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Physiological responses of resistant and susceptible pepper plants to exogenous proline application under *Phytophthora capsici* stress

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Abstract – *Phytophthora capsici* Leon. is the main pathogen that limits the production of peppers. In this study, the effects of 1 and 10 mM proline (Pro), prior to exposure of resistant (CM-334) and susceptible (SD-8) pepper seedlings to *P. capsici*, on some physiological parameters were investigated. A lower Pro concentration (1 mM) was found to be more effective than 10 mM Pro in increasing the stress tolerance of the CM-334 cultivar. Namely, in CM-334 cultivar, the highest chlorophyll *a*, chlorophyll *b*, carotenoid, glucose and fructose content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity percentage were detected on the seventh day after application of 1 mM Pro + *P. capsici*, while the lowest malondialdehyde (MDA) amount was measured on the third day in the same treatment. The highest ferric reducing antioxidant power (FRAP) increase was determined on the seventh day in the 10 mM Pro + *P. capsici* application. The effects of the same Pro treatments on the SD-8 cultivar somewhat differed; the highest amounts of chlorophyll *a*, chlorophyll *b*, anthocyanins, fructose, total protein and endogenous Pro were detected on the seventh day in the 1 mM Pro + *P. capsici* application, while the lowest MDA amount was measured on the third day after the 10 mM Pro + *P. capsici* application, the highest DPPH % and FRAP values were detected on the seventh day with 10 mM Pro + *P. capsici* application. Although some differences were detected between the cultivars, Pro application against the *P. capsici* stress in general resulted in a positive effect on photosynthetic pigments, soluble carbohydrates and antioxidant capacity in pepper. The exogenous application of Pro helped the non-resistant cultivar to overcome the stress.

Keywords: antioxidant capacity, lipid peroxidation, pepper, photosynthetic pigments, soluble carbohydrate

Introduction

Pepper (*Capsicum annuum* L.) is a vegetable that belongs to the *Solanaceae* family. It has both economic and high nutritional value. According to *Food and Agriculture Organization* (*FAO*) data for 2017, pepper was among the ten most cultivated vegetables in the world with a production of approximately 34 million tons. However, various diseases that threaten production of the pepper worldwide are a limiting factor. *Phytophthora capsici* Leon. is a widespread, destructive and invasive soil-borne oomycete pathogen that causes decomposition of root and root collar in pepper and therefore poses a serious threat to its production (Siddique et al. 2019). Increasing the plant's tolerance to *P. capsici*-imposed stress is vital in agriculture and horticulture. Stress tolerance is a complex trait that is controlled by multiple genes and includes different physiological and biochemical mechanisms (Zhang and Shi 2013, Sharma and Prasad 2017, Rabuma et al. 2021). It is essential to develop economically viable strategies to increase and improve plants' stress tolerance under adverse environmental conditions. Therefore, in the fight against *P. capsici*, various approaches have been developed to increase the genetic resistance of the host, as well as crop rotation, soil solarizations, application of fungicides, fumigation and cultural methods (Hausbeck and Lamour 2004, Jin et al. 2016, Xu et al. 2016, Kim et al. 2017, Rabuma et al. 2021). Application of amino acids such as proline (Pro), which is also synthesized by a wide variety of plants during abiotic and biotic stress, can be a promising alternative strategy for the management of root rot disease. Pro has many beneficial traits in the plant organism; it acts as an osmolyte, which accumulates in plant tissues exposed to stress; it is an antioxidant compound (Hoque et al. 2008) a source of carbon and nitrogen, both of which are essential for plant growth; it stabilizes protein structure and protects biological membranes and macromolecules from denatur-

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ation (Trovato et al. 2008). The effect of Pro depends on its concentration since an excessive amount of free Pro has adverse effects on cell growth and protein functions (Nanjo et al. 2003), application time, plant species and plant growth stage (Ashraf and Foolad 2007, Elewa et al. 2017). Thus, it is essential to determine optimal concentrations of exogenously applied Pro which has positive effects on plants exposed to stress.

There are many studies which reported the successful application of exogenous Pro for increase of stress tolerance in plants (Nounjan and Theerakulpisut 2012, Medeiros et al. 2015, Abdelaal et al. 2020, Hayat et al. 2021). While these studies mostly focused on abiotic stress, there is little information on the effects of exogenous application of Pro to plants exposed to biotic stress. The aim of this study was to determine the extent to which exogenous application of Pro could change important physiological parameters in pepper cultivars with different tolerances to *P. capsici*. The strain CM-334 is a hot pepper cultivar originating from Southern Mexico, which shows consistently high resistance to various pathogens, including *P. capsici*, pepper mottle virus and root-knot nematodes (Ortega et al. 1991; Kim et al. 2014). CM-334 is a genotype with very high resistance to multiple *P. capsici* strains (Foster and Hausbeck 2010). On the other hand, SD-8 is a sweet pepper cultivar commercially grown in Turkey and susceptible to *P. capsici* (Göçmen 2006). To establish the possible positive effect of exogenously applied Pro on pepper plants exposed to *P. capsici*, the content of soluble carbohydrates and starch, photosynthetic pigments, anthocyanins and total flavonoids as well as endogenous Pro and total protein were examined along with the 1,1-diphenyl-2- picrylhydrazyl (DPPH) scavenging activity, reducing antioxidant power and lipid peroxidation in pepper leaves on the 3rd, 5th and 7th day post inoculation. In the framework of the findings obtained, the relationship of Pro with the investigated metabolic pathways is discussed. According a survey of the literature, there is no record of the effect of exogenous Pro pre-applications on the investigated parameters in peppers exposed to *P. capsici*.

Materials and methods

Plant material

In this study, two pepper (*Capsicum annuum* L.) cultivars, Criollo de Morelos 334 (CM-334; resistant to *P. capsici*) and Sera demre-8 (SD-8; susceptible to *P. capsici*), have been used. After germination, pepper seedlings were grown in plastic pots containing a steam-sterilized soil/fertilizer/sand mix (1/1/1, v/v/v) in a growth chamber (Digitech GLO-PG42) under controlled environmental conditions (25±2 °C, 16-h light/8-h dark photoperiods and 60% humidity). At the end of the two months period, when seedlings reached the six-leaf stage, they were collected and the leaves were separated, frozen in liquid nitrogen, and stored at -80 °C until analysis.

