Net Journal of Agricultural Science
Vol. 5(2), pp. 58-65, June 2017
ISSN: 2315-9766
Full Length Research Paper

Changes in the activities of metabolic enzymes and antioxidant defense system in '*Candidatus* phytoplasma solani' infected pepper (C*apsicum annuum* L.) plants

Irada Huseynova*, Gulnara Balakishiyeva, Durna Aliyeva, Ulduza Gurbanova, Jamila Bayramova, Nurmammad Mustafayev and Jalal Aliyev

Institute of Molecular Biology and Biotechnology, Azerbaijan National Academy of Sciences, 2a Matbuat Avenue, AZ1073, Baku Azerbaijan.

Accepted 31 May, 2017

ABSTRACT

Phytoplasmas are pathogens of important annual crops as well as perennial cultures, causing different symptoms that ranges from yellowing to death of infected plants. The aim of the present work was to analyze the changes in the activity of metabolic and antioxidant enzymes, as well as in the content of total soluble sugars, tocopherols, and total phenolic compounds in field-grown pepper plants (*Capsicum annuum* L.) under the influence of phytoplasma infection. The activities of benzidine peroxidase (BPO) and guaiacol peroxidase (GPO) were observed to increase in infected pepper leaves in comparison with the healthy control. According to the native polyacrylamide gel electrophoresis (PAGE), the levels of studied antioxidant enzyme isoforms were enhanced under pathogenesis. Activities of aspartate aminotransferase (AsAT) and alanine aminotransferase (ALAT) were also increased in the infected plants compared with healthy plants. The amount of tocopherols, soluble sugars, and total phenols was significantly higher in leaves due to the phytoplasma infection. In conclusion, all of the observed alterations could be considered to be the response and the adaptation ability of pepper plants against pathogen infection.

Keywords: *Capsicum annuum* L., '*Candidatus* phytoplasma solani', metabolic enzymes, peroxidase, soluble sugars, tocopherols, phenolic compounds.

*E-mail: huseynova-i@botany-az.org. Tel: +994 12 538 1164. Fax: +994 12 510 2433.

INTRODUCTION

Phytoplasmas are associated with diseases in several hundreds of important plant species. These pathogens multiply within the phloem cells of the host plant and are transmitted from plant to plant by phloem-feeding insects and by vegetative multiplication of infected plant material (Weintraub and Beanland, 2006). Phytoplasmas spread through the plants by insect vectors during feeding activity and survive and multiply in the plant phloem and insect hemolymph (Bertaccini et al., 2014). In Azerbaijan, '*Candidatus* Phytoplasma solani' is the most common phytoplasma, which has a wider range of host plants (Balakishiyeva et al., 2010). '*Ca.* P. solani', is widespread in all European and Mediterranean areas, and it can seriously affect quality and quantity of production (Quaglino et al., 2013). This phytoplasma induces stolbur disease and infected plants show leaf discoloration, stunting and especially flower malformations, such as virescence and phyllody, leading to sterility (Bertaccini and Duduk, 2009). All these morphological changes affecting phenotypical expression closely related to biochemical alterations in infected plants. One of the earliest cellular responses of plants to pathogen infections is the intensification of the synthesis of reactive oxygen species (ROS). This is one of the major host

defenses against pathogenic bacteria. One of the main reasons for the formation of ROS, including hydrogen peroxide (H_2O_2) was found to be increasing intensity of photorespiration during pathogenesis. H_2O_2 is the most stable compound among ROS and it plays a signaling role in plant responses to stress (Van Breusegem et al., 2008). During the pathogen attack certain defense mechanisms in plants, such as alterations in antioxidant substances and enzyme synthesis, were induced (An et al., 2010). One of the pivotal enzymes providing plant defense against pathogens is peroxidase. Peroxidases (EC, 1.11.1.7) perform various functions in living organisms. They fulfil H_2O_2 detoxification along with catalase (EC 1.11.1.6) (Huseynova et al., 2015). Peroxidases, defending plants against oxidative stress, also control plant growth, differentiation and development and participate in the lignification of cell walls (Blee et al., 2003). Therefore, peroxidases can be considered as stress markers of the organism.

Soluble compounds (including sucrose) are accumulated in plants during pathogenic disease and participate in plant protection against stress (Rolland et al., 2006). Tocopherols and total phenolic compounds are low molecular weight antioxidants protecting plants against oxidative stress (Shalaby and Horwitz, 2015). Phenolic compounds may contribute to enhance the mechanical strength of host cell walls by the synthesis of lignin and suberin that are involved in the formation of physical barriers that can block the spread of pathogens. It is known that the presence of phenolic compounds in plants and their synthesis in response to infection is associated with resistance (Jayaraj et al., 2010).

The aim of the present work was to investigate the biochemical alterations in metabolic and antioxidant enzyme activities in field-grown pepper plants (*Capsicum annuum* L.) infected with '*Candidatus* Phytoplasma solani'.

