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RESEARCH ARTICLE

From Defense to Resilience: Exploring the Metabolic and Biochemical Responses of Beans to Halo and Common Blight Pathogens

Badel UYSAL SAHIN[1], Kubilay Kurtulus BASTAS[2], and Ercan CEYHAN[3]
[1]Mersin Agricultural Quarantine Directorate, Mersin/Turkey.
[2]Department of Plant Protection, Faculty of Agriculture, Selcuk University, Konya /Turkey.
[3]Department of Crop Fields, Faculty of Agriculture, Selcuk University, Konya /Turkey.

Abstract

B. UYSAL SAHIN, K. K. BASTAS, and E. CEYHAN. 2025. From Defense to Resilience: Exploring the Metabolic and Biochemical Responses of Beans to Halo and Common Blight Pathogens. Int. J. Agric. Nat. Resour. 40-54. In the context of bean production, the insidious presence of *Pseudomonas savastanoi pv. phaseolicola (Psp)* and *Xanthomonas axonopodis pv. phaseoli (Xap)* is a formidable challenge, exerting significant negative impacts in Türkiye. This study aimed to estimate the chlorophyll carotenoid contents and phenolic compounds to determine the effects of pathogen damage on bean pigment contents. Physiological and biochemical changes were observed after bacterial pathogens attacked susceptible and resistant bean plants. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were measured using a spectrophotometer 72 h after pathogen inoculation, and phenolic compounds were measured by high-performance liquid chromatography (HPLC) at 12 and 72 h after Xap and Psp inoculation in susceptible and resistant bean plants. Although the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the leaves of cultivar Aras-98 decreased after both pathogen treatments, there was no change in the leaves of the 36K genotype. HPLC revealed the presence of various amounts of phenolic compounds, namely, gallic, catechin, chlorogenic, caffeic, syringic, p-coumaric, rutin, q-coumaric, myricetin, and quercetin. In this study, the intricate ways in which pathogens influence metabolic processes within bean leaf tissue were investigated, with a focus on the resilient 36K and susceptible Aras98 genotypes. The insights obtained from our research are useful not only for our understanding of plant–pathogen interactions but also as a foundational cornerstone for future endeavors in molecular pathological breeding. These findings provide guidance, illuminating pathways for the cultivation of robust, disease-resistant bean varieties.

Keywords: Chlorophyll, carotenoid, phenolic compounds, Pseudomonas, Xanthomonas

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Corresponding author: kbastas@selcuk.edu.tr

Highlights

- Differential pigment responses: Pathogen inoculation (Xap and Psp) significantly reduced chlorophyll and carotenoid levels in the susceptible bean cultivar *Aras98*, whereas the resistant genotype *36K* maintained stable pigment content, highlighting its resilience to pathogen stress.

- Phenolic compound dynamics: HPLC analysis identified 10 phenolic compounds in both genotypes. The 36K genotype showed early and strong induction of defense-related compounds like gallic acid, catechin, chlorogenic acid, and quercetin post-inoculation.

- Gallic acid as a key defense marker: Gallic acid levels increased significantly after pathogen exposure—by 157% in *36K* and 53% in *Aras98*—suggesting a potential role in early immune signaling and pathogen resistance.

- Resilient recovery in 36K genotype: Although transient reductions in chlorophyll pigments occurred in 36K after bacterial inoculation, pigment levels recovered by 72 hours, unlike in Aras98, where reductions persisted.

- Breeding implications: The study underscores the utility of metabolic and biochemical profiling in identifying disease-resilient genotypes, offering valuable insights for future bean breeding programs to enhance bacterial pathogen resistance.

Introduction

The common bean *Phaseolus vulgaris* L. (*Leguminosae*) is a widely cultivated legume worldwide. It is highly valued for its rich nutritional content and economic significance (Ceyhan, 2004; Popović et al., 2012; Tekin & Ceyhan, 2023). Owing to its high levels of protein, starch, dietary fiber, vitamins, minerals, and natural antioxidants, it is widely regarded as one of the most important legumes in the world. Studies suggest that including common beans in our diet can potentially help lower blood cholesterol and reduce the risk of chronic diseases (Ceyhan et al., 2014; Tekin & Ceyhan, 2022; Tamüksek & Ceyhan, 2022).

Approximately 55 million tons of common bean are produced worldwide, and in Türkiye it is the third most cultivated legume after chickpeas and lentils, with a cultivation area of 108,000 hectares and a production of 305,000 tons (FAO, 2024). Common beans are an inexpensive source of protein in human nutrition, making them a popular choice for both farmers and consumers (Ceyhan, 2004; Ulker & Ceyhan, 2008).

Pseudomonas savastanoi pv. *phaseolicola* (Psp) and *Xanthomonas axonopodis* pv. *phaseoli* (Xap) are bacterial pathogens that cause serious diseases in bean crops. Psp causes bacterial spot disease, which is common in broad bean and other bean species. This disease can cause spotting on leaves, stems, and pods, necrosis, and eventually death of the plant. Xap causes bacterial leaf spot disease in broad bean plants. This disease can cause yellowing, spotting, and eventually defoliation of leaves, reducing plant productivity. Both pathogens usually spread under humid and hot weather conditions and can seriously affect plant health (Coyne et al., 1994; Kahveci & Maden, 1994; Güven et al., 2004). In bean cultivation, strategies such as appropriate agricultural practices, the use of resistant varieties, and chemical control are widely used for disease control (Singh & Schwartz, 2010).

