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Effect of *Candidatus Phytoplasma pyri* infection on fruit quality, total phenolic content and antioxidant capacity of 'Deveci' pear, *Pyrus communis* L.

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Abstract

Pear decline is an important threat for Turkish pear production. In this study, we attempt to compare several pomological characteristics, total phenolic content and total antioxidant capacities in *Candidatus Phytoplasma pyri* infected and noninfected 'Deveci' pear from Bursa, Turkey. Based on pear decline symptoms, the fruit samples were taken in October 2008 on harvest maturity from four infected and non-infected trees. Presence of *Candidatus P. pyri* was later confirmed by nested PCR tests. The result indicated that infection significantly reduced fruit size, width, length; and increased pH, color values of a, b and hue. Abortive and healthy seed numbers and weights, soluble solids and acidity did not change significantly. Similarly, the infection did not affect the flesh color. To investigate a possible differential response on skin and flesh of fruits, total phenolic (TP) and total antioxidant capacity (TAC) analyses were conducted on skin and flesh tissues separately. The results indicated that, infected skin tissue had higher total phenolic and total antioxidant capacity for both methods analyzed (TEAC and FRAP). TP content of skin increased from 806 to 923 µg gallic acid equivalents (GAE)/g fresh weight (fw) while TP content of flesh increased from 195 to 249 µg GAE/g fw. TAC also found to be enhanced on infected fruits. On average, non-infected trees had 32.4 and 28.3 µmol TE/g fw for TEAC and FRAP, respectively. Infection increased these averages to 35.4 and 32.3 µmol TE/g fw tabulating 18 and 12% increase in flesh tissue. Similarly, the TEAC and FRAP averages increased from 4.0 to 5.8 and 3.3 to 4.9 µmol TE/g fw, respectively.

Keywords: Abiotic stress, FRAP, pear decline, phytoplasma, TEAC

Introduction

Turkey is an important pear producing country with nearly 350,000 tones yearly production (FAO, 2009). Bursa is the leading province producing approximately 15% of total production. There have been recent surveys to identify phytoplasma diseases of several regions of Turkey (Sertkaya et al., 2005; Ulubaş Serçe et al., 2006; Gazel et al., 2007; Canik and Ertunç, 2007). These surveys revealed a high incidence of "Pear Decline" (PD), caused by '*Candidatus Phytoplasma pyri*' and suggested that PD is a big threat for pear production in Turkey. The PD presence were first realized by symptoms such as foliar reddening in late summer and fall, leaf roll, leaf curl, poor growth and slow or quick decline (Gazel et al., 2007). The causal agent was then confirmed by PCR. PD is transmitted by pear psylla (*Cacopsylla pyricola*, *C. pyri*). It also can be transmitted by grafting and budding. Decline is much more prevalent on trees with rootstocks of *P. ussuriensis* or *P. pyrifolia* than trees on domestic *P. communis* roots. PD is characterized by two phases; quick decline and slow decline. Trees may wilt, scorch, and die within a few weeks or loose vigor over several seasons during which foliage gets sparse with little or no terminal growth. Abnormal early red leaf coloration and reduced leaf size has been observed on effected trees.

It is known that many plant species increase secondary metabolites especially phenolics by their defense system in response to pathogen attacks (Bennett and Wallsgrow, 1994). Anthocyanine biosynthesis, usually with high antioxidant activity, is also associated with environmental stress including pathogens attacks (Chalker-Scott, 1999). It was recently demonstrated that an environmental stimulus, wounding, resulted in a 60% increase in total phenolic (TP) content and 85% increase in total antioxidant capacity (TAC) in the flesh tissues of wounded purple-colored potatoes when compared to control (Fernando and Cisneros-Zevallos, 2003). In the present study, we attempt to compare fruits from healthy (non-infected) pear trees and *Candidatus P. pyri* infected for several pomological characteristics, TP and TAC.