MATERIALS AND METHODS

Plant materials

The pepper (*Capsicum annuum* L.) leaves used in this study were collected from field-grown plants with symptoms reminiscent of phytoplasma infection located in experimental field of the Institute of Botany, Baku, Azerbaijan. Symptomless plant leaves were also used as control.

Phytoplasma detection and identification

Total DNAs were extracted from 1 g fresh leaf midribs of infected and healthy plants (as control) following CTAB extraction protocol (Maixner et al., 1995). Extracted DNA concentrations were determined by nanodrop reading. DNA samples were tested by 16S-rDNA nested PCR with the universal primers for phytoplasmas R16mF2 / R16mR1 and R16F2n / R16R2 (Gundersen and Lee, 1996). Polymerase chain reaction (PCR) was performed in mixtures (50 µl total volume) containing 100 ng of nucleic acid, PCR Buffer 1X, 2 mM $MgCl_2$, 1 µM of each primer, 200 µL of dNTP mix, and 2 units of *Taq* polymerase. Following conditions were used: denaturation at 94°C for 1 min (94°C for 2 min for the first cycle), annealing (hybridation) at 60°C during 2 min (55°C for second amplification in Nested PCR), and primer extension (elongation) at 72°C for 3 min and 10 min in the final cycle. 7 µl of Nested PCR products were analyzed by agarose gel electrophoresis.

Identification was performed by enzymatic restriction fragment polymorphism (RFLP). The obtained Nested PCR products (1.25 kbp) were digested with *Alu*I (Promega), and *Rsa*I (Promega) restriction endonucleases according to the manufacturer's protocol.

Estimation of total phenolic compounds

The amount of total phenol compounds in the plant tissue was estimated using the method proposed by Mallick and Singh (1980). Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent to produce a blue-coloured complex in alkaline medium, which can be estimated spectrophotometrically at 650 nm. Standard gallic acid solution (0.2 to 1.0 ml) corresponding to 2.0 to 10 µg concentrations were also treated as above. The concentration of total phenolic compounds is expressed as mg/g dry weight.

Estimation of soluble sugars

The amount of the soluble sugars in the plant tissue was determined using the anthrone method (Kochetkov et al., 1976). An aliquot of the extract was hydrolyzed in 5 ml of 0.4% anthrone solution (4 g anthrone in 1000 ml 95% H_2SO_4) in boiling waterbath for 15 min. After cooling, the sugar concentration was determined spectrophotometrically at 620 nm. Sucrose was used as standard. The concentration of soluble sugars is expressed as mg/g dry weight.

Estimation of tocopherol

Tocopherol was estimated in the plant samples by the Emmerie-Engel reaction as reported by Rosenberg (1992). The Emmerie-Engel reaction is based on the reduction of ferric to ferrous ions by tocopherols, which, with 2,2'-dipyridyl, forms a red colour. Tocopherols and carotenes are first extracted with xylene and read at 460 nm to measure carotenes. A correction is made for this after adding ferric chloride and read at 520 nm. Tocopherol was used as a standard. The concentration of tocopherol in the sample was calculated using the formula:

Tocopherols (µg/mg fresh weight) =
$((\text{Sample } A_{520}-A_{460})/\text{Standard } A_{520}) \times 0.29 \times 0.15$

Extractions and determination of the activity of aminotransferases

The plant material frozen in liquid nitrogen was thawed, and 200 mg of it was subjected to quickly extraction using a chilled mortar and pestle with 1 ml of medium containing 100 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 1 mM EDTA, 10 mM 2-Mercaptoethanol, 2 mM phenylmethylsulphonyl fluoride (PMSF) and 2% (w/v) insoluble polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12000 g for 5 min. Supernatant was used for the enzyme activity assays. Enzymatic activities of transferases were determined spectrophotometrically (Ultrospec 3300 pro, Amersham, USA).

Measurements were taken at 340 nm for 1 min and the obtained results were expressed as U/mg protein min.

Alanine aminotransferase activity (ALAT)

The ALAT (EC 2.6.1.2) enzyme activity was determined at 25°C in a continuous assay by coupling the reaction of lactate dehydrogenase (LDH) to nicotinamide adenine dinucleotide (NADH) oxidation (Horder and Rej, 1983). The reaction mixture contained 70 mM alanine, 10 mM 2-oxoglutarate, 5 µM pyridoxal 5-phosphate, 0.2 mM NADH, 2mM EDTA, 25 mM Tris-HCl (pH 8.5) and 3 units of LDH. Results were expressed as U/mg protein min.

Aspartate aminotransferase (ASAT) activity

ASAT (EC 2.6.1.1) activity was measured in 1 ml volume at 25 mM Tris-HCl (pH 8.5), containing 2mM EDTA, 2.5 mM 2-oxoglutarate, 5 µg/ml pyridoxal 5-phosphate, 10 mM DTT, 12 U/ml malate dehydrogenase, 0.1 mM NADH (Alfonso and Brüggemann, 2012). Results were expressed as U/mg protein min.