In Türkiye, halo blight caused by Psp and common bacterial blight caused by Xap are the most common foliar and bacterial diseases of beans that have also been reported (Benlioğlu et al., 1994; Demir & Gündoğdu, 1994; Kahveci & Maden, 1994; Güven et al., 2004; Bastas & Sahin, 2017). Bacterial diseases cause yield losses of up to 45%,

depending on bean variety and environmental conditions (Singh & Schwartz, 2010). Psp and Xap cause serious problems in bean production areas and are widespread in the field. Both pathogens cause severe seed-borne bacterial diseases, causing crop loss and disorders in terms of seed quality (Coyne et al., 1994).

The typical symptom of Psp is wet-looking lesions surrounded by a yellow halo produced by the release of phaseolotoxin. Over time, these lesions develop into small necrotic spots, ranging from 3 to 6 mm in diameter, on the leaf, pod, and stem. The yellow halos surrounding these necrotic areas can reach up to 2.5 cm in diameter (Bender et al., 1999; Arnold et al., 2011). Xap causes infection in all above-ground plant parts, but symptoms in leaves and pods are more severe. The irregular necrotic lesions on the leaf and the light lemon-yellow halo surrounding these lesions are typical symptoms. Severe infections, especially those caused by high humidity, high temperature, abundant rainfall, and subsequent air drying, can lead to more than 40% yield losses (Saettler, 1989).

Understanding plant defense mechanisms against pathogens is essential for safeguarding global food security and breeding disease-resistant crops (Freeman & Beattie, 2008). Plant disease resistance mechanisms play a vital role in sustainable agriculture by minimizing reliance on chemical treatments and promoting environmentally friendly farming practices (Waller et al., 2005).

Phenolic compounds are essential for the defense mechanism of plants and help to protect against various pests, including viruses, bacteria, and fungi (Bohn, 2014). Phenolic acids are produced in the cell walls of plants in response to pathogens through the phenylpropanoid pathway, leading to the creation of cinnamic acid and benzoic acid derivatives (de Ascensao & Dubery, 2003). The pigment content of leaves can provide important information about the physiological status of plants (Gitelson & Merzlyak, 2004). Among the different pigments, chlorophyll a and chlorophyll b are the most important in plants, and their levels can affect a plant's ability to produce energy through photosynthesis (Jin et al., 2019). Photosynthesis is a vital process in plant physiology and plays a crucial role in plant defense against biotic stress (Pérez-Bueno et al., 2019).

To date, research on various bean cultivars has focused on the effects of biotic and abiotic stress factors on plant biochemistry, particularly concerning the Psp and Xap pathogens. However, studies addressing bacterial pathogens have focused primarily on determining the responses of different cultivars in terms of metabolic and biochemical reactions. In these studies, the effects of pathogens on the activities of enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POX), have been investigated (Rudolph & Stahmann, 1964; Adam et al., 1995; Farahani & Taghavi, 2016).

This study aimed to explore the metabolic and biochemical responses of bean plants to diseases caused by halos and bacterial blight pathogens. Understanding the defense mechanisms of plants against these pathogens and their strategies for disease control may help in the development of more effective and sustainable approaches for plant disease management. The findings obtained may help us better understand the mechanisms of bean plants in disease control and to develop more effective protection strategies in future agricultural practices.

Materials and methods

Plant material and growth conditions

Seeds of 36K genotype (the tolerant genotype was obtained by Prof. Dr. Erdal Elkoca from Atatürk University, Faculty of Agriculture, Field Crops Department Seed Collection) and Aras98 (susceptible cultivar from Eastern Anatolia Agricultural Research

Institute) were used in the experiments. The seeds were soaked in a 2% sodium hypochlorite solution for 2 minutes for superficial sterilization and then washed three times with sterile distilled water. Six seeds were then planted in each pot (23.7 × 19.2 cm in diameter) with a mixture of sterile peat, soil, and sand (at a ratio of 1:1:1) and grown in a controlled climate chamber with a photoperiod of 16 hours of light and 8 hours of darkness at a temperature of 27–28 °C and a relative humidity of 65–70% (Gökmen & Ceyhan, 2015). Samples were taken from 2 bean plants (28 days old) after thinning. All samples were obtained from a uniform location on the plant to ensure consistency.

Bacterial growth and inoculation

The Xap strain 120-x and Psp strain 522-p pathogens were obtained from Dr. MF Dönmez, Igdır University, Turkey. Suspensions prepared from fresh 48-hour cultures of the pathogens grown on nutrient agar (NA) were applied to 6-week-old Aras 98 variety bean plants at the quantities and methods specified below to determine their virulence levels. The virulence factors were found to be Xap strain 120-x (84%) and Psp strain 522-p (82%). Owing to their high virulence, these strains were selected for use in the experiments. The strains were subsequently grown on NA media (peptone from meat, 5 g L^{-1}; meat extract, 3 g L^{-1}; agar extract, 12 g L^{-1}) for 48 h at 27–28 °C. A cell suspension (Spectrophotometric, OD_{660}: 0.15) of 10^8 CFU ml^{-1} was made individually from both bacterial pathogens. The bacterial suspensions were sprayed by a pressure hand sprayer on the leaves of bean plants on the 28th day until full wetting occurred, and on average, 50 ml of bacterial suspension was sprayed per plant. The plants in the control group were sprayed with sterile distilled water.