Materials and methods

Plant materials: A survey study resulted in the identification of numerous pear orchards infected by *Candidatus P. pyri* in Bursa and Yalova provinces of Marmara region in Turkey. The orchard located in Bursa was used in the present study. Seven years old pear trees cv 'Deveci' grafted on BA29 rootstock, four infected and noninfected trees were randomly sampled based on the plant and fruit symptoms. Fruits were harvested when they reached the harvesting

maturity in October. From each experimental tree, three subsamples of 10 fruits were randomly sampled and transferred to the laboratory for analysis. The pomological analysis were completed within 24 hrs.

Pomological analysis: Several pomological characteristics were determined on infected and noninfected fruit samples of 'Deveci' pear. Fruit weight was measured by using a digital balance with a sensitivity of 0.001 g (Scaltec, SPB31). Linear dimensions, length and width of fruits were measured by using a digital caliper gauge with a sensitivity of 0.01 mm. External and internal colors were measured with a Minolta Chroma Meter CR-400 (Minolta-Konica, Japan) having a measuring area of 8 mm in diameter for readings of small samples without cut-off. For the soluble solid contents (SSC), pH and acidity determinations, the samples were homogenized and samples were taken from this slurry. SSC was determined by a digital refractometer (Atago-1, Atago, Japan) at 20 °C. pH was determined by potentiometric measurement at 20 °C with a pH meter. The acidity was determined by titration of 0.1 N NaOH to pH 8.1, expressing malic acid (%).

Confirmation of *Candidatus P. pyri* infection by PCR: To confirm *Candidatus P. pyri* infections on the trees based on plant and fruit characteristics, PCR reactions were carried out according to Ulubaş Serçe et al. (2006). Phytoplasma specific primers P1 (Deng and Hiruki, 1991) and P7 (Smart et al., 1996) followed by R16F2n/R2 universal primers designed to amplify a 1200 bp portion of 16S rRNA gene (Lee et al., 1993) were used.

Sample extraction procedures: A single extraction procedure designed to measure total phenolic content (TP) (Singleton and Rossi, 1965) was used to determine TP and TAC of all samples. Briefly, 100 g samples were homogenized in a blender. A 3 g of aliquot was then transferred to polypropylene tubes and extracted with 15 mL of extraction buffer containing acetone, water, and acetic acid (70:29.5:0.5 v/v) for 24 h. After filtration, samples were concentrated and all laboratory procedures were performed on sub-sample duplicates of each replicate extraction.

Determination of total phenolic content (TP): To determine the amount of total phenolic compounds of the extract, Folin-Ciocalteu's phenol reagent and water 1:1:5 (v/v) were combined and incubated for 8 min followed by the addition of 2.5 mL of 7 % (w/v) sodium carbonate. After 2 h, the absorbance of each sample was measured at 750 nm on a spectrophotometer (Model T60U, PG Instruments). Gallic acid was used as standard. The results are expressed as µg gallic acid equivalent (GAE)/g fw (fresh weight).

Total antioxidant capacity (TAC): TAC was estimated by two standard procedures FRAP and TEAC assays as suggested by Ozgen et al. (2006).

The Ferric Reducing Ability of Plasma (FRAP): FRAP was determined according to the method of Benzie and Strain 1996. Assay was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid (1000:3.3 v/v), and 20 mmol/L ferric chloride. These solutions were prepared and stored in darkness under refrigeration. Stock solutions were combined (10:1:1 v/v/v) to get the FRAP reagent just prior to analysis. Absorbance at 593 nm was determined after 10 min 3 mL reagent and 50 µL peel and 200 µL fruit flesh extract was mixed. After 10 minutes, the absorbance of the reaction mixture was determined at 593 nm on a UV-VIS spectrophotometer (Model T60U, PG Instruments). The absorbance of each sample was compared with those obtained from the standard curve made from Trolox (10 - 100 µmol/L). Trolox was used as standard and the results are expressed as µmol TE/g fw basis.