Preparation of *P. capsici* zoospore suspension

Phytophthora capsici strain 22 (*P. capsici*-22) was obtained from the fungal culture collection of Ankara University, Faculty of Agriculture, Ankara, Turkey. Zoospore production and spore concentrations were determined as described previously (Jones et al. 1974). *P. capsici*-22 was grown on V8 agar (200 mL V8 juice, 20 g agar, 3 g $CaCO_3$ and tap water to 1 L) plates at 25 °C in the dark. Zoospores were produced from mycelia. Drops of mycelial suspension was placed onto the surface of water-agar plates using a sterile syringe and the cultures were incubated for an additional 3 days at 25 °C under fluorescent lights (40 W daylight). The release of zoospores was induced by incubating the culture plates in sterile water at 4 °C at room temperature for 1 h. The zoospores were collected and filtered through a Whatman No. 54 filter to remove sporangial cases. The concentration of 10^4 zoospores mL^{-1} was the desired inoculum concentration and the optimal zoospore concentration for inducing disease in pepper (Koç et al. 2011).

Proline application and plant inoculation

Pro application and plant inoculation were performed according to Koç (2017). For each pepper cultivar four applications were used: 1 - control (no *P. capsici* or Pro), 2 - *P. capsici* alone, 3 - 1 mM Pro + *P. capsici* and 4 - 10 mM Pro + *P. capsici*. For both cultivars, each application was repeated three times. In all, 30 seedlings were used for each repetition of each application. The roots of the seedlings were washed with tap water and disinfected with 0.75% (v/v) sodium hypochlorite for 1-2 min and then washed with sterile distilled water several times. The seedlings were placed into a sterile glass bottle containing 400 mL of liquid Hoagland medium. Pro was applied to the plants once before the *P. capsici* inoculation by spraying the leaf surface of pepper seedlings. For the seedlings in the control group, sterile distilled water was used instead of Pro application and then control group and Pro-applied pepper seedlings were transferred back to the growth chamber and incubated for 3 days at 25±2 °C, 16-h light/8-h dark photoperiods and 60% humidity. Inoculation of *P. capsici* zoospores was performed 72 hours after the Pro application. 100 mL of zoospore suspension (10^4 zoospores mL^{-1}) was placed into 250 mL beakers in which seedling roots were dipped for 1 h. Afterwards, seedlings were placed into sterile glass bottles containing 400 mL of Hoagland solution and kept in the growth chamber. For control seedlings, sterile water was used instead of *P. capsici* suspension. Samples were taken on the 3rd, 5th and 7th day post inoculation (dpi) according to the random blocks design model. The leaves were harvested and homogenized in liquid nitrogen and stored at -70 °C until the analysis. All chemicals and reagents used in the analyses were of analytical grade. Distilled water was used throughout the study.

Determination of pigments and flavonoid content

For chlorophyll extraction, 0.1 g of fresh leaf sample was ground with 8 mL of 80% acetone with a mortar and pestle.

The mixture was centrifuged at 5000 rpm for 10 minutes. The absorbance of the resulting supernatant was recorded at 664 and 647 nm using an UV-visible spectrophotometer (Cecil 5000, Cecil Instruments, Milton, UK) with 80% acetone as blank. Chlorophyll *a* and *b* amounts were calculated using the equations given by Porra et al. (1989).

The extraction of carotenoids (xanthophyll + β-carotene) was done with 0.1 g fresh leaf material using 1 mL of 100% acetone. The mixture was centrifuged at 3500 rpm for 10 minutes. The absorbance of the resulting supernatant was recorded at 470 nm using an UV-visible spectrophotometer. The carotenoids amount was calculated using the equations given by Lichtenthaler (1987).

Anthocyanin content was determined using the method described by Mancinelli et al. (1975). Fresh leaf sample (0.1 g) was extracted with 1 mL of a solution containing 79% (v/v) methanol, 20% distilled water and 1% (v/v) HCl. Absorbances were measured at 530 and 657 nm wavelengths. The amount of anthocyanin was expressed as mg mL^{-1}. Flavonoid content was determined with the method of Mirecki and Teramura (1984). Absorbance was measured at 300 nm. Flavonoid content was expressed as the percentage of content of control plants. Values obtained in the control plants at 3rd dpi were set as 100%, and all other values were calculated in reference to this value.

Determination of soluble carbohydrates, starch and protein content

Glucose and fructose content were determined according to Halhoul and Kleinberg (1972). Fresh leaf sample (0.1 g) was extracted with 2 mL of 80% (v/v) ethyl alcohol and the supernatant was transferred into an Erlenmeyer flask and filled to 100 mL with distilled water after the alcohol had evaporated. Glucose and fructose contents were analyzed by mixing 1 mL of the extract with 2 mL of anthrone solution. For measurement of glucose content, the mixtures were placed water bath at 95 °C for 15 minutes, while for measurement of fructose content, mixtures were placed in a water bath at 40 °C for 30 min and the reaction was finished in an ice bath. Five minutes later, absorbance was measured at 620 nm and calculated as ppm g^{-1} fresh weight (FW) against the glucose (Merck K13654437) and fructose (Merck K04317907) standards.

Starch content was determined using the method described by McCready et al. (1950). Fresh leaf sample (0.1 g) was extracted with 1.6 mL of 52% (v/v) perchloric acid and 1 mL of extract was mixed with 2 mL of anthrone solution. The reaction mixture was incubated in a boiling water bath for 5 minutes and the reaction was finished in an ice bath. Absorbance was measured at 520 nm and starch content was calculated as ppm g^{-1} FW against the glucose standard.

Total soluble proteins extraction was done according to Kurkela et al. (1988). Fresh leaf sample (0.1 g) was extracted with 1.5 mL of 50 mM Tris HCl buffer pH 6.8 containing 1% (v/v) 2-β mercaptoethanol (Sigma-Aldrich M6250) and 50 mg L^{-1} phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich P7626). Protein contents were measured according to Bradford method (Bradford 1976) using the bovine serum albumin (BSA) (Sigma-Aldrich B4287) as a standard protein.

Determination of proline and MDA content

Free Pro extraction and determination were made according to Bates et al. (1973). Fresh leaf sample (0.1 g) was extracted with 3 mL of 3% (w/v) sulfosalicylic acid. Two mL of extract was mixed with 2 mL of ninhydrin solution and 2 mL of glacial acetic acid. The reaction mixture was incubated in a boiling water bath for 1 h and the reaction was finished in an ice bath. Four mL of toluene was added to the reaction mixture and the absorbance of the toluene phase was measured at 520 nm in a UV-visible spectrophotometer and calculated as μg g^{-1} FW against the proline (Sigma-Aldrich P5607) standard.

To determine the level of lipid peroxidation, the malondialdehyde (MDA) method was used. After 0.1 g of fresh leaf sample was homogenized with 1.5 mL of 1% (w/v) trichloroacetic acid (TCA), a solution containing 20% (w/v) TCA and 0.5% (w/v) thiobarbituric acid (TBA) was added to the extract obtained. Subsequently, the solution was incubated in a water bath at 95 °C and absorbance was measured in a spectrophotometer at 532 and 600 nm. MDA content was measured according to thiobarbituric acid reaction and content was calculated using extinction coefficient of 155 mM^{-1} cm^{-1} (Devasagayam et al. 2003).