Sample collection

Two-gram leaf samples from twenty-eight-day-old seedlings with 8–10 leaves were collected from both pathogen-inoculated and noninoculated control plants at the critical 12 and 72 h to assess defense reactions. The samples were then stored at -80 °C until all analyses were performed.

For the experiments and analyses, three replications were performed for each treatment, with two bean plants used per replication.

Estimation of chlorophyll and carotenoid contents

The procedure used to extract chlorophyll and carotenoids from the leaf samples involved grinding the leaves with a mortar and pestle with 4 ml of 80% acetone. The resulting mixture was centrifuged at 6000 rpm for 10 minutes, and the clear liquid obtained from the centrifugation was stored. The solid residues were re-extracted with 4 ml of 80% acetone. The two extracts were mixed, and the total volume was increased to 10 ml with 80% acetone. These extracts were then used to estimate chlorophyll and carotenoid concentrations. The levels of chlorophyll and carotenoids were measured using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan), with 80% acetone used as a blank, and the absorbance was measured at 645, 663, and 470 nm. In accordance with Arnon (1949), formulae were used to determine the concentrations of chlorophyll a, b, and total chlorophyll, whereas in accordance with Lichtenthaler and Wellburn (1983), the formula was used to calculate carotenoid concentrations. The resulting amounts of chlorophyll a (Ca), chlorophyll b (Cb), total chlorophyll (C), and carotenoids (Car) were expressed in micrograms per microliter.

$$C_a\ (\mu g\ ml^{-1}) = 12.7\ D_{663} - 2.69\ D_{645} \quad (1)$$

$$C_b\ (\mu g\ ml^{-1}) = 22.9\ D_{645} - 4.68\ D_{663} \quad (2)$$

$$C\ (\mu g\ ml^{-1}) = 20.2\ D_{645} + 8.02\ D_{663} \quad (3)$$

$$Car\ (\mu g\ ml^{-1}) = (1000A_{470} - 3.27[chl\ a] - 104[chlb])/227 \quad (4)$$

Extraction and analysis of phenolic compounds

The modified method used for the extraction and analysis of phenolic compounds in leaf samples

was based on the method developed by Aaby et al. (2007). First, 5 grams of each leaf sample was crushed and mixed with 10 mL of a solvent consisting of 50% water and 50% acetonitrile. The mixture was then centrifuged at 15,000 rpm and 4 °C for 15 minutes. After centrifugation, the supernatant was collected for further analysis of phenolic extracts using high-performance liquid chromatography (HPLC). The equipment used for this study included an LC-20 AT pump, a CTO-20A column oven, and a SPDM20A prominence diode-array detector, along with a SIL-20A HT autosampler from Shimadzu Corp. in Kyoto, Japan. Data collection and processing were performed using LabSolutions LC software from the same company, with readings taken at 273 and 370 nm. Before injection, each extract was filtered through a 0.45 µL nylon filter from Millipore Corp. in Billerica, USA. Chromatographic separations were carried out on an Inertsilr ODS-3V column (250 mm × 4.6 mm i.d., 5 µm particle size) from GL Sciences in Tokyo, Japan, at a temperature of 40 °C. The mobile phases used were (A) acetic acid/water (2:98, v/v), (B) 50% aqueous acetonitrile/0.5% aqueous acetic acid (1:1, v/v), and (C) acetonitrile. The gradient program described in Table 1 (Pehluvan et al., 2015) was followed, with a flow rate of 1.2 mL min^{-1} and a total running time per sample of 60 minutes. The quantification of individual phenolic acids (chlorogenic acid, caffeic acid, syringic acid, q-coumaric acid, p-coumaric acid) and flavonoids (catechin, myricetin, quercetin, rutin) was performed using regression curves calculated for authentic standards purchased from Sigma–Aldrich in Steinheim, Germany. The calibration curves showed a good linear relationship, with correlation coefficients above 0.999. The concentration data are presented as mg kg^{-1} fresh weight (FW). Identification was carried out based on retention times and UV spectra.

Table 1. Mobile phase gradient program for HPLC analysis of phenolic extracts from leaves of susceptible and resistant bean plants.

Time (min)	Solutions (%)		
	A	B	C
0	95	5	0
5	95	5	0
8	80	20	0
10	78	22	0
17	75	25	0
19	73	27	0
30	60	40	0
35	55	45	0
40	35	65	0
45	0	10	90
50	0	0	100
52	95	5	0
60	95	5	0

Solution A: Acetic acid/water (2:98, v/v), Solution B: %50 aqueous acetonitrile/0.5% aqueous acetic acid (1:1, v/v), Solution C: acetonitrile.