Extraction and determination of the activity of peroxidases

To obtain total cell extract, pepper leaves were homogenized in a medium containing 1 mM EDTA, 2 mM PMSF, 1% PVP, 100 mM Na-phosphate buffer (pH 7.8), 0.1% Triton X-100, then filtered and centrifuged for 20 min at 15,000 g. The resulting supernatant was used for analysis of peroxidase enzymes. The activities of the studied enzymes in leaves of pepper were assessed spectrophotometrically at a linear reaction and results were expressed as µmol/mg protein min.

Guaiacol-type peroxidase activity (GPO)

Guaiacol-type peroxidase activity (EC 1.11.1.7) was determined by the change in optical density of the reaction mixture for 3 min at 470 nm (Mahalingam et al., 2005). GPO activity was calculated according to the quantity of oxidized guaiacol µmol / (mg protein min) considering the extinction coefficient, $\varepsilon = 26.6\ mM^{-1}cm^{-1}$.

Activity of the benzidine-type peroxidase (BPO)

Benzidine-type peroxidase (EC 1.11.1.7) activity was measured by the increase in optical density of the reaction mixture for 1 min at 590 nm (Gechev et. al., 2002). The activity was calculated from the amount of expended benzidine in µmol / (mg protein min) considering extinction coefficient, $\varepsilon = 39\ mM^{-1}cm^{-1}$.

Determination of the isoenzyme content of peroxidase enzymes

Qualitative changes in the enzyme activities were determined using a vertical native 7% PAGE according to the method of Davis (Davis, 1964). Electrophoresis was carried out for 3 h at 4°C with a steady current of 30 mA, using the device SE 250 (Amersham Biosciences, USA). Staining of guaiacol peroxidase lines was performed by the method of Radotic et al. (2000) and benzidine peroxidase by the method of Cuypers et al. (2002).

Statistical analysis

The significance of differences between control and experimental groups was analyzed using the unequal variance two-tailed Student's *t* –test. Data with $P \leq 0.05$ were considered statistically significant. Three different samples for each treatment were taken and analysed twice.

RESULTS

Phytoplasma detection and identification

The infected pepper plants have shown typical symptoms, including sterility of the flowers, leaf yellowing and leaf rolling. The presence of the phytoplasmas was confirmed by 16S-rDNA nested PCR with the universal primers R16mF2 / R16mR1 and R16F2n / R16R2 (Figure 1). While healthy control gave no amplification, an expected 1250 bp fragments were obtained from all symptomatic pepper plant DNA samples (Figure 1).

Taxonomic characterization was performed by RFLP analysis of 16 S Nested PCR products with *Alu*I and *Rsa*I restriction enzymes. Results of RFLP analysis indicated that all the pepper samples were infected by '*Candidatus* Phytoplasma solani'.

The changes in the activity of metabolic and antioxidant enzymes, as well as in the content of total soluble sugars, tocopherols, and total phenolic compounds in field-grown pepper plants (*Capsicum annuum* L.) were analyzed under the influence of '*Ca.* P. solani' infection.

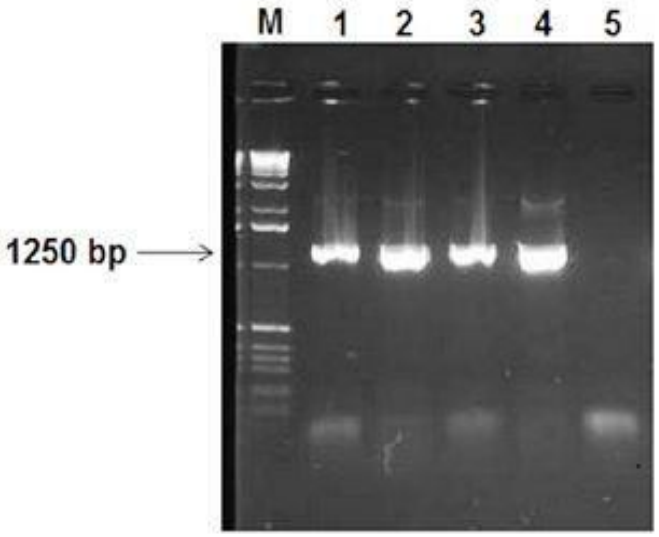

Figure 1. Nested PCR amplification of phytoplasma 16S rDNA with the universal primer pairs R16mF2 / R16mR1 and R16F2n / R16R2. Lane M - 1Kb DNA ladder (Invitrogen), lanes 1, 2 and 3 are infected pepper samples, lane 4- positive control, lane 5- negative control.

Aminotransferase activity

Aspartate aminotransferase (ASAT) plays an important role in the primary nitrogen assimilation, in the transport of reducing equivalents and also in the exchange of carbon (C) and nitrogen (N) among subcellular compartments of the cell. The activity of ASAT was higher in leaves of infected plants compared with healthy plant samples and the highest activity was observed in infected samples N3. The activity of ASAT increased 1.7, 1.3 and 2.6 times in infected leaf samples N1, N2 and N3, respectively (Figure 2).