Evaluation of antioxidant capacity

The measurement of the 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activity was performed according to a methodology described by Blois (1958). The percentage of antioxidant activity of methanol extracts of pepper cultivars was assessed by DPPH free radical assay. A fresh leaf sample (0.1 g) was incubated overnight at room temperature in 2 mL methanol, 1.5 mL of 0.1 mM DPPH (Sigma-Aldrich D9132) was mixed with 100 μL of extract and the samples were incubated in a water bath for 30 min at 24 °C. The reduction of DPPH radicals was determined by measuring the absorption at 517 nm. Percent inhibition was calculated using the formula: DPPH scavenging activity (% Inhibition) = [(Ac – As)/ Ac] × 100, where "Ac" is the absorbance of the control reaction (absorbance of the DPPH solution), while "As" is the absorbance of the extracts.

Ferric reducing antioxidant power (FRAP) of extracts was determined by the method of Vijayalakshmi and Ruckmani (2016). To determine reducing power, 100 mg of fresh leaves was extracted with 1.5 mL of methanol. The reaction mixture, which consisted of 250 μL of extract, 1.25 mL of 0.2 M phosphate buffer pH 6.6 and 1.25 mL of 1% (m/v) potassium hexacyanoferrate ($K_3Fe(CN)_6$) (Sigma-Aldrich P8131), was incubated at 50 °C for 20 min. The reaction was stopped by the addition of 1.25 mL of 10% (m/v) TCA and centrifuged at 3000 g for 10 min. 1.25 mL of the supernatant upper layer was mixed with 1.25 mL of distilled water and

250 µL of 0.1% (m/v) of ferric chloride ($FeCl_3$) and incubated at 24 °C for 10 min. The absorbance was measured at 700 nm. A higher absorbance value of the reaction mixture indicated greater reducing power. The FRAP value of the extracts was calculated and expressed as ascorbic acid equivalent (AAE) (µg AAE g^{-1} FW) through the calibration curve of ascorbic acid (Sigma-Aldrich A4544) (10-100 µg/mL).

Statistical analysis

Variance analysis (ANOVA-factorial design: cultivar* day*application) was conducted using a test arrangement in which data analysis was completely random. The trials were arranged to create an experimental design with three repetitions in randomized blocks. Variance analyses were conducted using SPSS 24 software package. A 5% significance level was used in the Tukey test and in the interpretation of the results. The statistical significance is indicated by appropriate letters within the tables.

Results

As a result of variance analysis for chlorophyll *a*, chlorophyll *b*, carotenoid, flavonoid, fructose, starch, Pro, MDA, DPPH and FRAP content, cultivar*day*application triple interaction was found to be statistically significant ($P < 0.01$). As a result of variance analysis for glucose and protein content, cultivar*day*application triple interaction was found to be statistically significant ($P < 0.05$).

Pigments and flavonoid content

Chlorophyll *a, b* and carotenoid contents of the leaves of control seedlings of resistant CM-334 cultivar on the 3rd, 5th and 7th dpi were found to be higher than in the control leaves of susceptible SD-8 cultivar ($P < 0.05$). Chlorophyll *a* and *b* content mostly decreased due to *P. capsici*-imposed stress in both cultivars compared to control (Tab. 1). In the CM-334 cultivar, the highest values of chlorophyll *a* and *b* as well as of carotenoids were determined on the 5th dpi upon 1 mM Pro + *P. capsici* application. Although *P. capsici* application generally induced an increase in the amount of anthocyanin and flavonoids in the CM-334 cultivar compared to the control group, the highest values were obtained upon applications of both Pro concentrations + *P. capsici* ($P < 0.05$) (Tab. 1).

When the cultivars were compared, the highest amounts of chlorophyll *a* and *b* were found in the SD-8 cultivar upon 1 mM Pro + *P. capsici* application on the 7th dpi ($P < 0.05$). In the SD-8 cultivar, the highest amounts of chlorophyll *a* and *b* as well as of anthocyanin were detected after exposures to both Pro concentrations + *P. capsici* on the 7th dpi, while the highest carotenoid amount was detected upon 1 mM Pro + *P. capsici* application on the 3rd dpi. In the SD-8 cultivar, the highest chlorophyll *a* and *b* increases were determined respectively as 247% and 170% after exposure to 1 mM Pro + *P. capsici* on the 7th dpi ($P < 0.05$) as compared to exposure to *P. capsici* alone. A significantly higher flavonoid level was detected in CM-334 cultivar than in all corresponding controls and treatments of the SD-8 cultivar. The highest flavonoid increases were determined in CM-334 cultivar upon exposures to both Pro concentrations + *P. capsici* ($P < 0.05$) when compared to control and treatment with *P. capsici* alone (Tab. 1).

Soluble carbohydrates, starch and protein content

Glucose content in the leaves of SD-8 cultivar control seedlings was significantly higher than in the CM-334 cultivar ($P < 0.05$) at all dpi (Tab. 2). In the SD-8 cultivar exposed to *P. capsici*, glucose amount was similar to the control value on the 3rd and 5th dpi, but significant reduction was recorded on the 7th dpi; however, upon exposure to the combined treatments with both Pro concentrations, the values were significantly elevated and even exceeded the control value. Moreover, the highest increase in glucose content was approximately 63.7% and 57.8% on the 7th dpi with 1 and 10 mM Pro + *P. capsici* applications respectively ($P < 0.05$) when compared to the values obtained after exposure to *P. capsici* alone. In the CM-334 cultivar, the highest glucose increase of 7.7% was recorded on the 5th dpi upon 1 mM Pro + *P. capsici* application ($P < 0.05$) when compared with *P. capsici* application alone.

Fructose content in the leaves of SD-8 cultivar control seedlings was significantly higher at the 3rd and 5th dpi ($P < 0.05$) than in the CM-334 cultivar (Tab. 2). The greatest, significant changes in fructose content were recorded in SD-8 cultivar on the 7th dpi between control and all treatments. In the CM-334 cultivar, the highest fructose increase of 40% was detected on the 7th dpi upon exposure to 1 mM Pro + *P. capsici* ($P < 0.05$) when compared with *P. capsici* application alone (Tab. 2).

The starch content was found to be higher in the leaves of the control seedlings of the SD-8 cultivar than in the CM-334 cultivar at all dpi ($P < 0.05$) (Tab. 2), while the highest value was detected in the SD-8 cultivar upon infection with of *P. capsici* on the 3th dpi ($P < 0.05$). In the CM-334 cultivar, the starch content increased at all dpi after infection with *P. capsici* alone compared to the control, while combined treatments with both Pro concentrations resulted in values that were not significantly different from control values, with the exception of the combined treatment with 10 mM Pro + *P. capisici* (Tab. 2). In the SD-8 cultivar, the most prominent increase in starch content was detected upon *P. capsici* application on the 3rd dpi ($P < 0.05$). Combined treatments with both Pro concentrations and *P. capisici* failed to increase the starch content to the control value, which was particularly pronounced on the 5th and the 7th dpi (Tab. 2).