Statistical analysis

The pot experiments were established in a completely randomized design, and the pots of each treatment were set in triplicate. The statistical program used for data analysis was R version 4.0.2, which included an analysis of variance to compare mean differences. The significant treatment differences were determined using the means. MSTAT software was used to perform the data analysis, whereas the Tukey multiple range test was used to determine treatment differences at a significance level of $p<0.05$.

Results

Chlorophyll and carotenoid contents in bean leaves after bacterial pathogen inoculation

This study revealed that the presence of Xap and Psp pathogens had no effect on the levels of chlorophyll a, chlorophyll b, total chlorophyll, or carotenoid in the leaves of 36K genotype plants compared with those in healthy plants. However, in the leaves of cultivar Aras98, significant differences in the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content were detected between the control group and infected plants. The chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were

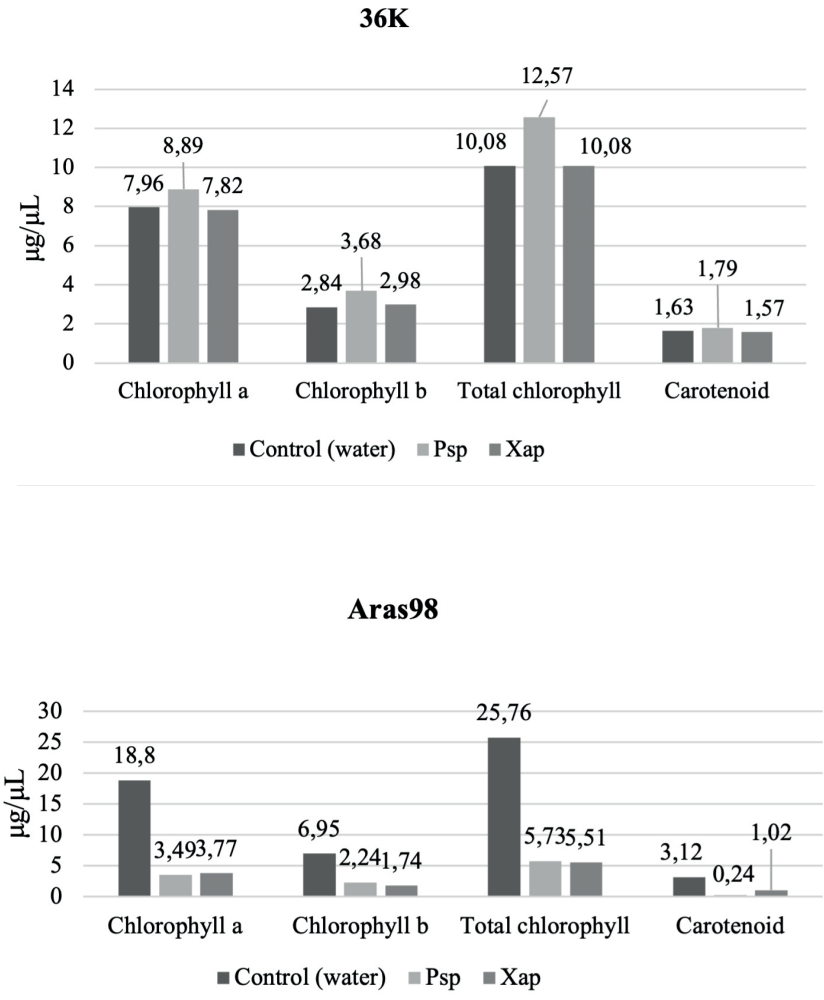

Figure 1. (a) Effects of Psp and Xap inoculation on chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents in the leaves of the 36K genotype after 72 hours; (b) effects of Psp and Xap inoculation on chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents in the leaves of the cultivar Aras98 after 72 hours

Table 2. The chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents changed 72 hpi after the inoculation of cv. 36K genotype and cv. Aras-98 leaves with Psp and Xap.

Compound	36K			Aras-98		
	Control (water)	Psp	Xap	Control (water)	Psp	Xap
Chlorophyll a	7,96±0,01 **a**	8,89±1,38 **a**	7,82±0,04 **a**	18,8±1,79 **a**	3,49±0,6 **b**	3,77±0,04 **b**
Chlorophyll b	2,84±0,01 **a**	3,68±0,64 **a**	2,98±0,05 **a**	6,95±0,75 **a**	2,24±0,96 **b**	1,74±0,01 **b**
Total chlorophyll	10,08±0,01 **a**	12,57±2,02 **a**	10,08±0,01 **a**	25,76±2,55 **a**	5,73±1,56 **b**	5,51±0,02 **b**
Carotenoid	1,63±0,01 **a**	1,79±0,3 **a**	1,57±0,01 **a**	3,12±0,29 **a**	0,24±0,21 **b**	1,02±0,3 **b**

reduced in the susceptible bean leaves at 72 h after inoculation with the Xap and Psp pathogens (Table 2; Figure 1).

Phenolic compounds in bean leaves

After bacterial pathogen inoculation, ten phenolic acids were identified in both susceptible and resistant bean cultivars (Tables 3 and 4, Figures 2, 3 and 4).

