30	30	100.00	E	100.00	E
Ekşi	26	26	100.00	E		
Williams	25	25	100.00	E	100.00	E
Williams	22	22	100.00	E		
Santa Maria	15	10	66.67	E	83.34	E
Santa Maria	13	13	100.00	E		
Moonglow	10	10	100.00	E	62.50	E
Moonglow	40	10	25.00	C		
Magness	9	1	11.11	B	55.56	D
Magness	17	17	100.00	E		
Kieffer	32	0	0.00	A	50.00	D
Kieffer	20	20	100.00	E		
Taş	37	37	100.00	E	100.00	E
Taş	28	28	100.00	E		
Güz	28	0	0.00	A	50.00	D
Güz	19	19	100.00	E		
Ankara	24	10	41.67	D	23.96	C
Ankara	16	1	6.25	A		
I-16-18 (Magness x Ankara)	25	0	0.00	A	1.82	A
I-16-18 (Magness x Ankara)	55	2	3.64	A		
I-16-17 (Magness x Ankara)	30	1	3.33	A	8.33	A
I-16-17 (Magness x Ankara)	30	4	13.33	B		
I-16-28 (Magness x Ankara)	29	0	0.00	A	3.33	A
I-16-28 (Magness x Ankara)	15	1	6.67	A		
II-14-37 (Magness x Kieffer)	45	0	0.00	A	2.43	A
II-14-37 (Magness x Kieffer)	103	5	4.85	A		

Table 3. SSC, pH, TA, vitamin C, antioxidant capacity, and total phenol content in fruit juice samples from fire blight-resistant and susceptible cultivars and genotypes

		SSC (%)	pH	TA (mg/100 ml)	Vitamin C (mg/100 mL)	Antioxidant Capacity (% İnhibisyon)	Total Phenol (mg gallik asit/ L)
Susceptible Cultivars	Santa Maria	13.00	3.86	0.43	2.73	56.00	466.00
	Akça	11.20	4.10	0.23	2.69	57.00	443.00
	Williams	10.50	3.85	0.40	2.03	65.00	326.00
	Ekşi	13.00	3.69	0.30	2.51	55.00	314.00
	Mean	**11.92±0.6 B**	**3.88±0.08 B**	**0.34±0.04 ns**	**2.49±0.16 ns**	**58.25±2.3 ns**	**387.30±39 ns**
Resistant Genotypes	I-16-18	16.90	4.75	0.13	2.47	43.00	344.00
	I-16-17	18.00	4.63	0.25	2.82	43.00	401.00
	I-16-28	17.50	4.86	0.23	2.73	45.00	329.00
	II-14-37	18.40	4.78	0.18	2.60	46.00	*565.00*
	Mean	**17.70±0.32 A**	**4.76±0.04 A**	**0.20±0.02 ns**	**2.65±0.07 ns**	**44.25±0.75 ns**	**410.00±54 ns**
	P-Value	**0.001**	**0.001**	**0.057**	**0.408**	**5.82**	**0.750**

ns: Not significant

Although the total phenolic value was slightly higher in resistant genotypes (410 mg gallic acid/L) than in susceptible cultivars (387.30 mg gallic acid/L), the difference was not statistically significant (Table 3). However, genotype II-14-37 among the resistant genotypes was found to have a significantly higher total phenolic content than other cultivars and genotypes, with a value of 565 mg gallic acid/L. The absence of statistically significant differences in total phenolic content suggests that resistance to fire blight may not be directly related to the total amount of phenolic compounds. Nevertheless, the observation of high total phenolic values in some resistant genotypes (particularly II-14-37) indicates that resistance may be related to the profile and biological activity of specific phenolic compounds rather than the total amount (Nicholson and Hammerschmidt, 1992; Lin et al., 2016; Xu and Wang, 2025).

The total flavonoid content was found to be slightly higher in susceptible cultivars (572.20 mg catechin/L) compared to resistant genotypes (536 mg catechin/L). However, this difference was not statistically significant (Table 4). This suggests that total flavonoid content alone may not be a criterion that determines resistance to diseases or environmental stresses. Flavonoids should be examined not only in terms of their total content but also in terms of their composition and types, as specific flavonoid compounds may directly affect pathogen resistance (Mierziak et al., 2014). However, the high flavonoid content (705.56 mg/L) of the II-14-37 genotype, which belongs to the resistant group, reveals that the variation between genotypes is quite significant. Changes in the flavonoid profile between different genotypes suggest that some compounds may be associated with resistance; this is more explanatory than the total flavonoid level (Ramaroson et al., 2025).

HPLC analyses revealed that the chlorogenic acid content was higher in susceptible cultivars (average 13.28 mg/L) than in resistant genotypes (10.87 mg/L). However, this difference was not statistically significant (Table 4). Nevertheless, the high chlorogenic acid content (23.02 mg/L) in the II-14-37 genotype in the resistant group suggests that this compound may contribute to defense mechanisms in some genotypes. Studies have reported that chlorogenic acid plays an antifungal and antioxidant role against pathogens, and such individual genotype differences may be important in tolerance to biotic stress (Martínez et al., 2017; Kundu and Vadassery, 2019).