Total protein levels increased in leaves of both cultivars on the 7th dpi in control and *P. capsici*-exposed plants ($P < 0.05$) (Tab. 2). Compared to the values obtained in treatment with *P. capsici* alone, the highest total protein increase was detected after exposure to 1 mM Pro + *P. capsici* on 7th dpi with approximately 23% in CM-334 cultivar ($P < 0.05$). Likewise, an 3.5% increase was detected in the 1 mM Pro +

Tab. 1. The content of pigments and flavonoids in leaves of CM-334 and SD-8 pepper cultivars exposed to proline (Pro) and *Phytophthora capsici*. Type of applications (App): 1 - control (no Pro, no *P. capsici*), 2 - *P. capsici* alone, 3 - 1 mM Pro + *P. capsici*, 4 - 10 mM Pro + *P. capsici*. Mean ± standard deviation of 3 replicates is presented. Flavonoid values obtained in the control plants at 3rd day post inoculation (dpi) were set as a 100%, and all flavonoid contents were calculated in reference to this value. Different letters indicate significant differences at $P < 0.05$ by the Tukey test. Capital letters represent differences in applications for the same cultivar and the same day. Lower case letters represent differences in days for the same cultivar and the same application. Superscript lower case letters represent differences in cultivars for the same day and the same application. FW – fresh weight.

Cultivar	dpi	App	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	Anthocyanins (mg mL^{-1})	Flavonoid (% of control)
CM-334	3	1	1.157 ± 0.050 Aa^{a}	0.791 ± 0.031 Aa^{a}	0.924 ± 0.179 Aa^{a}	0.043 ± 0.002 Bc^{a}	100.0 ± 0.00 Dc^{a}
		2	0.925 ± 0.266 Ba^{a}	0.653 ± 0.156 Aa^{a}	0.901 ± 0.048 Aa^{a}	0.042 ± 0.002 Bc^{b}	115.3 ± 4.12 Cc^{a}
		3	1.041 ± 0.200 Ab^{a}	0.735 ± 0.151 Ab^{b}	0.820 ± 0.046 Ab^{b}	0.085 ± 0.001 Bc^{a}	263.6 ± 5.57 Aa^{a}
		4	0.795 ± 0.026 Bc^{b}	0.568 ± 0.264 Ab^{b}	0.900 ± 0.077 Aa^{a}	0.213 ± 0.027 Aa^{a}	183.8 ± 0.38 Bb^{a}
	5	1	1.206 ± 0.500 BCa^{a}	0.905 ± 0.099 ABa^{a}	0.870 ± 0.029 Ba^{a}	0.051 ± 0.001 Ca^{b}	123.5 ± 5.54 Cb^{a}
		2	0.972 ± 0.072 Ca^{a}	0.735 ± 0.194 Ba^{a}	0.882 ± 0.026 Ba^{a}	0.089 ± 0.000 Ba^{a}	207.9 ± 3.62 Ba^{a}
		3	1.657 ± 0.164 Aa^{a}	1.134 ± 0.086 Aa^{a}	0.965 ± 0.026 Aa^{a}	0.112 ± 0.000 Ab^{a}	234.3 ± 9.28 Aa^{a}
		4	1.346 ± 0.023 Ba^{a}	0.974 ± 0.109 $ABab^{a}$	0.449 ± 0.032 Cc^{b}	0.109 ± 0.010 Ab^{a}	237.8 ± 3.49 Aa^{a}
	7	1	1.155 ± 0.123 Aa^{a}	0.825 ± 0.046 Ba^{a}	0.767 ± 0.033 Aa^{a}	0.048 ± 0.010 Db^{b}	133.1 ± 0.94 Ca^{a}
		2	1.120 ± 0.051 Aa^{a}	0.770 ± 0.059 Ba^{a}	0.770 ± 0.053 Ab^{b}	0.082 ± 0.000 Cb^{a}	196.1 ± 1.89 Bb^{a}
		3	1.126 ± 0.094 Ab^{b}	0.793 ± 0.039 Bb^{b}	0.690 ± 0.022 Ac^{a}	0.120 ± 0.000 Ba^{b}	227.4 ± 24.71ABa^{a}
		4	1.271 ± 0.002 Ab^{b}	1.034 ± 0.034 Aa^{b}	0.720 ± 0.017 Ab^{a}	0.152 ± 0.000 Aa^{a}	231.9 ± 6.510Aa^{a}
SD-8	3	1	0.893 ± 0.281 Ba^{b}	0.765 ± 0.033 BCa^{a}	0.850 ± 0.012 Bb^{b}	0.040 ± 0.010 Dc^{a}	100.0 ± 0.00 Aa^{a}
		2	0.663 ± 0.135 Bc^{b}	0.652 ± 0.014 Ca^{a}	0.797 ± 0.004 Cc^{b}	0.050 ± 0.002 Cb^{a}	91.49 ± 10.09Ab^{b}
		3	0.759 ± 0.192 Bc^{b}	0.920 ± 0.042 Ab^{a}	0.970 ± 0.004 Aa^{a}	0.074 ± 0.000 Ab^{b}	103.1 ± 2.15 Aa^{b}
		4	1.043 ± 0.378 Ab^{a}	0.795 ± 0.012 Bb^{a}	0.840 ± 0.001 Ba^{a}	0.062 ± 0.006 Bc^{b}	98.87 ± 8.35 Aa^{b}
	5	1	0.921 ± 0.175 Ba^{b}	0.685 ± 0.035 Bb^{b}	0.882 ± 0.021 Aa^{a}	0.062 ± 0.005 Bb^{a}	98.06 ± 4.30 Aa^{b}
		2	1.003 ± 0.053 Aa^{a}	0.623 ± 0.114 Ba^{a}	0.871 ± 0.002 Aa^{a}	0.068 ± 0.014 Bb^{b}	107.2 ± 3.57 Aab^{b}
		3	1.330 ± 0.102 Ab^{b}	1.110 ± 0.095 Ab^{a}	0.565 ± 0.027 Bc^{b}	0.060 ± 0.004 Bc^{b}	99.98 ± 6.98 Aa^{b}
		4	1.626 ± 0.495 Aa^{a}	1.106 ± 0.111 Aab^{a}	0.588 ± 0.031 Bc^{a}	0.111 ± 0.005 Ab^{a}	106.2 ± 0.85 Aa^{b}
	7	1	0.833 ± 0.029 Ca^{b}	0.705 ± 0.034 Cab^{b}	0.804 ± 0.005 Abc^{a}	0.075 ± 0.000 Ba^{a}	102.3 ± 0.10 Ba^{b}
		2	0.821 ± 0.041 Cb^{b}	0.667 ± 0.013 Ca^{a}	0.827 ± 0.003 Ab^{a}	0.112 ± 0.027 Aa^{a}	111.2 ± 3.31 Aa^{b}
		3	2.849 ± 0.223 Aa^{a}	1.803 ± 0.121 Aa^{a}	0.730 ± 0.013 Bb^{a}	0.151 ± 0.010 Aa^{a}	108.8 ± 2.77 ABa^{b}
		4	2.081 ± 0.371 Ba^{a}	1.359 ± 0.195 Ba^{a}	0.739 ± 0.060 Bb^{a}	0.139 ± 0.016 Aa^{a}	104.9 ± 3.02 ABa^{b}

