Bulletin of Insectology ***64*** *(Supplement): S167-S168, 2011*
ISSN 1721-8861

Proteome responses of *Vitis vinifera* L. to 'flavescence dorée' phytoplasma infection

Paolo MARGARIA, Sabrina PALMANO
Istituto di Virologia Vegetale- CNR, Torino, Italy

Abstract

'Flavescence dorée' is a serious phytoplasma disease affecting grapevine in several European countries. We are currently studying the interaction of 'flavescence dorée' phytoplasma with its natural plant host, by monitoring the effects of infection on the protein expression profile. The red-berried *V. vinifera* cv. Nebbiolo and Barbera were the source of plant material, as they are widely grown in Piedmont and produce wines of high quality. Moreover, the two varieties show a different sensitivity to the disease: 'Nebbiolo' is generally considered more tolerant to 'flavescence dorée' infection and shows milder symptoms than 'Barbera'. Total proteins from midrib tissues were separated by two dimensional gel electrophoresis, and differentially expressed spots ($p \leq 0.05$, $| \text{ratio} | > 2$) were identified by mass spectrometry (MALDI-TOF-TOF) analysis. The proteins were further analyzed by Blat2GO software, in order to study their function and involvement in biological processes. By this way, we could develop interaction maps, showing the possible involvement of the proteins in several biological pathways.

Key words: grapevine, 2-DE, recovery, biological pathways.

Introduction

'Flavescence dorée' (FD) is a serious disease of the cultivated grapevine (*Vitis vinifera* L.), causing significant reduction in yield and fruit quality. The disease is caused by an obligate biotrophic phytopatogenic bacterium in the class Mollicutes, 'flavescence dorée' phytoplasma (FDp). FD may cause epidemics and is subject to quarantine restrictions in Europe. Two typical cultivars of Piedmont, 'Barbera' and 'Nebbiolo', naturally infected by FDp, were considered. Barbera was chosen for its high sensitivity to FDp infection, which can frequently result in whole plants turning purple. Nebbiolo is generally more tolerant to infection and shows milder symptoms. Often, a spontaneous remission of symptoms (known as recovery) may occur and interestingly, beside the high incidence to FD, 'Barbera' shows a high recovery phenotype after the initial infection with FDp (Morone *et al*., 2009).

In the last decade, 2-DE coupled to peptide analysis by mass spectrometry has become important in the study of plant biology and plant pathology. So far, proteomic studies in grapevine have been mostly applied to berry quality, and to uncover proteome changes during plant development, ripening or response to abiotic stress (Giribaldi and Giuffrida, 2010).

In this study, we are using 2-DE to explore the proteome changes in the leaves of *V. vinifera* infected by FDp or recovered. We have so far performed the analysis on not-infected and infected plants. The analysis on recovered plants is in progress. Our goals are: i) obtainment of 2-DE maps from total protein extracts of not-infected, infected and recovered plants; ii) comparison of not-infected, infected and recovered proteome profiles of the two cultivars; iv) identification of differentially expressed spots; iv) development of maps showing the participation of the proteins in biological pathways.

Materials and methods

A vineyard of 'Nebbiolo' grapevines located in Vezza d'Alba and a vineyard of 'Barbera' grapevines in Cocconato (Cuneo province, Piedmont, northern Italy), in August 2008 and 2009, respectively were monitored. The vineyards had already been monitored for 3 years for phytoplasma infection, and a map of infected, recovered (i.e. symptomless-plants found positive for FDp in previous years) and healthy plants was already available at the beginning of the study. The sanitary status of the collected material was checked by molecular assays: each sample was tested for phytoplasma infection ('flavescence dorée' and 'bois noir' phytoplasma) and for other grapevine viruses (i.e., GLRaV-1, GLRaV-2, GLRaV-3, GFLV, GFkV, GVA, GVB, ArMV). FDp strains were characterized and corresponded to subgroup FD-C. Not-infected and FDp-infected samples were selected. At least three biological replicates/thesis (not-infected versus infected) were considered. Total proteins were extracted from midribs isolated from symptomatic or "healthy" leaves following a TCA/acetone protocol (Margaria and Palmano, 2011) and quantified using BSA as standard.

2-DE, gel analysis and MS. Isoelectrofocusing (IEF) was carried out with 400 µg of protein extract using immobilized pH gradient (IPG) strips 17 cm-long, pH interval 4-7 (ReadyStrip IPG Strips, BioRad). Sample loading was performed by passive rehydration for 1 h followed by active rehydration for 12 h at 50 V in a Protein IEF cell apparatus (BioRad). IEF migration was performed at 20°C using a scalar gradient until reaching 10.000 V; the final value was 60.000 Vh. Second dimension SDS-PAGE was performed after strips equilibration, in 12% acrylamide gels using the PowerPac Universal apparatus (BioRad) in a buffer containing 25 mM Tris, 0.192 M glycine and 0.1% SDS. Running conditions were: 30 min at 16 mA per gel and 6 hours at

35mA per gel. Gels were stained using a colloidal Coomassie brilliant blue (CBB) G-250 procedure and scanned with Versadoc Imaging System (BioRad). Image elaboration and analysis were carried out with the PDQuest Software (Biorad). Spots showing significant variation between healthy and infected samples were selected according to the results of Student's *t*-tests ($p \leq 0.05$). Molecular weights and p*I* of spots were predicted according to migration of 2-D standards (Bio-Rad). Gel fragments were excised and analyzed at the Genomics & Proteomics Laboratories Technology Facility, University of York.