After 12 h of Xap and Psp inoculation, gallic, catechin, chlorogenic, rutin, and quercetin increased compared with those of healthy plants, whereas caffeic acid decreased in leaves of 36K genotype. Syringia increased after Psp but decreased after Xap at 12 h in leaves of 36K genotype plants. q-Coumaric acid and myricetin increased after Psp, and there was no change after Xap inoculation at 12h in leaves of 36K genotype plants. Compared with that in healthy plants, p-coumaric capacity in 36Kgenotypeplants was lower at 12 h after inoculation with Psp, whereas it was greater after Xap inoculation in 36K genotype plants. At 72h, the levels of catechin, chlorogenic, caffeic, and quercetin in the 36K genotype were reduced after the inoculation of both bacterial pathogens compared with those in healthy plants. Syringic acid and p-coumaric acid levels in the 36K genotype at 72 h were reduced after Psp inoculation, but there was no change after Xap inoculation. Rutin increased after the inoculation of Psp but decreased after Xap inoculation in the 36K genotype at 72 h. The amount of Q-coumaric acid present at 72

Table 3. Changes in the contents of phenolic compounds in the leaves of cv. 36Kgenotype inoculated with Psp or Xap

Compound	12hpi			72hpi		
	Control (water)	Psp	Xap	Control (water)	Psp	Xap
Gallic	18,93±0,01 e	47,24±0,01 **a**	20,25±0,01**d**	22,35±0,05 **c**	30,73±0,19 **b**	15,32±0,04 **f**
Catechin	54,70±11,40 **c**	60,57±0,89 **c**	92,24±3,64 **b**	167,97±10,06 **a**	116,30±4,57 **b**	107,62±6,44 **b**
Chlorogenic	274,98±0,52 **e**	308,28±1,24 **d**	370,39±1,20 **b**	452,27±0,59 **a**	14,96±0,00 **f**	336,83±1,56 **c**
Caffeic	10,64±0,06 **c**	8,65±0,16 **cd**	8,69±0,10 **b**	11,04±0,07 **a**	8,29±0,03 **f**	8,36±0,01 **de**
Syringic	11,60±0,00 **b**	16,98±0,39 **a**	9,44±2,14 **bc**	7,78±0,07 **c**	11,85±0,09 **b**	7,70±1,04 **c**
p-coumaric	8,00±0,01 **c**	7,51±0,01 **d**	9,24±0,03 **a**	8,24±0,02 **b**	6,52±0,00 **e**	8,26±0,00 **b**
Rutin	6,89±0,02 **e**	12,19±0,17 **b**	8,60±0,89 **d**	10,68±0,34 **c**	26,19±0,35 **a**	8,59±0,06 **d**
q-coumaric	4,33±0,30 **c**	18,02±0,99 **a**	4,97±0,09 **c**	10,91±2,41 **b**	11,14±1,76 **b**	7,71±1,05 **bc**
Myricetin	26,90±0,13 **b**	3,99±0,59 **c**	24,10±7,00 **b**	4,83±0,79 **c**	7,62±0,02 **c**	79,45±8,58 **a**
Quercetin	23,30±1,33 **bc**	41,01±1,53 **a**	33,22±0,19 **ab**	38,01±1,00 **a**	120,13±8,84 **c**	31,66±0,11 **ac**

Table 4. Changes in the contents of phenolic compounds in the leaves of cultivar Aras-98 inoculated with Psp or Xap

Compound	12hpi			72hpi		
	Control (water)	Psp	Xap	Control (water)	Psp	Xap
Gallic	21,50±0,14 **b**	33,09±0,23 **a**	19,53±0,01 **c**	17,59±0,53 **d**	18,41±0,01 **d**	18,32±0,02 **d**
Catechin	61,93±0,44 **b**	41,10±2,70 **bc**	30,58±0,24 **c**	58,01±0,19 **bc**	145,77±19,25 **a**	31,68±0,03 **c**
Chlorogenic	13,29±0,19 **b**	16,88±0,04 **a**	8,12±0,07 **e**	7,91±0,33 **e**	11,64±0,13 **c**	9,67±0,05 d
Caffeic	6,88±0,04 **b**	12,77±0,15 **a**	6,85±0,07 **b**	7,22±0,02 **b**	8,04±1,35 **b**	6,82±0,06 **b**
Syringic	6,60±0,00 **e**	14,95±0,06 **a**	7,21±0,22 **d**	7,58±0,01 **c**	11,08±0,09 **b**	10,88±0,05 **b**
p-coumaric	6,61±0,01 **b**	12,84±0,02 **a**	6,92±0,02 **b**	6,94±0,02 **b**	7,79±0,01 **a**	6,72±0,01 **b**
Rutin	8,84±0,02 **c**	38,68±0,64 **a**	18,60±0,63 **b**	11,68±0,08 **c**	8,23±0,37 **c**	41,04±2,57 **a**
q-coumaric	11,08±1,50 **cd**	14,78±2,82 **bc**	81,99±1,38 **a**	18,91±0,01 **b**	6,72±0,72 **d**	10,03±0,09 **cd**
Myricetin	15,27±1,81 **b**	10,68±0,03 **bc**	37,58±4,87 **a**	4,14±0,24 **c**	40,48±0,42 **a**	9,29±0,05 **bc**
Quercetin	28,30±0,13 **d**	66,62±1,21 **a**	36,49±0,89 **c**	29,85±0,92 **d**	27,02±1,78 **d**	49,26±0,05 **b**