Tab. 2. Soluble carbohydrate, starch and total protein amount in leaves of CM-334 and SD-8 pepper cultivars exposed to proline (Pro) and *Phytophthora capsici*. Type of applications (App): 1 - control (no Pro, no *P. capsici*), 2 - *P. capsici* alone, 3 - 1 mM Pro + *P. capsici*, 4 - 10 mM Pro + *P. capsici*. Mean ± standard deviation of 3 replicates is presented. Different letters indicate significant differences at $P < 0.05$ by Tukey test. Capital letters represent differences in applications for the same cultivar and the same day. Lower case letters represent differences in days for the same cultivar and the same application. Superscript lower case letters represent differences in cultivars for the same day and the same application. FW – fresh weight, dpi – day post inoculation.

Cultivar	dpi	App	Glucose (ppm g^{-1} FW)	Fructose (ppm g^{-1} FW)	Starch (ppm g^{-1} FW)	Total protein (mg g^{-1} FW)
CM-334	3	1	12.607 ± 0.158 Aa^{b}	22.178 ± 1.244 Aa^{b}	8.658 ± 0.113 Cb^{b}	36.335 ± 1.608 Aa^{a}
		2	11.659 ± 0.236 Aa^{b}	24.373 ± 0.572 Aa^{a}	12.744 ± 0.596 Ac^{b}	38.333 ± 0.644 Aa^{a}
		3	15.897 ± 2.601 Aa^{a}	24.071 ± 3.163 Aa^{a}	10.192 ± 0.361 Bb^{b}	37.766 ± 1.154 Ac^{a}
		4	14.391 ± 3.191Aa^{b}	20.765 ± 0.653 Ab^{b}	9.435 ± 0.378 BCb^{b}	36.385 ± 0.515 Aa^{b}
	5	1	12.238 ± 1.020 Ba^{b}	19.499 ± 0.700 Ca^{b}	8.767 ± 1.192 Bb^{b}	36.170 ± 1.115 Ba^{b}
		2	12.505 ± 0.776 Ba^{b}	21.013 ± 0.786 Bb^{b}	17.865 ± 0.350 Aa^{a}	39.623 ± 1.145 ABa^{a}
		3	17.226 ± 2.731 Aa^{a}	29.404 ± 2.800 Aa^{a}	9.166 ± 0.642 Bb^{b}	42.671 ± 0.654 Ab^{a}
		4	11.403 ± 0.855 Ba^{b}	25.803 ± 3.734 $ABab^{a}$	10.845 ± 0.984 Bb^{a}	38.900 ± 3.078 ABa^{b}
	7	1	12.369 ± 0.277 Aa^{b}	23.085 ± 2.621 Ba^{a}	11.119 ± 0.941 Ba^{b}	41.760 ± 3.326 Ba^{a}
		2	12.318 ± 0.669 Aa^{b}	24.909 ± 1.328 ABa^{b}	14.497 ± 0.946 ABb^{a}	42.685 ± 10.49 Ba^{b}
		3	13.255 ± 1.009 Aab^{b}	29.892 ± 3.242 Aa^{b}	12.196 ± 0.993 Ba^{b}	52.528 ± 1.407 Aa^{a}
		4	10.001 ± 0.584 Ba^{b}	27.909 ± 1.472 ABa^{b}	17.650 ± 2.726 Aa^{a}	40.956 ± 2.114 Ba^{b}
SD-8	3	1	15.476 ± 1.301 Bb^{a}	25.398 ± 1.230 Aa^{a}	20.690 ± 0.539 Ba^{a}	34.528 ± 0.143 Ab^{a}
		2	15.136 ± 0.653 Bb^{a}	24.568 ± 0.057 Ab^{a}	31.548 ± 2.392 Aa^{a}	39.671 ± 3.711 Ab^{a}
		3	15.562 ± 0.540 Bc^{a}	24.771 ± 0.790 Ab^{a}	19.810 ± 0.220 Ba^{a}	38.052 ± 3.928 Ab^{a}
		4	20.918 ± 3.431 Aa^{a}	25.858 ± 1.180 Ab^{a}	18.593 ± 0.508 Ba^{a}	41.813 ± 2.338 Aa^{a}
	5	1	17.948 ± 1.261 Aab^{a}	24.955 ± 0.111 Aa^{a}	18.693 ± 0.539 Ab^{a}	44.099 ± 0.756 Aa^{a}
		2	17.919 ± 0.197 Aa^{a}	24.689 ± 0.743 Ab^{a}	12.243 ± 0.726 Bc^{b}	37.763 ± 4.145 Bb^{a}
		3	18.595 ± 0.555 Ab^{a}	25.066 ± 1.128 Ab^{b}	11.169 ± 0.124 BCc^{a}	44.575 ± 0.297 Ab^{a}
		4	22.705 ± 5.509 Aa^{a}	26.377 ± 0.541 Ab^{a}	10.212 ± 0.579 Cc^{a}	43.385 ± 2.351 ABa^{a}
	7	1	20.612 ± 2.155 Ba^{a}	24.385 ± 1.480 Ba^{a}	17.451 ± 0.121 Ab^{a}	42.385 ± 2.571 Ba^{a}
		2	15.208 ± 0.119 Cb^{a}	32.637 ± 1.619 Aa^{a}	16.291 ± 0.955 ABb^{a}	53.480 ± 1.501 Aa^{a}
		3	24.906 ± 0.567 Aa^{a}	34.673 ± 2.632 Aa^{a}	14.866 ± 0.446 Bb^{a}	55.355 ± 3.661 Aa^{a}
		4	24.003 ± 2.203 ABa^{a}	33.107 ± 2.729 Aa^{a}	13.601 ± 0.602 Cb^{b}	45.671 ± 0.868 Ba^{a}

Tab. 3. Proline and malondialdehyde (MDA) content, 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activity (%) and ferric reducing antioxidant power (FRAP) in leaves of CM-334 and SD-8 pepper cultivars exposed to proline (Pro) and *Phytophthora capsici*. Type of applications (App): 1 - control (no Pro, no *P. capsici*), 2 - *P. capsici* alone, 3 - 1 mM Pro + *P. capsici*, 4 - 10 mM Pro + *P. capsici*). Mean ± standard deviation of 3 replicates is presented. Different letters indicate significant differences at $P < 0.05$ by Tukey test. Capital letters represent differences in applications for the same cultivar and the same day. Lower case letters represent differences in days for the same cultivar and the same application. Superscript lower case letters represent differences in cultivars for the same day and the same application. AAE – ascorbic acid equivalent, dpi – day post inoculation, FW – fresh weight.

