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Preliminary proteomic analysis of pear leaves in response to pear decline phytoplasma infection

Sonia DEL PRETE[1], Viviana DE LUCA[1], Clemente CAPASSO[1], Maria PASTORE[2], Marco DEL VAGLIO[2], Antonio CAPASSO[1], Vincenzo CARGINALE[1]
[1]*CNR Institute of Protein Biochemistry, via P. Castellino, 111, Naples, Italy*
[2]*CRA Unità di Ricerca per la Frutticoltura di Caserta , via Torrino, 3, Caserta, Italy*

Abstract

Pear decline phytoplasma, '*Candidatus* Phytoplasma pyri', belongs to the apple proliferation (AP) group and causes serious diseases in *Pyrus communis* fruiting cultivars in many areas around the world. It induces two types of symptoms, depending on the rootstock: 'slow' decline occurs on trees with tolerant or resistant rootstocks; 'quick' decline occurs on trees with sensitive rootstocks. The most common symptoms are leaf curl and a premature reddening and loss of foliage in the autumn. To better understand the pathogen-stress response of pear (*Pyrus communis* L.) to pear decline phytoplasma, we have initiated a comparative proteomic analysis of infected and healthy pear leaves. The proteins so far identified are mainly involved in carbohydrate metabolism and photosynthesis.

Key words: LC/ES/MSMS; host-pathogen interactions; stress response; plant pathogens.

Introduction

Phytoplasmas are small (0.2-0.8 μm), wall-less, pleomorphic prokaryotes responsible for numerous economically important plant diseases. They are characterized by a very small genome and are obligate parasites of plants and some insects that act as vectors (Lee *et al*., 2000; Bertaccini *et al*., 2007).

Pear decline is associated with '*Candidatus* Phytoplasma pyri' that is transmitted by pear psylla, also known as pear sucker. Two types of decline symptoms are recognized: quick decline and slow decline or leaf curl. The degree to which decline symptoms are expressed is governed by the sensitivity of the rootstock, age, and location of tree planted. In the quick decline, fruits cease to develop and both fruits and leaves wilt rapidly. This may be followed by some leaf scorching and leaf death. Trees generally die within a few weeks. In the slow decline there is a progressive weakening of the tree, which may fluctuate with severity. Terminal growth is reduced or may cease completely. Leaves are few, small, leathery and light green, with slightly uprolled margins; they become abnormally red in autumn and drop prematurely. Although blossoming is heavy in the early stages of attack, later on, fewer flowers are produced, fruit set is reduced and fruit does not attain the normal size (Garcia-Chapa *et al*., 2003).

Due to inability in culturing phytoplasmas *in vivo*, our knowledge about their physiology, biochemistry, and molecular biology is limited. Only recently, the introduction of molecular methods into plant mycoplasmology have made it possible to determine the phylogenetic and taxonomic relationships between phytoplasma strains, and those between phytoplasmas and other prokaryotes (Lee *et al*., 1998). So, these organisms remain one of the most poorly characterized plant pathogens, and the molecular mechanisms involved in their pathogenicity are still poorly understood. Following a pathogen attack, the host plant activates a response involving radical changes in the pattern of gene expression (Carginale *et al*., 2004; Xianling *et al*., 2009).

Proteomic analysis is a key molecular tool for the analysis of gene expression and has successfully identified novel protein components in plants in response to cold, salt, fungi, bacteria, and viruses. In our study, the protein profiles of healthy and diseased pear leaves were compared, and protein bands showing quantitative changes were subjected to liquid chromatography/electrospray tandem mass spectrometry (LC/ES/MSMS) for identification.

Materials and methods

Pears (*Pyrus communis* L.) were harvested in the orchard of the Fruit Tree Research Institute in Caserta, Italy. Pooled leaves from healthy and PD-infected pear samples were immediately frozen in liquid nitrogen and grinded thoroughly with a prechilled mortar and pestle. The powder obtained was suspended in 10% TCA/acetone. After centrifugation (16,000 g for 3 min), the pellets were washed first with 80% methanol/0.1 M ammonium acetate, and then with 80% acetone. Protein pellets were vacuum-dried at room temperature, and then suspended in 1:1 phenol (pH 8)/SDS buffer. After a thorough mixing (1 hr), the phenol phases were collected and precipitated by methanol/ammonium acetate. The pellets were then washed once with 100% methanol and once with 80% acetone. The resulting pellets were finally suspended in SDS sample buffer. Protein was quantified using the 2-D Quant kit, following the manufacturers' instructions.

Purified proteins from both healthy and infected samples were run on SDS-polyacrylamide gel. Gels were visualized by Coomassie or silver nitrate staining. Protein bands showing significant changes in abundance between healthy and infected samples were selected and excised for protein identification by in-gel trypsin digestion.

Table 1. Proteins identified by LC/ESMSMS.