As an index of metabolic alterations caused by phytoplasma infection in pepper plants, alanine aminotransferase activity was also studied comparatively in infected and healthy plant leaves. ALAT activity was found to increase 3.8, 2.2 and 8.4 times in phytoplasma infected samples N1, N2 and N3, respectively in comparison with the healthy control samples (Figure 3). The rate of the general metabolic processes was higher in infected sample N3.

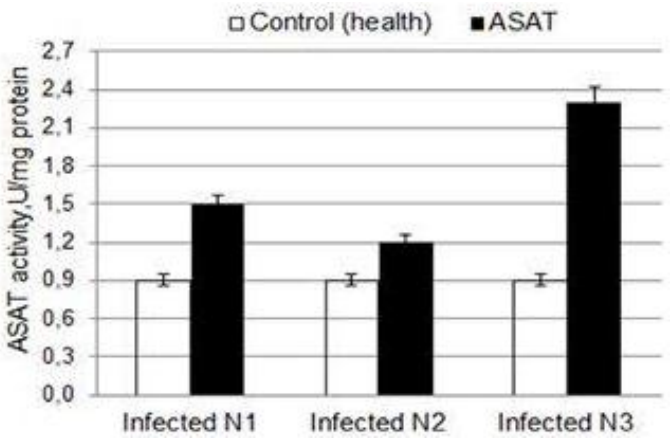

Figure 2. Changes of aspartate aminotransferase activity in healthy and phytoplasma infected pepper leaves.

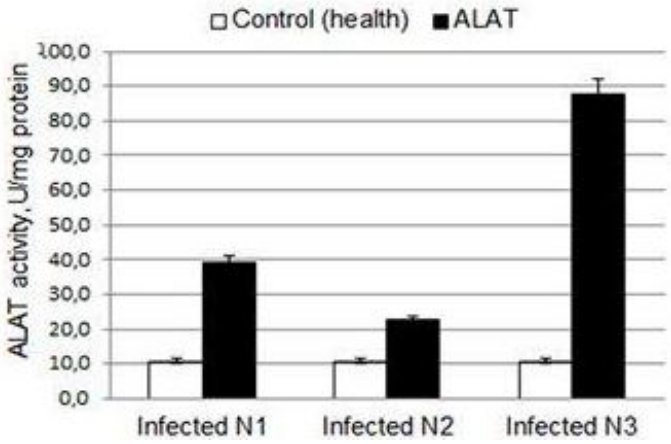

Figure 3. Changes of alanine aminotransferase activity in healthy and phytoplasma infected pepper leaves.

Total soluble sugars, tocopherols and total phenolic compounds

Synthesis of polysaccharides which takes an active part in the stabilization of biomembranes was intensified under stress. An increase in the sucrose amount was observed in all the infected pepper samples. So, compared with the healthy leaves in the first, second and third samples, 21.7, 8.7 and 30.9% increases in sucrose amounts were observed, respectively (Table 1).

Phenolic compounds were considered as a major group of compounds that contributed to the antioxidant activity. Significant amounts of total phenolic compounds (TPC) were detected in all pepper samples infected by '*Candidatus* Phytoplasma solani'. The content of TPC of the different pepper leaf samples ranged from 55.19 to 200.11 mg GA g^{-1} DW (Table 1). Higher amount of TPC was detected in the infected sample N3 (~4 times more), and significantly lower content in N2 compared with the healthy sample.

Tocopherols are a group of powerful antioxidants having additional roles in signaling and gene expression. In our investigations, tocopherols content (vitamin E) in the infected pepper leaves ranged from 0.673 to 1.287 µg/mg fresh weight (Table 1).

Peroxidase activity

In the present work, two types of substrates were used to determine the activities of peroxidases: anionic peroxidases express higher specificity to benzidine, while cationic peroxidases are more specific to guaiacol. Peroxidase activity was observed to change in the infected pepper leaves compared with control (healthy) ones. Peroxidase activity increase occurred in the infected leaves of all studied plants and was dependent on the degree of leaf damage. Guaiacol peroxidase activity increased 4, 1.5 and 3 times in the variants N3, N1 and N2, respectively (Table 2). The sharp enhancement of the guaiacol peroxidase activity during stress may be related to the increased content of phenolic compounds.

Pathogen infection causes rapid activation of peroxidases, which is often accompanied by the emergence of multiple new forms and disappearance of other forms of the enzyme. Native gel electrophoresis was performed to determine isoenzyme content of peroxidase in phytoplasma-infected pepper leaves (Figure 4). According to the electrophoregram, the

Table 1. Changes of total phenolic compounds, tocopherols and sucrose contents in healthy and phytoplasma infected pepper leaves.