Cultivar	dpi	App	Proline (μg g^{-1} FW)	MDA (nmol g^{-1} FW)	DPPH scavenging activity (%)	FRAP (μg AAE g^{-1} FW)
CM-334	3	1	288.379 ± 3.8911 Bb^{b}	77.798 ± 1.316 Ba^{a}	31.346 ± 1.452 Ca^{a}	638.55 ± 21.309 Bb^{a}
		2	359.407 ± 12.053 Bc^{b}	78.080 ± 3.432 Bc^{a}	33.098 ± 0.886 Ca^{a}	657.30 ± 10.229 Ba^{a}
		3	979.033 ± 119.23 Aa^{b}	76.940 ± 0.495 Bc^{a}	43.021 ± 0.833 Bb^{b}	991.08 ± 45.159 Aa^{a}
		4	260.342 ± 26.521Bb^{b}	90.100 ± 2.972 Ac^{a}	51.723 ± 2.910 Aa^{a}	922.16 ± 59.626 Ab^{b}
	5	1	326.697 ± 42.978 Bab^{b}	77.798 ± 0.990 Ca^{a}	32.320 ± 2.221 Da^{a}	720.27 ± 8.6326 $BCab^{a}$
		2	1013.92 ± 215.88 Aa^{b}	89.233 ± 2.624 Bb^{b}	34.428 ± 1.264 Ca^{a}	696.19 ± 36.592 Ca^{b}
		3	333.115 ± 5.1065 Bb^{b}	89.241 ± 3.432 Bb^{a}	43.075 ± 1.815 Bb^{b}	786.62 ± 53.697 Bb^{b}
		4	366.884 ± 9.4541 Ba^{b}	150.456 ± 4.319 Aa^{a}	54.761 ± 1.696 Aa^{a}	1020.27 ± 15.78 Aa^{a}
	7	1	364.080 ± 6.5642 Ba^{b}	79.799 ± 2.574 Da^{a}	32.378 ± 0.370 Da^{a}	778.60 ± 56.945 Ba^{a}
		2	746.946 ± 31.724 Ab^{b}	122.417 ± 1.786 Ba^{b}	33.935 ± 0.971 Ca^{a}	677.21 ± 5.5530 Ba^{b}
		3	306.759 ± 12.017 Cb^{b}	112.713 ± 1.981 Ca^{a}	62.533 ± 1.355 Aa^{a}	734.62 ± 5.6135 Bb^{b}
		4	254.423 ± 24.768 Cb^{b}	128.995 ± 1.310 Ab^{a}	46.069 ± 0.663 Bb^{b}	1061.47 ± 94.71 Aa^{b}
SD-8	3	1	690.872 ± 23.370 Ca^{a}	73.790 ± 3.934 Ab^{a}	27.727 ± 1.708 Ba^{b}	664.25 ± 25.808 Cb^{a}
		2	1188.725 ± 52.07 Bb^{a}	76.940 ± 1.310 Ab^{a}	26.474 ± 1.455 Bc^{b}	663.74 ± 23.241 Cc^{a}
		3	1173.150 ± 312.49 Bb^{a}	5.720 ± 0.495 Bc^{b}	54.882 ± 3.495 Ab^{a}	763.88 ± 16.223 Bb^{b}
		4	1780.842 ± 27.06 Ab^{a}	3.4320 ± 1.486 Bc^{b}	54.742 ± 2.464 Ab^{a}	1186.01 ± 8.371 Aa^{a}
	5	1	678.099 ± 37.480 Ba^{a}	78.370 ± 2.758 Bab^{a}	28.033 ± 1.344 Ca^{b}	679.53 ± 6.5622 Cab^{b}
		2	1504.302 ± 216.6 Aab^{a}	191.240 ± 4.726 Aa^{a}	37.029 ± 2.098 Ba^{a}	861.47 ± 26.293 Bb^{a}
		3	1374.930 ± 277.3 Aab^{a}	21.162 ± 1.310 Db^{b}	58.967 ± 1.462 Aa^{a}	1171.62 ± 27.81 Aa^{a}
		4	1269.376 ± 9.047Ac^{a}	41.471 ±2. 621 Cb^{b}	39.047 ± 0.987 ABc^{b}	761.79 ± 58.927 Cb^{b}
	7	1	659.407 ± 275.75 Ca^{a}	86.691 ± 5.573 Ca^{a}	28.295 ± 1.762 Da^{b}	721.65 ± 24.686 Ca^{a}
		2	1592.429 ± 9.345 Ba^{a}	187.896 ± 0.828 Aa^{a}	35.394 ± 2.491 Cb^{a}	1033.23 ± 27.445Ba^{a}
		3	2016.025 ± 116.8 Aa^{a}	37.468 ± 5.172 Da^{b}	43.794 ± 1.149 Bc^{b}	1100.91 ± 39.245Ba^{a}
		4	1999.594 ± 110.3 ABa^{a}	119.139 ± 7.539 Ba^{a}	64.571 ± 3.295 Aa^{a}	1377.21 ± 133.63Aa^{a}

P. capsici application on the 7th dpi when compared with *P. capsici* in SD-8 cultivar, but the difference was not found to be significant. When the two cultivars were compared, the highest total protein level was detected in the SD-8 cultivar after application of 1 mM Pro + *P. capsici* on the 7th dpi with 55.385 ± 3.661 mg g^{-1} FW, but the difference was not found to be significant (Tab. 2).

Proline and MDA content

The content of endogenous Pro in all controls and Pro treatments of SD-8 cultivar was higher than in the CM-334 cultivar ($P < 0.05$) (Tab. 3). Although exposure to *P. capsici* alone caused an increase in the amount of endogenous Pro in both cultivars on all dpi, Pro application before inoculation increased the endogenous Pro accumulation ability only in the SD-8 cultivar ($P < 0.05$). In the CM-334 cultivar, the application of 1 mM Pro + *P. capsici* on the 3rd dpi resulted in the highest Pro increase of 239% when compared with the control group and 172.2% compared to exposure to *P. capsici* alone ($P < 0.05$) (Tab. 3). In the SD-8 cultivar, the highest Pro increase of 205% was determined on the 7th dpi upon exposure to 1 mM Pro + *P. capsici* ($P < 0.05$) when compared with the control group. In addition, a significant increase of approx 49.8% in Pro amount was detected on the 3rd dpi after exposure to 10 mM Pro + *P. capsici* compared to treatment with *P. capsici* alone ($P < 0.05$) (Tab. 3).