Trolox Equivalent Antioxidant Capacity (TEAC): For the standard TEAC assay, ABTS was dissolved in acetate buffer and prepared with potassium persulfate as described in Ozgen et al. (2006). The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability (Ozgen et al., 2006). For the spectrophotometric assay, 3 mL of the ABTS⁺ solution and 50 µL peel or 200 µL fruit flesh acetone extract was mixed and incubated in 10 min and the absorbance was determined at 734 nm by a UV-VIS spectrophotometer (Model T60U, PG Instruments). Trolox was used as standard and the results are expressed as µmol trolox equivalent TE/g fw (fresh weight) basis.

Results and discussion

PCR analysis confirmed the *Candidatus Phytoplasma pyri* infections (Figure 1). The phytoplasma specific primers amplified a 1200 bp DNA fragment on four symptomatic trees confirming phytoplasma presence while no amplification products were observed on asymptomatic trees.

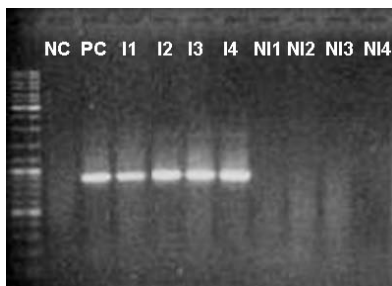


Fig. 1 The confirmation of *Canditatus Phytoplasma pyri* infection on 'Deveci' pear using P1 and P7 primers. NC = negative control, PC = positive control, I = infected, NI = noninfected.

The two fruit groups, infected vs. noninfected, were compared for several pomological characteristics. Fruit size, width, length, pH, a, b and hue values of skin color significantly differed between groups (Table 1). *Canditatus Phytoplasma pyri* infection resulted in reductions in fruit size variables. For example, fruit weight, width and length dropped from 939.2 g to 301.2 g; 92.4 to 84.6 mm, and, 90.3 to 81.9 mm, respectively making 31, 9 and 10% reductions. Infection resulted in an increase in pH from 4.0 to 4.3. *Canditatus Phytoplasma pyri* infections also had an effect on skin color; the infected fruits had higher a, b and hue averages for their skin color than those of noninfected fruits. Abortive and healthy seed numbers and weights, SSC and acidity did not change significantly. Similarly, the infection did not affect the flesh color.

Tab. 1 Several pomological characteristics of noninfected and infected (by *Canditatus Phytoplasma pyri*) of 'Deveci' pear.

Character	Noninfected	Infected	% Change	Significance
Fruit weight (g)	939.2	301.2	-31	0.001
Fruit width (mm)	92.4	84.6	-9	0.002
Fruit length (mm)	90.3	81.9	-10	0.049
Abortive seed no. (no./fruit)	2.56	5.00	49	0.067
Healthy seed no. (no./fruit)	1.19	0.75	-58	0.409
Abortive seed weight (g/fruit)	0.02	0.03	32	0.203
Healthy seed weight (g/fruit)	0.09	0.05	-97	0.203
Soluble solids (%)	13.35	12.38	-8	0.158
Acidity	0.35	0.32	-9	0.341
pH	4.0	4.3	7	0.001
Skin color (L)	74.5	74.8	0	0.688
Skin color (a)	-2.0	-2.8	29	0.036
Skin color (b)	9.8	12.5	22	0.026
Skin color (hue)	10.0	12.8	22	0.026
Skin color (C)	101.4	102.4	1	0.168
Flesh color (L)	62.1	64.9	4	0.211
Flesh color (a)	-13.2	-10.2	-29	0.090
Flesh color (b)	37.9	39.2	3	0.548
Flesh color (hue)	40.4	41.3	2	0.650
Flesh color (C)	105.7	104.1	-2	0.700

Significant comparisons, at 0.05, are bolded.