Plant samples	Total phenolic compounds (mg/g dry weight)	Tocopherols (µg/mg fresh weight)	Sucrose (mg/g dry weight)
Control (health)	43.75 ± 2.18	0.59 ± 0.03	15.01 ± 0.75
Infected N1	75.21 ± 3.76	0.80 ± 0.04	18.27 ± 0.91
Infected N2	55.19 ± 2.50	0.67 ± 0.03	16.32 ± 0.81
Infected N3	200.11 ± 10.00	1.29 ± 0.06	19.65 ± 0.98
P values			
Control/N1	***	**	**
Control/N2	**	*	ns
Control/N3	***	***	**
N1/N2	**	*	ns
N1/N3	***	***	ns
N2/N3	***	***	*

Note: *** – p<0.001; ** – p<0,01; * – p<0.05; ns – no significance;
Means ± SD of three replicates.

Table 2. Changes of guaiacol and benzidine peroxidase activities in healthy and phytoplasma infected pepper leaves.

Plant samples	GPO (µmol/mg protein min)	BPO (µmol/mg protein min)
Control (healthy)	0.013 ± 0.001	0.943 ± 0.090
Infected N1	0.020 ± 0.002	2.357 ± 0.230
Infected N2	0.039 ± 0.004	1.836 ± 0.180
Infected N3	0.053 ± 0.005	1.422 ± 0.140
P values		
Control/N1	**	***
Control/N2	***	**
Control/N3	***	**
N1/N2	**	*
N1/N3	***	**
N2/N3	*	*

Note: *** – p < 0.001; ** – p < 0.01; * – p < 0.05; ns – no significance;
Means ± SD of three replicates
GPO - guaiacol peroxidase
BPO - benzidine peroxidase.

phytoplasma infection in pepper plants did not cause the emergence of new forms of guaiacol peroxidase (Figure 4a). Intensification of stained bands testified an enhancement of GPO activity in these plants upon pathogen infection. Apparently, peroxidase activation occurred due to the activation of isoperoxidase existing already in the cell. The increase in enzyme activity with the increasing degree of leaf damage may have occurred due to peroxidase synthesized by the pathogen itself. The electrophoregram of benzidine peroxidase showed an increase in the amounts of isoforms (Figure 4b). The emergence of three new forms of benzidine peroxidase (BPO3, BPO4 and BPO5) with high electrophoretic mobility was detected in all infected samples and one more high-molecular form (BPO1) in sample N1. Apparently, peroxidase activation occurs due to *de novo* synthesis of peroxidases. This suggests that those isoenzymes are most likely involved in defense mechanism in relation to pathogenesis of '*Candidatus* Phytoplasma solani' in pepper plants.

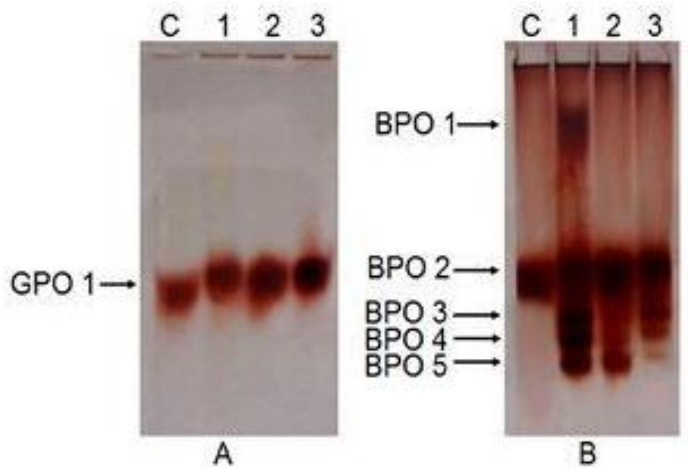

Figure 4. Native gel analysis for GPO (a) and BPO (b) activity in healthy and phytoplasma infected pepper leaves. Lane C – control, healthy pepper sample; lanes 1-, 2-, 3- phytoplasma infected pepper samples, respectively. Arrowheads indicate a novel isoenzymes induced by phytoplasma infection.

DISCUSSION

Phytoplasmas cause many different diseases, damaging the physiological and biochemical processes in plants. However, several recent studies have improved the knowledge on the effect of phytoplasma infection on host metabolism (Lepka et al., 1999; Musetti et al., 2000; Abdollahi et al., 2012; Margaria et al., 2013). Physiological and biochemical aspects of phytoplasma-plant interaction are still required to be investigated. Studies of biochemical alterations in plants induced by phytoplasma infection are important contribution to clarifying plant-pathogen interaction and understanding how plants react to phytoplasma infections. In our investigations, the effect of '*Ca.* P. solani' infection on the activity of metabolic and antioxidant enzymes, content of total soluble sugars, tocopherols, and total phenolic compounds in field-grown pepper plants was studied.