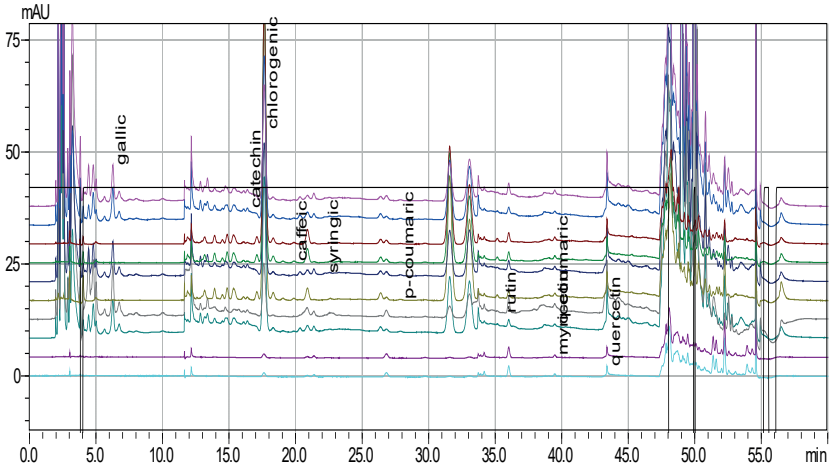

Figure 2. Retention times of phenolic compounds in bean plants after pathogen inoculation

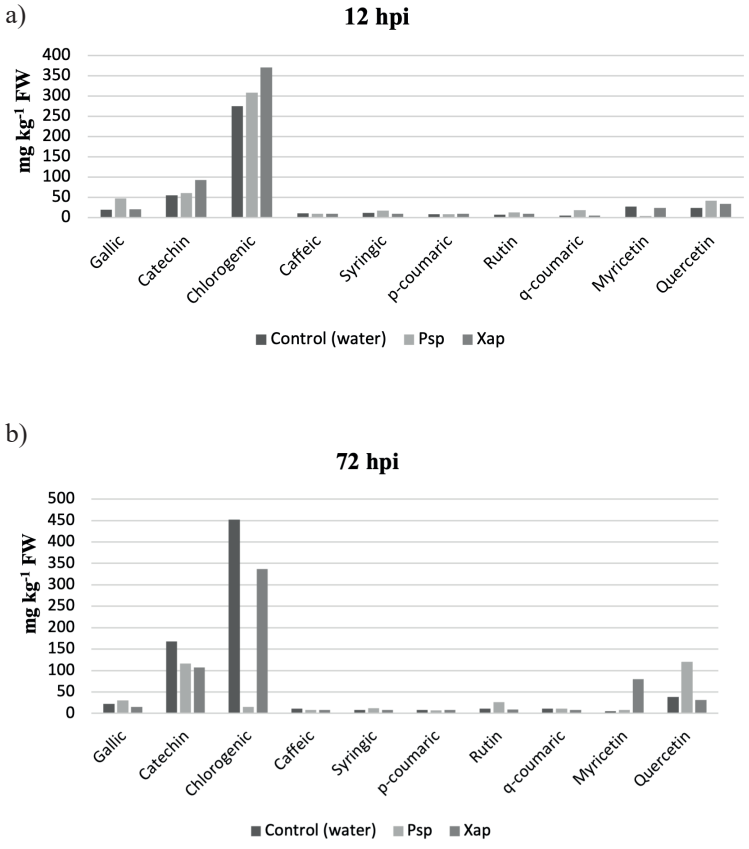

Figure 3. (a) Changes in the content of phenolic compounds in leaves of the 36K genotype inoculated with Psp and Xap after 12 hours. (b) Changes in the content of phenolic compounds in leaves of the 36K genotype inoculated with Psp and Xap after 72 hours.