When comparing the average catechin content between groups, the average for resistant cultivars (13.30 mg/L) was higher than that for susceptible cultivars (10.27 mg/L), but this difference was not statistically significant again for this character, too (P = 0.778) (Table 4). However, as observed in the last three criteria, genotype II-14-37 (40.41 mg/L) also has a significantly higher catechin content than other cultivars and genotypes, suggesting that catechin may be associated with defense in some resistant genotypes. Furthermore, catechin is defined as one of the defense metabolites that can accumulate in plants in response to pathogens, and higher levels in some genotypes have been associated with stress tolerance (Ullah et al., 2017). Therefore, the high catechin content in the II-14-37 genotype suggests that this genotype may have a more effective capacity to produce defense metabolites against biotic stress.

Table 4. Total flavonoid, chlorogenic acid, catechin, and arbutin amounts in leaf samples from fire blight-resistant and susceptible cultivars and genotypes

		Total Flavonoid (mg Catechin /L)	**Chlorogenic acid (mg/L)**	**Catechin (mg/L)**	**Arbutin (mg/L)**
Susceptible Cultivars	Santa Maria	616.67	3.18	18.96	256.00
	Akça	650.00	14.57	13.60	265.00
	Williams	516.67	18.94	8.42	183.00
	Ekşi	505.56	16.44	TE	217.00
	Mean	**572.20± 36 ns**	**13.28± 3.5 ns**	**10.27± 4.0 ns**	**230.50± 19 ns**
Resistant Genotypes	I-16-18	394.44	3.71	5.97	268.00
	I-16-17	505.56	4.48	6.64	383.00
	I-16-28	538.89	12.26	TE	246.00
	II-14-37	*705.56*	*23.02*	*40.41*	*442.00*
	Mean	**536.00± 64 ns**	**10.87± 4.5 ns**	**13.30± 9.2 ns**	**334.90± 47 ns**
	P-Value	**0.650**	**0.688**	**0.778**	**0.129**

ns: Not significant

A similar result to catechin was obtained in terms of arbutin content; the average arbutin content of resistant genotypes (334.90 mg/L) was found to be higher than the average of susceptible cultivars (230.50 mg/L). Although this difference was not statistically significant, genotype II-14-37 again had a higher value than all other cultivars and genotypes for this trait, with a value of 442 mg/L (Table 4). Arbutin is among the phenolic compounds associated with defense in pear and similar species, and high arbutin content is thought to contribute to resistance. It is known that arbutin is stored in the plant as a phenolic glycoside and is hydrolyzed to free phenol (hydroquinone) for defense in case of infection. Hydroquinone has been shown to inhibit bacterial growth. Therefore, the high concentration of arbutin in resistant genotypes may also represent a "pre-defense" potential against infection (Hildebrand and Schroth, 1963; Boyraz and Sürel, 2004; Xu et al., 2025).

However, the fact that total phenol, total flavonoid, and chlorogenic acid levels are higher or statistically similar in resistant cultivars indicates that the quality of phenolic compounds (which specific phenolics are present) and the rate of release of free phenols from glycosides are more decisive factors in resistance than the total amount of phenolic compounds. For example, the high antioxidant capacity in susceptible cultivars may indicate that compounds with high antioxidant activity but low direct toxicity against *E. amylovora* are more prevalent in these cultivars (Bhattacharya et al, 2010; Skłodowska et al., 2018; Wallis and Galarneau, 2020).

CONCLUSION

In conclusion, fire blight-resistant pear genotypes are significantly different from susceptible cultivars in terms of fruit quality characteristics, particularly SSC and pH. Accordingly, it has been noted that these parameters are the primary parameters that can be used in comparing the resistance of varieties. However, biochemical parameters such as antioxidant capacity, vitamin C, and total phenols alone are insufficient to explain resistance. These findings indicate that fire blight resistance is a multifactorial trait and that further studies should focus on detailed analysis of phenolic compound profiles, enzymatic defense systems, and molecular-level mechanisms.

Additionally, no statistically significant differences were found in the phenolic compounds examined between susceptible and resistant cultivars and genotypes; however, a notable trend emerged in resistant genotypes, where arbutin and certain individual phenolic compounds were found at higher levels. This suggests that resistance may be related to the presence of specific phenolic compounds and their genotype-specific distribution rather than the total phenolic content. Therefore, future studies examining the phenolic profile under a wider range of genotypes, different environmental conditions, and stress treatments will contribute to a clearer understanding of the resistance mechanisms in pears.

The II-14-37 genotype stands out for having the highest values in all phenolic parameters examined (total phenols, total flavonoids, chlorogenic acid, catechin, and arbutin). This indicates that this genotype possesses a superior chemical defense profile compared to other genotypes in terms of both quantity and potential efficacy, making it a valuable resource for breeding programs.

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