Phytoplasma infections adversely affect amino acid transport in host plants. An increase in the amounts of amino acids was detected in leaves of *C. roseus* and tobacco plants infected with the phytoplasma species "Ash yellows" and "*Ca*. P. mali", respectively (Lepka et al., 1999). Phytoplasma infection causes limitations in amino acid transport in phloem tubes of plant leaves, resulting in their accumulation (Carginale et al., 2004). Significant increase in aminotransferases activities have been observed in this study. Subbaiah and Sachs (2003) have also reported the occurrence of alterations in alanine aminotransferase activity in maize plants under the influence of pathogens. Alanine aminotransferase (ALAT, EC 2.6.1.2) catalyzes the conversion of alanine and 2-oxoglutarate into pyruvate and glutamate. This peroxidase-dependent enzyme plays a pivotal role in plant metabolism, especially in primary carbon metabolism and amino acid synthesis (Kendziorek et al., 2012; Mello, 2015). ALAT also plays an important role in the formation of the plant response to various pathogenes (Kim et al., 2005). Aspartate aminotransferase (ASAT, EC 2.6.1.1) plays a major role in the primary nitrogen assimilation, transport of reducing equivalents and exchange of carbon and nitrogen supplies among cellular subcompartments (Torre et al., 2014; Gaufichon et al., 2015). The evaluation of the role of ASAT and ALAT in plant responses to adverse environmental conditions and biotic stress is considered as the most urgent issue.

The result showed that the content of total soluble sugars increased in infected pepper leaf samples in comparison with healthy control plants. An increase in the sucrose amount can be explained by its anti denaturating effect on protein-lipid components exposed to denaturation under biotic stress. This is consistent with the results obtained by Lepka and co-authors (Lepka et al., 1999). The authors showed that phytoplasma infection resulted in an increase in the soluble carbohydrate and starch in source leaves of tobacco and periwinkle plants. In sweet orange (*Citrus sinensis*) leaves infected with '*Candidatus liberibacter asiaticus*', sucrose and glucose remained at high levels compared to healthy leaves, whilst no accumulation of fructose was observed; in contrast, the maltose content decreased (Fan et al., 2010).

In our investigations, content of total phenolic compounds and tocopherols in infected pepper leaves was increased in comparison with healthy control. Plant phenolic compounds are known to protect plants against tissue injuries, high levels of oxygen, free radicals and reactive oxygen species (Shalaby and Horwitz, 2015). Having high activity due to the aromatic rings and free hydroxyl groups in their composition, these compounds easily react with free radicals and scavenge ROS formed during stress. This function of phenolic compounds is more important for leaves compared with other organs of plants during pathogen infection, as chloroplasts where the formation of H_2O_2 and other ROS occur are located in the leaves (Foyer and Noctor, 2003). Tocopherols prevent the oxidation of unsaturated fatty acids in cell membrane, thus maintaining their structure (Mohamed et al., 2012). Phenolic compounds have different rates of accumulation depending on whether plant-pathogen interaction is compatible or incompatible. The results of this study were similar to those reported by Zhao et al. (2008). Results of other studies point to an involvement of phenolic compounds in the defensive reactions of banana plants against various pathogens (Ewané et al., 2012). The defense response of the tomato tissue was

seen in an increase in soluble phenol, and/or the deposit of lignin (Zhu and Yao, 2004). Therefore, it seems reasonable to speculate that the increment in tocopherol levels in infected pepper leaf tissues may enhance resistance to '*Candidatus* Phytoplasma solani' by protecting cell membrane from oxidative damage associated with infection. Thus, an increase in the quantity of low molecular weight antioxidants in the studied samples under stress is considered to be the adaptation ability of pepper plants against phytoplasma infection.

Results also showed that the peroxidase activities were significantly higher in infected pepper leaves than in healthy control. These results have shown increase of peroxidase activity under phytoplasma infection is in compliance with Agrios (2005). According to the literature, peroxidase activation is an index of early plant response to pathogen exposure and plant tolerance (Kuvalekara et al., 2011). Peroxidase is highly sensitive to environmental factors and biotic and abiotic stresses cause the alterations in enzyme activity. Therefore, peroxidase actively participating in self-regulation of plant metabolism under infection is considered to be one of the most important catalytic systems among biochemical factors of plant protection against pathogen microorganisms. Our investigations also showed that new molecular forms of the peroxidases are synthesized under the influence of phytoplasma infection. It can be concluded that the phytoplasma infection is accompanied by changes in peroxidase activity and, quantitative and qualitative alterations of its multiple molecular forms. The determination of peroxidase activity allows the assessment of plant tolerance against pathogen at early stages of plant development and hence, it is possible to purposefully speed up selection work by the exclusion of sensitive varieties.