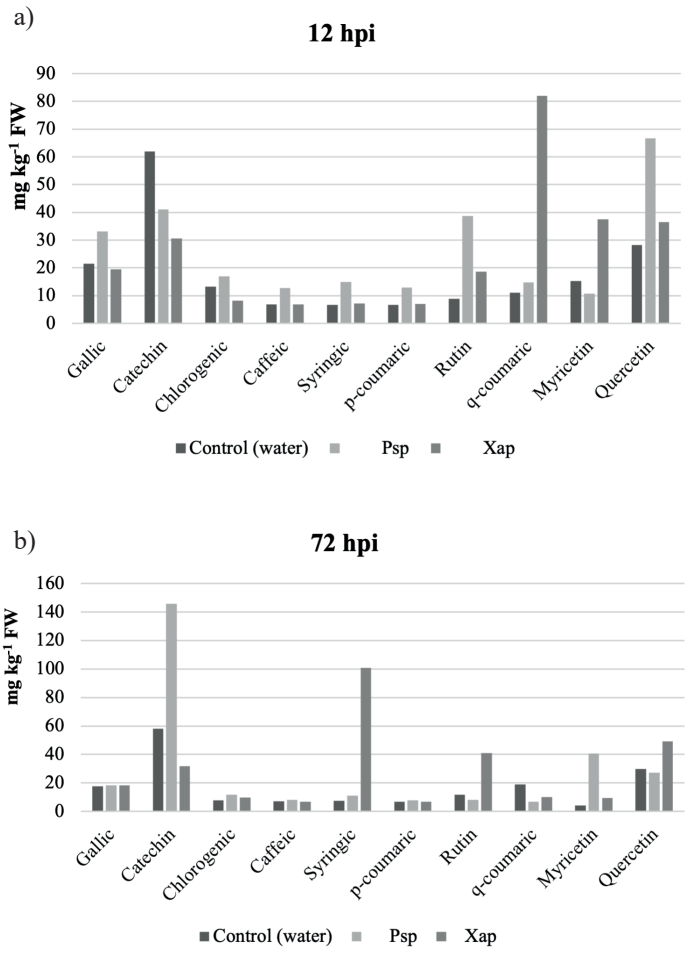

Figure 4. (a) Changes in the content of phenolic compounds in leaves of cultivar Aras98 inoculated with Psp and Xap after 12 hours; (b) Changes in the content of phenolic compounds in leaves of cultivar Aras98 inoculated with Psp and Xap after 72 hours.

a, b, Comparison of pathogen levels

h in the 36K genotype did not change after the inoculation of Psp but decreased after Xap inoculation. The amount of myricetin produced at 72 h in the 36K genotype did not change after Psp inoculation but increased after Xap inoculation (Tables 3 and 4, Figures 2, 3, and 4).

At 12 h, the syringic, rutin, and q-coumaric contents increased after Xap and Psp inoculation in the Aras98 cultivar. Compared with those of healthy plants, the contents of gallic acid, chlorogenic acid, and quercetin increased after the inoculation of Psp, whereas they decreased after Xap inoculation. Compared with that in healthy plants, the catechin content in Aras98 plants was reduced at 12 h after inoculation with both bacterial pathogens. Caffeic and p-coumaric acid contents increased after inoculation with Psp, but there was no change after Xap inoculation. The level of myricetin in the 12th cultivar decreased after the inoculation of Psp but decreased after Xap inoculation. At 72 h, the amount of gallic and caffeic acid did not differ from that of healthy plants. Compared with those of healthy plants, the

amounts of chlorogenic, syringic, and myricetin increased after the inoculation of both bacterial pathogens. The p-coumaric amount increased after inoculation with Psp, but there was no change after Xap inoculation. The rutin and quercetin contents did not change after Psp inoculation, but they increased after Xap inoculation. Compared with that in healthy plants, the Q-coumaric content in Aras98 plants was reduced at 72 h after inoculation with both bacterial pathogens (Tables 3 and 4, Figures 2, 3, and 4).

Discussion

Crop losses caused by bacterial pathogens are quite significant, as effective chemical and biological control methods are limited. However, genetic resistance plays a crucial role in controlling plant diseases caused by bacterial pathogens. The development of resistant cultivars is the most effective and environmentally friendly strategy for controlling this disease. Thus, the primary objective of many bean breeding programs is to develop resistant cultivars.

Plants undergo metabolic and biochemical changes in response to biotic stress. Chlorophyll and carotenoids are vital for providing resistance to crop plants. Chlorophyll pigments a and b play significant roles in light absorption during photosynthesis (Lobato et al., 2009). Potent stress factors, such as Xap and Psp, cause important anatomical, morphological, and physiological biochemical disorders in plants (Smith & McLaughlin, 1999). Berova et al. (2007) compared four *P. vulgaris* varieties and reported that chlorophyll a and b were negatively affected by inoculation with *X. campestris* and *P. syringae*. Lobato et al. (2009) reported that anthracnose infection is detrimental to chlorophyll pigments in beans. However, Balai et al. (2017) reported that the host plant was sometimes stimulated to synthesize these compounds after infection. Rasoulnia et al. (2018) reported that bacterial pathogen infection, despite its negative effect on chlorophyll pigment content, improved physiological conditions. Siddiqui et al. (2019) reported that inoculating plants with *Pectobacterium carotovorum*, *Xanthomonas hotorum* pv. *carotae*, and the fungi *Rhizoctonia solani*, *Fusarium solani*, and *Alternaria dauci* reduced the chlorophyll and carotenoid contents compared with those of the uninoculated controls.

In this study, the bacteria Xap and Psp were found to affect the content of photosynthetic pigments in plants. When Xap was inoculated into the 36K genotype, which is resistant, the chlorophyll content decreased. Conversely, both pathogens caused a decrease in the chlorophyll content of the susceptible bean cultivar Aras98 at 72 h. In the 36K genotype, which is a robust plant, it is believed that the damage to chlorophyll-a can be overcome by the end of 72 h. The content of chlorophyll-b decreased at 24 and 36 h after both bacterial pathogens were inoculated into the 36K genotype, but it increased at 72 hours. On the other hand, a decrease was observed in the susceptible cultivar Aras98. According to the results of the photosynthetic pigments, although the chlorophyll a and b contents and the total chlorophyll and carotenoid contents of the resistant cultivar sometimes decreased at 24 and 36 h, the plants recovered at 72 h. However, in the susceptible cultivar Aras98, the contents of these pigments decreased compared with those in the control. Pathogens primarily affect photosynthesis, a key physiological process in plants. The occurrence of chlorosis suggests that pathogens hinder photosynthesis through chlorophyll or chloroplast degradation (Bastiaans et al., 1991; Silva et al., 2020). Pathogens may reduce photosynthesis by degrading chlorophyll or chloroplasts, as indicated by the occurrence of chlorosis. In a previous study by Silva et al. (2020), the Xapf infection concentration of photosynthetic pigments decreased, but such a decrease was significantly greater for the susceptible cultivar than for the resistant cultivar. Silveira et al. (2015) reported that photosynthetic pigment concentrations decreased in tomato plants inoculated with *Xanthomonas gardneri*.