To investigate a possible differential response on skin and flesh of fruits, TP and TAC of skin and flesh tissues analyzed separately. The results indicated that, as expected, skin tissue had higher TP and TAC for both methods analyzed (Figure 2). The infection increased TP content in both tissues. Skin TP content increased from 806 to 923 $\mu\text{g GAE/g fw}$ while flesh TP content increased from 195 to 249 $\mu\text{g GAE/g fw}$. The proportion of increase was similar for both tissues (13 and 12%). TEAC and FRAP was also found to be enhanced on infected fruits. On average, noninfected trees had 32.4 and 28.3 $\mu\text{mol TE/g fw}$ for TEAC and FRAP, respectively. Infection raised these averages to 35.4 and 32.3 $\mu\text{mol TE/g fw}$ tabulating 18 and 12% increase. Although TAC was lower in flesh tissue in comparison to skin, the increase by the infection was more profound (32% by both methods). The TEAC and FRAP averages increased from 4.0 to 5.8 and 3.3 to 4.9, respectively.

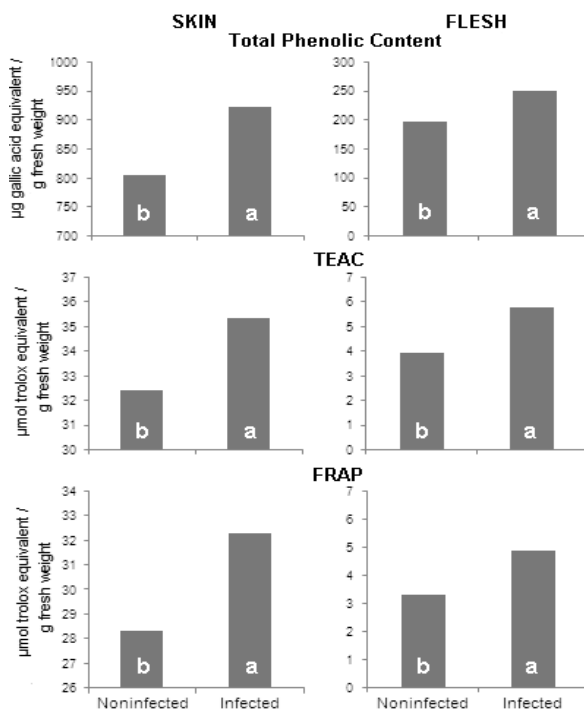


Fig. 2 Total phenolic content and total antioxidant capacity (determined by TEAC, Trolox Equivalent Antioxidant Power, and FRAP, Ferric Reducing Antioxidant Power) of skin and flesh tissues of 'Deveci' pear either noninfected or infected by *Candidatus* Phytoplasma pyri. Different letters indicated statistical significance at 0.05.

Pear has many phenolic compounds on their different tissues. For example, Andreotti et al. (2006) determined the several phenolic compounds in leaves of five pear cultivars grown in orchards, glasshouse and *in vitro*. Similar to our findings, they found differential level of several compounds affected by the environment and the age of the leaves. Indeed, specific changes on phenolics by a phytoplasma infection were examined in some of the plant species. For example, Choi et al. (2004) profiled Madagascar periwinkle (*Catharanthus roseus* L. G. Don) infected by 10 types of phytoplasmas, including apple proliferation group 16SrX-A. They identified many metabolites with different levels in phytoplasma-infected and noninfected plants. The infections increased the metabolites of chlorogenic acid, loganic acid, secologanin, and vindoline. avari et al. (2004) reported an elevated total alkaloid concentration in phytoplasma infected plants compared to controls, in particular in vinblastine. These two studies concluded that the biosynthetic pathway of some phenolics is stimulated by the infection with phytoplasmas having an important role on secondary metabolism of the diseased plants, modifying both the total content of alkaloids and their ratio.

The production of defense-related phenolic compounds in response to pathogenic or insect attack was previously described (Vidhyasekaran, 1997; Sadasivam and Thayumanavan 2003). With this in mind, Brandt and Molgaard (2001) argue that these defense-related secondary metabolites may constitute the most significant nutritional advantage of these products. In our study this plant defense reaction caused increase phenolics in both flesh and skin tissue of pear fruits. Since antioxidant activity of fruits closely related to phenolic contents (Ozgen et al., 2006), antioxidant activity of infected fruits was also increased.