CONCLUSION

In summary, the activities of nitrogen metabolism enzymes and antioxidant defence enzymes, content of soluble sugars, low molecular weight antioxidants were found to increase in diseased pepper plants compared with healthy ones under the influence of phytoplasma infection. Alterations in the activities of metabolic enzymes, which provide the response to various abiotic and biotic stresses, are considered to be the plant response to the phytoplasma infection. The increase in the quantity of low molecular weight antioxidants such as flavonoids, phenolic acid and tocopherols exerts protective effects against oxidative stress. So, it could be considered to be the adaptation ability of pepper plants against pathogen infection. Further research should aim to investigate the antioxidant defence enzymes in the most productive pepper cultivars in comparison with the susceptible and resistant genotypes.

ACKNOWLEDGEMENTS

This work was supported by the Science Development Foundation under the President of the Republic of Azerbaijan (EİF-2014-9(24)-KETPL-14/11/3).

REFERENCES

Abdollahi F, Niknam V, Ghanati F, Masroor F, Noorbakhsh SN, **2012**. Biological effect of weak electromagnetic field on healthy and infected lime (*Citrus aurantifolia*) trees with phytoplasma. Sci World J, 716929.

Agrios GN, **2005**. Plant Pathology, 5th ed. Elsevier Academic Press, San Diego.

Alfonso SU, **Brüggemann** W, **2012**. Photosynthetic responses of a C3 three C4 species of the genus *Panicum* (s.l.) with different metabolic subtypes to drought stress. Photosynth Res, 112: 175-191.

An Y, Kang S, Kim K-D, Hwang BK, Jeun YC, **2010**. Enhanced defense responses of tomato plants against late blight pathogen *Phytophthora infestans* by pre-inoculation with rhizobacteria. Crop Prot, 29: 1406-1412.

Balakishiyeva G, Danet JL, Qurbanov M, Mamedov A, Khey-Pour A, Foissac X, **2010**. First report of phytoplasma infections in several temperate fruit trees and vegetable crops in Azerbaijan. J Plant Pathol, 92(S4): 115.

Bertaccini A, **Duduk** B, **2009.** Phytoplasma and phytoplasma diseases: a review of recent research. Phytopathol Mediterr, 48: 355-378.

Bertaccini A, Duduk B, Paltrinieri S, Contaldo N, **2014**. Phytoplasmas and phytoplasma diseases: A severe threat to agriculture. Am J Plant Sci, 5: 1763-1788.

Blee KA, Choi JW, O'Connell AP, Schuch W, Lewis NG, Bolwell GP, **2003**. A lignin-specific peroxidases in tobacco whose antisense suppression leads to vascular tissue modification. Phytochemistry, 64:163–176.

Carginale V, Capasso MG, Ionata E, la Cara F, Pastore M, Bartaccini A, Capasso A, **2004**. Identification of genes expressed in response to phytoplasma infection in leaves of *Prunus armeniaca* by messenger RNA different display. Gene, 332: 29-34.

Cuypers A, Vangronsveld J, Ciijsters H, **2002**. Peroxidases in roots and primary leaves of *Pharseolus vulgaris* copper and zinc phytotoxcity: a comparison. J Plant Physiol, 159: 869-876.

Davis B, **1964**. Disc electrophoresis. I. Method and application to human serum proteins. Ann New York Acad Sci, 121: 404-427.

Ewané CA, Lepoivre P, de Bellaire L, Lassois L **2012**. Involvement of phenolic compounds in the susceptibility of bananas to crown rot. Biotechnol Agron Soc Environ, 16(3): 393-404.

Fan J, Chen C, Brlansky RH, Gmitter FG, Lia Z-G, **2010**. Changes in carbohydrate metabolism in *Citrus sinensis* infected with *'Candidatus liberibacter asiaticus'*. Plant Pathol, 59: 1037–1043.

Foyer CH, **Noctor** G, **2003**. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxiosomes and mitochondria. Physiol Plant, 119: 355-364.

Gaufichon L, Steven JR, Akira S, **2016**. Asparagine metabolic pathways in Arabidopsis. Plant Cell Physiol, 57(4): 675-689.

Gechev T, Gadjiev I, van Breusagem E, Inzé D, Dukiandjiev S, Toneva V, Minkov I, **2002**. Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. Cell Mol Life Sci, 59: 708-714.

Gundersen DE, **Lee** IM, **1996**. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathol Mediterr, 35: 114-151.

Hørder M, **Rej** R, **1983**. Alanine aminotransferase. In: Methods of enzymatic analysis. 3rd ed. Bergmeyer HU, Bergmeyer J, Grabl M (Eds.), Chemie, Weinheim, Verlag, Germany, pp. 444-456.

Huseynova IM, Aliyeva DR, Mammadov ACh, Aliyev JA, **2015**. Hydrogen peroxide generation and antioxidant enzyme activities in the leaves and roots of wheat cultivars subjected to long-term soil drought stress. Photosynth Res, 125(1-2): 279-289.

Jayaraj J, Bhuvaneswari R, Rabindran R, Muthukrishnan S, Velazhahan R, **2010**. Oxalic acid-induced resistance to *Rhizoctonia solani* in rice is associated with induction of phenolics, peroxidase and pathogenesis-related proteins. J Plant Interact, 5: 147–157.