Phenolic compounds are the main polyphenols produced by plants. These compounds play diverse multifunctional roles and are highly important in plant–pathogen interactions, and they include a wide array of secondary metabolites synthesized to provide resistance to plants (Kousar et al., 2020). Phenolic compounds act as signaling molecules in plants and can act as agents in plant defense (Mandal et al., 2010). Many phenolic compounds with antibiotic activity act as phytoalexins in plant tissues during their interactions with phytopathogens (VanEtten et al., 1994). A previous study reported that phenolic metabolites are associated with biotic stress resistance phenomena in plants (Hammerschmidt, 2005). Petkovsek et al. (2011) reported that the accumulation of phenolic compounds was a postinfection response and possibly a prerequisite for further transformation and plant resistance.

Phenolic compounds found in plants help defend against bacterial pathogens by damaging the bacterial membrane, inhibiting virulence factors such as enzymes and toxins, and suppressing bacterial biofilm formation. According to Li et al. (2017), gallic acid [C_6H_2 (OH) 3COOH] is a trihydroxybenzoic acid, and gallic acid is a natural polyphenol that has antifungal and antibacterial properties and can be found in various plant species.

This study used HPLC analysis to identify and quantify 10 phenolic compounds in the leaves of healthy and infected bean plants. The roles of these compounds in plant defense mechanisms were investigated. The level of defense varies depending on the plant. After the leaves of the 36K genotype and Aras98 cultivar were inoculated with Psp (at 12 hpi), significant increases in gallic acid levels were observed. Specifically, the levels in the 36K genotype and Aras98 cultivar increased by 157% and 53%, respectively, compared with the respective control (water) values.

A previous study by Treutter (2001) revealed that p-coumaric acid plays a crucial role in the biosynthetic pathways of phenolics in apples. On the other hand, Li et al. (2017) reported that p-coumaric acid can inhibit bacterial growth by reducing the expression of type III secretion system genes. However, in our study, there was no significant difference in the concentration of p-coumaric acid between the two varieties of beans and the control group.

Caffeic acid is a type of carboxylic acid that is found in many plants, including cinnamon (Rice, 1995). According to Bowles and Miller (1994), it has been shown to inhibit bacterial growth. In this study, the accumulation of cafeic, syringic, rutin, and myricetin was greater in the Aras98 cultivar than in the 36K genotype and control cultivars.

Compared with those in the Aras98 cultivar and the control, the catechins accumulated earlier, and the levels of CGAs and quercetin were greater in the 36K genotype. The greater and earlier induction of gallic acid, chlorogenic, and quercetin in resistant genotypes than in susceptible genotypes indicates their significant role in this process.

This study revealed that Psp or Xap infection specifically affected the contents of phenolic compounds in each bean variety. Gallic acid, catechin, chlorogenic acid, and quercetin were positively related to 36K genotype Psp or Xap resistance.

In conclusion, the findings of this study underscore the critical role of genetic resistance and the intricate interplay of metabolic and biochemical responses in plants facing bacterial pathogen challenges. Despite the significant crop losses attributed to bacterial pathogens, the development of resistant cultivars has emerged as a promising and environmentally safe strategy for disease control.

This investigation revealed dynamic changes in photosynthetic pigments and phenolic compounds upon infection with Xap and Psp in bean plants. While susceptible cultivars presented notable reductions in chlorophyll and carotenoid contents, resistant genotypes demonstrated resilience,

often recovering to near-control levels, which was particularly evident in the 36K genotype. This resilience suggests the effectiveness of genetic resistance mechanisms in mitigating the detrimental effects of bacterial pathogens on photosynthetic processes.

This analysis of phenolic compounds highlighted their multifaceted roles in plant defense mechanisms. The significant increases in gallic acid levels following pathogen inoculation suggest its pivotal role in eliciting defense responses, particularly in the 36K genotype. Additionally, the varied accumulation patterns of other phenolic compounds underscore the complexity of plant–pathogen interactions, with certain compounds showing higher and earlier induction in resistant genotypes, potentially contributing to increased resistance to bacterial infection.

This study provides valuable insights into the molecular mechanisms underlying plant defense against bacterial pathogens. The identification of specific phenolic compounds associated with resistance underscores the potential for targeted breeding strategies aimed at enhancing pathogen resistance in bean cultivars. By elucidating these intricate defense mechanisms, our findings contribute to ongoing efforts to develop sustainable and resilient agricultural practices, ultimately assisting in the protection of crop yields against devastating bacterial diseases.

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