Acc. n° NCBI and identified protein	Biological function	Acc. n° NCBI and identified protein	Biological function
gi/131385 Oxygen-evolving enhancer protein 1, chloroplastic	Photosynthesis	gi/194708200 Actin	Structural
gi/7525028 Photosystem II D2 protein	Photosynthesis	gi/115800 Chlorophyll a-b binding protein 3, chloroplastic	Photosynthesis
gi/81301580 Cytochrome f	Photosynthesis	gi/403160 Ribulose 1,5-bisphosphate carboxylase small s.u.	Photosynthesis
gi/17981607 Sorbitol 6-phosphate dehydrogenase	Carbohydrate metabolism	gi/28630975 Photosystem II D1 protein	Photosynthesis
gi/5031279 Porin	Transport	gi/27311547 Unknown protein	Unknown
gi/119905 Ferredoxin-NADP reductase, leaf isozyme, chloroplastic	Photosynthesis	gi/136057 Triosephosphate isomerase, cytosolic	Carbohydrate metabolism
gi/119656699 Photosystem II 32 KDa protein	Photosynthesis	gi/8272386 Endo-chitinase class III	Carbohydrate metabolism
gi/19184 Type 1 CP29 polypeptide	Photosynthesis	gi/16175 Adenylate translocator	Carrier
gi/2254440674 Hypothetical protein	Unknown	gi/132270 Rubber elongation factor	Metabolism

The peptide fragments obtained were then subjected to LC/ESMSMS analysis. Database searching, peptide mass fingerprinting (PMF), and MS/MS were performed using MASCOT 2.1 against the NCBI non-redundant *Viridiplantae*-specified protein sequences.

Results and discussion

Proteomic analysis represents a useful tool to gain insight into the plant host responses to stresses. To investigate the effects of PD phytoplasma on the pear protein profile SDS-PAGE on leaf proteins from infected and healthy plants showed differentially expressed protein bands that were excised from the gel and analyzed by LC/ESMSMS. The accession numbers and names of the identified proteins are listed in table 1. Among the 18 proteins identified, two were annotated as unknown, the others are involved in diverse processes including photosynthesis, carbohydrate metabolism, and metabolite transport.

These findings support the data deriving from physiological and biochemical analyses showing that infection with phytoplasmas is associated with increase in soluble carbohydrate and starch content, and decrease in the photosynthesis rate, carboxylation efficiency, and pigment content of leaves (Xianling *et al*., 2009). In a study on gene expression profile of PD infected periwinkles, genes involved in plant defense/stress responses, protein metabolism and transport, transcriptional regulation, vesicle trafficking, and carbohydrate metabolism were identified (De Luca *et al*., 2011). Proteomic analysis showed that the expression of many proteins changed during phytoplasma infection. These changes may alter many physiological and biochemical processes, and result in diverse and severe symptoms in infected plants.

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References

BERTACCINI A., 2007.- Phytoplasmas: diversity, taxonomy, and epidemiology.- *Frontiers in Bioscience*, 12: 673-689.

CARGINALE V., MARIA G., CAPASSO C., IONATA E., LA CARA F., PASTORE M., BERTACCINI A., CAPASSO A., 2004.- Identification of genes expressed in response to phytoplasmal infection in leaves of *Prunus armeniaca* L. by messenger RNA differential display.- *Gene*, 12(332): 29-34.

DE LUCA V., CAPASSO C., CAPASSO A., PASTORE M., CARGINALE V., 2011.- Gene expression profiling of phytoplasma-infected Madagascar periwinkle leaves using differential display.- *Molecular Biology Reports*, in press, DOI 10.1007/s11033-010-9964-x.

GARCIA-CHAPA M., LAVINA A., SANCHEZ I., MEDINA V., BATTLE A., 2003.- Occurrence, symptom expression and characterization of phytoplasma associated with pear decline disease in Catalonia (Spain).- *Journal of Phytopathology*, 151: 584–590.

LEE I-M., GUNDERSEN-RINDAL 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.- *International Journal of Systematic and Evolutionary Microbiology*, 48: 1153-1169.

LEE I-M., DAVIS R. E., GUNDERSEN-RINDAL D. E., 2000.- Phytoplasma: phytopathogenic Mollicutes.- *Annual Review of Microbiology*, 54: 221-255.

TESSITORI M., MARIA G, CAPASSO C., CATARA G., RIZZA S., DE LUCA V., CATARA A., CAPASSO A., CARGINALE V., 2007.- Differential display analysis of gene expression in Etrong citron leaves infected by citrus viroid III.- *Biochimica et Biophysica Acta*, 1769: 228-235.

XIANLING J., YINGPING G., CHENGCHAO Z., ZHIMEI M., 2009.- Comparative proteomic analysis provides new insights into mulberry dwarf responses in mulberry (*Morus alba* L.).- *Proteomics*, 9: 5328-5339.

Corresponding author: Vincenzo CARGINALE (e-mail: v.carginale@ibp.cnr.it), CNR Institute of Protein Biochemistry, via P. Castellino, 111, Naples, Italy.