Kendziorek M, Paszkowski A, Zagdanska B, **2012**. Differential regulation of alanine aminotransferase homologues by abiotic stresses in wheat (*Triticum aestivum L.)* seedlings. Plant Cell Reports, 31: 1105-1117.

Kim KJ, Park CJ, An JM, Ham BK, Lee BJ, Paek KH, **2005**. CaAlaAT1 catalyzes the alanine: 2-oxoglutarate aminotransferase reaction during the resistance response against Tobacco mosaic virus in hot pepper. Planta, 221: 857-867.

Kochetkov NK, Bochkov AF, Dmitriyev BA, Usov AI, Chijov OS, Shibayev BN, **1976**. Chemistry of carbohydrates. Chemistry, Moscow, pp. 672 (in Russian).

Kuvalekara A, Redkara A, Gandheb K, Harsulkara A, **2011**. Peroxidase and polyphenol oxidase activities in compatible host pathogen interaction in *Jasminum officinale* and *Uromyces hobsoni*: Insights into susceptibility of host. N. Z. J Bot, 49(3): 351-359.

Lepka P, Stitt M, Moll E, Seemüller E, **1999**. Effect of phytoplasmal infection on concentration and translocation of carbohydrates and amino acids in periwinkle and tobacco. Physiol Mol Plant Pathol, 55(1): 59-68.

Mahalingam R, Shah N, Scrymgeour A, Fedoroff N, **2005**. Temporal evolution of the *Arabidopsis* oxidative stress response. Plant Mol Biol, 57: 709-730.

Maixner M, Ahrens U, Seemuller E, **1995**. Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. Eur J Plant Pathol, 101: 241–250.

Mallick CP, **Singh** MB, **1980**. Plant enzymology and histoenzymology. Kalyani publishers, New Delhi, pp. 286.

Margaria P, Abbà S, Palmano S, **2013.** Novel aspects of grapevine response to phytoplasma infection investigated by a proteomic and phospho-proteomic approach with data integration into functional networks. BMC Genomics, 14: 38.

Mello JPFD, **2015**. Amino acids in higher plants. CABI Publishing, pp. 632.

Mohamed H, EL-Hady AA, Mansour M, El-Samawaty AE, **2012**. Association of oxidative stress components with resistance to flax powdery mildew. Trop Plant Pathol, 37(6): 386-392.

Musetti R, Favali MA, Pressacco L, **2000.** Histopathology and polyphenol content in plants infected by phytoplasmas. Cytobios., 102: 133–147.

Quaglino F, Zhao Y, Casati P, Bulgari D, Bianco PA, Wei W, Davis RE, **2013**. '*Candidatus* Phytoplasma solani', a novel taxon associated with Stolbur- and Bois Noir-related diseases of plants. Int J Syst Evol Microbiol, 63: 2879-2894.

Radotic K, Ducic T, Mutavdzic D, **2000**. Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentrations of cadmium. Envir and Exp Bot, 44: 105–113.

Rolland F, Baena GE, Sheen J, **2006**. Sugar sensing and signalling in plants: conserved and novel mechanisms. Annu Plant Biol, 57: 675-709

Rosenberg HR, **1992**. Chemistry and physiology of the vitamins. Interscience Publishers, New York, pp. 452–453.

Shalaby S, **Horwitz** BA, **2015**. Plant phenolic compounds and oxidative stress: integrated signals in fungal-plant interactions. Curr Genet, 61(3): 347-357.

Subbaiah CC, **Sachs** MM, **2003**. Molecular and cellular adaptations of maize to flooding stress. Ann Bot, 91: 119-127.

Torre F, Cañas RA, Pascual BM, Avila C, Cánovas FM, **2014**. Plastidic aspartate aminotransferases and the biosynthesis of essential amino acids in plants. J Exp Bot, 65(19): 5527-5534.

Van Breusegem F, Bailey-Serres J, Mittler R, **2008**. Unravelling the tapestry of networks involving reactive oxygen species in plants. Plant Physiol, 147: 978–984.

Weintraub PG, **Beanland** L, **2006**. Insect vectors of phytoplasmas. Annu Rev Entomol, 51: 91-111.

Zhao H, Fan W, Dong J, Lu J, Chen J, Shan L, Lin Y, Kong W, **2008**. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chem, 107: 296–304.

Zhu HH, **Yao** Q, **2004**. Localized and systemic increase of phenols in tomato roots induced by *Glomus versiforme* inhibits *Ralstonia solanacearum*. J Phytopathol, 152: 537-542.

Citation: Huseynova I, Balakishiyeva G, Aliyeva D, Gurbanova U, Bayramova J, Mustafayev N, Aliyev J, 2017. Changes in the activities of metabolic enzymes and antioxidant defense system in '*Candidatus* phytoplasma solani' infected pepper (*Capsicum annuum* L.) plants. Net J Agric Sci, 5(2): 58-65.