MDA content increased due to *P. capsici*-imposed stress in both cultivars on all dpi compared to the control group, although the difference was found to be statistically significant on the 5th and 7th dpi ($P < 0.05$) (Tab. 3). When the cultivars were compared, the lowest MDA amounts were found in the SD-8 cultivar in both Pro + *P. capsici* applications ($P < 0.05$). Compared to exposure to *P. capsici* alone in the CM-334 cultivar, the application of 1 mM Pro before inoculation was more effective against lipid peroxidation than 10 mM Pro ($p<0.05$). In the SD-8 cultivar, both Pro applications had a significant effect on the MDA amount when compared with treatment with *P. capsici* alone, while the highest MDA decrease of 95.5% was determined on the 3th dpi in the 10 mM Pro + *P. capsici* application ($P < 0.05$) (Tab. 3).

Antioxidant capacity

DPPH radical scavenging activity (%) increased in both cultivars on all dpi upon exposure to both combined treatments with Pro + *P. capsici* compared to the control group and treatment with *P. capsici* alone ($P < 0.05$) (Tab. 3). In CM-334 cultivar, the highest increase in DPPH radical scavenging activity of approximately 84% was recorded after the treatment with 1 mM Pro + *P. capsici* on the 7th dpi in comparison to *P. capsici* application alone. In SD-8 cultivar, the highest increase in DPPH radical scavenging activity of approximately 82% was observed upon exposure to 10 mM Pro + *P. capsici* on the 7th dpi ($P < 0.05$) in comparison to *P. capsici* application alone (Tab. 3).

FRAP values in control groups at all dpi were of similar values in the cultivars. In the CM-334 cultivar, antioxidant power increased on all dpi upon exposure to both combined treatments with Pro + *P. capsici* compared to control and treatment with *P. capsici* alone (Tab. 3). Similar results were obtained in the SD-8 cultivar as well with the exception of the treatment with 10 mM Pro + *P. capisici* on the 5th dpi. When the two cultivars were compared, the highest FRAP value was detected on the 7th dpi in the 10 mM Pro + *P. capsici* application in the SD-8 cultivar ($P < 0.05$), while the highest FRAP increase of 56.6% was determined in the treatment with 10 mM Pro + *P. capsici* in comparison to *P. capsici* applied group in CM-334 cultivar ($P < 0.05$) (Tab. 3).

Discussion

In this study the possible beneficial effects of exogenously applied Pro on physiological parameters in pepper plants exposed to infection with *Ph. capisici* were investigated. Pro is considered an important indicator of environmental stress caused by either abiotic or biotic factors (Claussen 2005, Hayat et al. 2012, Liang et al. 2013). Verslues and Sharma (2010) reported evidence that Pro plays a role in programmed cell death and development in plant-pathogen interactions. Penetration of *P. capsici* usually takes place in the host cell wall, a passive barrier that limits the access of pathogens to plant cells. Pro is an important source of cell wall matrix (hydroxyproline and proline-rich proteins), which indicates that it is necessary in the first line of defence against fungal pathogens (Lehmann et al. 2010, Kavi Kishor et al. 2015). Moreover, biotic stress results in increased production of *reactive oxygen species* (ROS) molecules, which play important roles in protecting plants against harmful pathogens. However, nucleic acid damage, oxidation of proteins and lipids and degeneration of chlorophyll pigments may occur due to excessive ROS accumulation, which increases depending on the severity and duration of the stress (Huang et al. 2019). Proline plays an important role in protection against oxidative damage as a powerful ROS scavenger as well as in stabilising the 3D structure of membranes and proteins (Hossain et al. 2014), protecting organelles such as mitochondria and chloroplasts (Ashraf and Foolad 2007) and by induction of stress-sensitive genes (Kahraman et al. 2019).

In the current study, *P. capsici* infection caused a decrease in chlorophyll *a* and *b* content, which was accompanied by an increase in leaf starch content on all days following the application in the resistant CM-334 cultivar. The increase in starch accumulation in infected leaves may have led to a decrease in the photosynthesis rate, which in turn caused the observed decrease in the amount of chlorophyll *a* and *b* in the leaves. The main reason for the decrease in chlorophyll content in plants exposed to stress may be disorganisation of thylakoid membranes due to the formation of proteolytic enzymes such as chlorophyllase, rather than chlorophyll synthesis (Sharma et al. 2019). The present results corroborate the findings of Mandal et al. (2009) and Llave (2016). Namely, Llave (2016) reported that viral infection caused an increase in starch accumulation in leaves and

a decrease in photosynthesis, while Mandal et al. (2009) found that downy mildew infection caused an increase in the amount of starch, a decrease in the amount of soluble sugars and in the rate of photosynthesis in leaves of the *Plantago ovata* Forsk. Both studies reported that the increase in the amount of starch in the infected leaves may be the reason for the decrease in photosynthesis. Moreover, decrease in the amount of chlorophyll content was accompanied with the decrease in the amount of glucose in infected leaves of SD-8 cultivar as well as with the increase in the amount of Pro. Proline's multiple roles as an osmolyte, ROS scavenger, signalling molecule and energy source are similar to glucose's multiple roles acting as a carbon and energy source (Trovato et al. 2008). Dawood et al. (2014) found that Pro application caused significant increases in photosynthetic pigments in faba bean plants under seawater stress, while Kaushal et al. (2011) reported that exogenous Pro protected the chlorophyll content and activity of Rubisco and antioxidant enzymes against heat stress in chickpea. In my previous study, the pre-application of Pro in pepper exposed to *P. capsici* caused an increase in the activities of antioxidant enzymes peroxidase (POX) and catalase (CAT) and a decrease in the amount of hydrogen peroxide (H_2O_2) (Koç 2017). Therefore, the increase in the chlorophyll *a* and *b* content in peppers exposed to *P. capsici* stress can be attributed to the stimulation of chlorophyll biosynthesis or inhibition of its degradation and the more efficient removal of stress-induced increased ROS by Pro. Moreover, application of 1 mM Pro significantly increased carotenoid concentration in SD-8 pepper leaves in the early days (3rd dpi) after infection. Ramel et al. (2012) reported that carotenoids play a role in the protection of the photosynthetic apparatus by directly deactivating singlet oxygen, which causes photoinhibition damage. This study, with the detection of an increase in the amount of anthocyanin and flavonoids in parallel with the increase in the amount of fructose due to *P. capsici* stress, also supports the view of Landi et al. (2013) that fructose is necessary for the biosynthesis of many defence compounds such as anthocyanin and phenolic compounds. In the SD-8 cultivar, Pro, exogenously applied under *P. capsici* stress, increased both endogenous Pro and soluble sugar content, and these results support Moustakas et al. (2011)'s conclusion that the Pro signalling pathway interacts with the soluble sugar signalling pathway. The increase in soluble sugar content in Pro + *P. capsici* applications revealed the positive effect of Pro on photosynthetic activity. In addition, soluble sugars participate in the defence by their capacity to directly or indirectly scavenge ROS in chloroplasts by stimulating antioxidative defence systems, as well as acting as an energy source (Van den Ende and Valluru 2009). However, exposure to 10 mM Pro + *P. capsici* caused a decrease in glucose content in the CM-334 cultivar. This result indicates that the high concentration of exogenously applied Pro may have negatively affected Rubisco activity. The same application caused an increase in glucose content only on the 7th day in SD-8 cultivar. There is information that the exogenously applied Pro can cause damage in some plants and have a stimulating effect on the defence (Hayat et al. 2012). The reason for this difference can be explained by the fact that genotypes react differently to the applied Pro concentration.