Conclusions

In a wide sense phenolics play a role in plant defense against pathogens. We only understand part of the mechanism. Considerably more studies are needed to investigate in detail physiology, biochemistry and biology of this action. Limited data from our study may help future studies to understand and use this natural mechanism to overcome *Candidatus Phytoplasma pyri*.

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Literature

- Andreotti, C., Guglielmo, C.A., Dieter, T. 2006. Composition of phenolic compounds in pear leaves as affected by genetics, ontogenesis and the environment. *Sci. Hortic.* 109:130-137.
- Bennett, R.N., Wallsgrow, R.M. 1994. Secondary metabolites in plant defence mechanism. *New Phytol.* 127:617-633.
- Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* 239:70-76.
- Brandt, K., Molgaard, J.P. 2001. Organic agriculture: Does it enhance or reduce the nutritional value of plant foods? *J. Sci. Food. Agric.* 81:924-931.
- Canik, D., Ertunç, F., 2007. Distribution and molecular characterization of apple proliferation phytoplasma in Turkey. *Bulletin of Insectology* 60 (2), 335-336.
- Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. *Photochem. Photobiol.* 70: 1-9.
- Choi, Y.H., Tapias, E.C., Kim, H.K., Lefeber, A.W., Erkelens, C., Verhoeven, J.T., Brzin, J., Zel, J., Verpoorte, R. 2004. Metabolic discrimination of *Catharanthus roseus* leaves infected by phytoplasma using 1H-NMR spectroscopy and multivariate data analysis. *Plant Physiol.* 135:2398-410.
- Deng, S., Hiruki C., 1991. Genetic relatedness between two non-culturable mycoplasmalike organisms revealed by nucleic acid hybridization and polymerase chain reaction. *Phytopathology* 81:1475-1479.
- FAO, 2009. www.faostat.org
- Favali, M. A., Musetti, R., Benvenuti, S., Bianchi, A., Pressacco, L. 2004. *Catharanthus roseus* L. plants and explants infected with phytoplasmas: alkaloid production and structural observations. *Protoplasma* 223:45-51.
- Fernando, R.L., Cisneros-Zevallos, L. 2003. Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). *J. Agric. Food Chem.* 51:5296-5300.
- Gazel, M., Ulubaş Serçe, C., Çağlayan, K., Öztürk, H. 2007. Detection of '*Candidatus phytoplasma pyri*' in Turkey. *Bull. Insec.* 60:125-126.
- Lee I.M., Gundersen D.E., Davis R.E., Bartoszyk I.M., 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *Int. J. Syst. Bact.* 48:1153-1169.
- Ozgen, M., Reese, R.N., Tulio, A.Z., Miller, A.R., Scheerens, J.C., 2006. Modified 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) Methods. *J. Agric. Food Chem.* 54:1151-1157.
- Sadasivam, S., Thayumanavan, B. 2003. *Molecular host plant resistance to pests.* Marcel Dekker, Inc., New York.
- Sertkaya G., Martini M., Ermacora P., Musetti R., Osler R., 2005. Detection and characterization of phytoplasmas in diseased stone fruits and Ppear by PCR-RFLP analysis in Turkey. *Phytoparasitica* 33:380-390.
- Singleton, V.L., Rossi, J.L., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Amer. J. Enol. Viticult.* 16:144-158.
- Smart, C.D., Schneider B., Blomquist C.L., Guerra L.J., Harrison N.A., Ahrens U., Lorenz K.H., Seemuller E., Kirkpatrick B.C., 1996. Phytoplasma-specific PCR primers based on sequences of 16S-23SrRNA spacer region. *Appl. Envir. Microb.* 62: 2988-2993.
- Ulubaş Serçe, Ç., Gazel, M., Çağlayan, K., Baş, M. Son, L. 2006. Phytoplasma diseases of fruit trees in germplasm and commercial orchards in Turkey. *J. Plant Path.* 88:179-185.
- Vidhyasekaran, P. 1997. *Fungal pathogenesis in plants and crops. Molecular biology and host defense mechanisms.* Marcel Dekker, Inc., New York.