The peroxidation of lipids in biological membranes is the most obvious symptom of oxidative stress and MDA is a marker of stress-induced oxidative lipid damage. In this study, an increase in the amount of endogenous Pro was determined in parallel with the increase in the amount of MDA under *P. capsici* stress in both cultivars, but it seems that this endogenous Pro accumulation was not sufficiently effective in reducing the lipid peroxidation damage caused by *P. capsici* stress. The exogenous 1 mM Pro pre-application was determined as the most effective joint application in stabilising the protein and membrane structure by causing an increase in the amount of endogenous Pro and total protein and a significant decrease in the amount of MDA in both cultivars, although there was no decrease in MDA on the 5th dpi for CM-334. The increased level of endogenous Pro in pepper exposed to exogenous Pro can be attributed to the ROS scavenging function of Pro. Roychoudhury and Chakraborty (2013) reported that low concentrations of exogenous Pro may activate cytosolic Pro biosynthesis from glutamate and induce Pro catabolism in mitochondria. Despite the positive effects of applied Pro in inducing plant stress tolerance, there are some reports on the inhibitory effect of Pro (Ashraf and Foolad 2007). In this study, the high concentration of 10 mM Pro application caused a decrease in the endogenous Pro and total protein accumulation and an increase in the MDA in the CM-334 cultivar when compared with *P. capsici* infected plants without previous Pro application, thus supporting the results of Ashraf and Foolad (2007). In addition, Pro degradation can provide carbon, nitrogen, and energy sources. Perhaps, despite the negative effect of 10 mM Pro application, endogenous Pro oxidation may also have been used as an energy source for repair of stress-induced damage. The same application caused an increase in the amount of proline and total protein in general and a significant decrease in the amount of MDA in the sensitive SD-8 cultivar. These different defence responses are attributed to the different genotypes. Hao et al. (2016) reported that the resistance and susceptibility levels of genotypes to *P. capsici* may be due to their different genetic structures.

DPPH is a free radical that easily damages the cell membrane. The high radical scavenging activity of the organism is directly proportional to its protective effect against oxidative damage. It has been reported that the antioxidant activity in plants is mostly due to phenolic compounds (Subramanian et al. 2013). Phenolic compounds are effective hydrogen donors, which makes them good antioxidants. Krishnan et al. (2015) reported that besides phenolic compounds, flavonoid compounds that tend to accumulate under stress conditions also contribute to total antioxidant activity. In my study, the effect of Pro applied through leaf on CM-334 and SD-8 cultivars under *P. capsici* stress was different based on cultivars. DPPH scavenging activity in the

leaves of SD-8 cultivars reached the highest level in 1 and 10 mM Pro applications, and in 1 mM Pro application in CM-334 cultivar. Findings show that the DPPH scavenging activity is positively associated with the amount of flavonoids and the exogenous Pro application directly contributes to the antioxidant activity. In this study, *P. capsici* stress applied alone caused an increase in the amount of flavonoid content, especially in the CM-334 cultivar. When Pro was applied before inoculation, it was observed that it caused further increases in flavonoid levels in the CM-334 cultivar. The results are consistent with the findings of Zhang et al. (2014), which stated that Pro application stimulates flavonoid synthesis. The rapid increase in the synthesis of anthocyanins (3rd day), a class of flavonoids, which are secondary metabolites after infection, indicated that it is among the earliest defence responses against the pathogen. Anthocyanins are synthesized through the phenylpropanoid pathway. Phenylalanine is the precursor of this synthesis, and the conversion from phenylalanine to anthocyanins occurs as a result of reactions catalyzed by enzymes. A previously conducted study determined that Pro application before inoculation significantly increased phenylalanine ammonia lyase (PAL) enzyme activity in CM-334 cultivar (Koç 2017). Findings from this study show that this increase in anthocyanin is associated with an increase in PAL enzyme activity. In this context, the results corroborate the findings of Elewa et al. (2017) and Zhang et al. (2014), who reported that flavonoids and anthocyanins tend to accumulate under stress conditions. In addition, Pro contributed to hydrogen bonding removal of the DPPH radical as a hydrogen donor (Zou et al. 2016) and demonstrated its antioxidant property by acting as a free radical inhibitor or scavenger.

The reducing capacity of a compound is considered an important indicator of its potential antioxidant activity. Antioxidant compounds can donate electrons to reactive radicals, reducing them to more stable and non-reactive species (Santos-Sánchez et al. 2019). A higher absorbance indicates a higher ferric reducing power. In this study, although Pro + *P. capsici* applications showed different effects in the periods following the inoculations in both cultivars, the fact that there was an overall increase in FRAP indicates that exogenous Pro may have stimulated the production of metabolites responsible (flavanoids, anthocyanins, Pro etc.) for the reductive mechanisms of plants under the *P. capsici* stress. 1 and 10 mM Pro pre-applications caused an increase in the amount of flavonoid and anthocyanin in CM-334, and endogenous Pro in SD-8 cultivar. Also, the findings show that the CM-334 cultivar responds earlier (3rd dpi) to the *P. capsici* infection in some parameters such as flavonoid, anthocyanins, DPPH radical scavenging activity than the SD-8 cultivar. Comparing the two Pro concentrations used, in terms of plant activity performance, in CM-334, application of 1 mM of Pro was more effective than 10 mM Pro in alleviating the damage caused by *P. capsici*. The low concentration of 1 mM Pro applied exogenously increased stress tolerance in the CM-334 cultivar. In the SD-8 cultivar, 1 mM Pro + *P. capsici* application on the 5th dpi and 10 mM Pro + *P. capsici* on the 7th dpi were more effective in parameters such as DPPH radical scavenging activity and FRAP value.

In this study and in previous studies, it was seen that different plants have diverse responses to different Pro concentrations applied exogenously. Thus, it seems important to determine the optimal exogenous Pro concentrations for each plant species when used as a stress tolerance-inducing stimulant. Despite the positive effect of Pro on stress tolerance, its toxic effects at high concentrations can cause problems.

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