The protein data were organized into functional categories according to the Blast2GO program version 2.4.6 (Conesa *et al*., 2005) with default parameters. The same software was used for building up biological pathway maps.

Results and discussion

The high potential of proteomics offers a high throughput tool to study the specific interaction grapevine-FDp from a wide point of view. We successfully performed a proteomic analysis of the differentially expressed spots in FDp-infected 'Nebbiolo' and 'Barbera' grapevines.

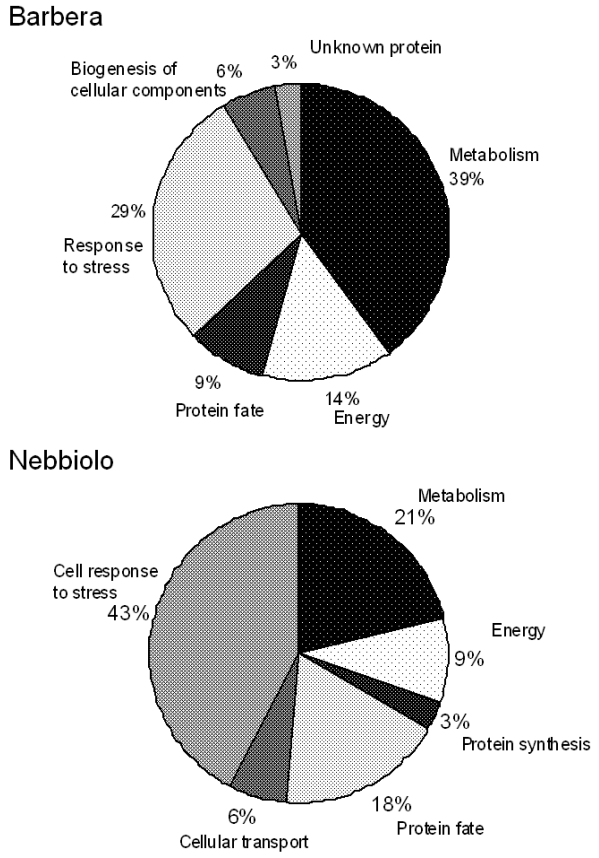

Figure 1. Pie charts representing the functional category distribution of the spots which were differentially expressed in 'flavescence dorée' phytoplasma-infected 'Barbera' (upper pie) and 'Nebbiolo' (lower pie) grapevines, in comparison to not-infected plants.

According to the set parameters and to Student's *t*-test, a mean of 5% of the detected proteins was differentially were up-regulated in infected tissues. Proteins were grouped in several functional categories, including metabolism, energy, photosynthesis, protein destination, protein synthesis, response to stress. Pie charts representing the distribution of the spots into functional categories are given in figure. 1. Beside common spots, some proteins were exclusively expressed in one cultivar: potentially they could be associated to the differential response to FD. However, further work will be necessary to assess whether the differences in the proteome profiles are effective or instead related to other factors, including the sampling date or variations in protein expression during the season.

Our comparative analysis between the three sanitary status considered (not-infected, infected, recovered), on cultivars showing different levels of sensitivity to FDp, could be critical for better understanding the grapevine responses to phytoplasmas infection and, in a wide view, the complex mechanisms of susceptibility in plants. Moreover, the work could give important information to address the impact of FDp-infection on the quality of the final product, given that some proteins involved in the race between host and pathogen are major allergens in grapes and wine (Pastorello *et al*., 2003).

Acknowledgements

The project was funded by a grant from ''Servizio fitosanitario regionale'', Regione Piemonte, Italy.

References

CONESA A., GOTZ S., GARCIA-GOMEZ J. M., TEROL J., TALON M., ROBLES M., 2005.- Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research.- *Bioinformatics*, 21(18): 3674-3676.

GIRIBALDI M., GIUFFRIDA G. M., 2010.- Heard it through the grapevine: Proteomic perspective on grape and wine.- *Journal of Proteomics*, 73(9): 1647-1655.

MARGARIA P., PALMANO S,. 2011.- Response of the *Vitis vinifera* L. cv. 'Nebbiolo' proteome to 'flavescence dorée' phytoplasma infection.- *Proteomics*, 11(2): 212-224.

MORONE C., BOVERI M., GIOSUÈ S., GOTTA P., ROSSI V., SCAPIN I., MARZACHÌ C. 2007.- Epidemiology of 'flavescence dorée' in vineyards in northwestern Italy.- *Phytopathology*, 97(11): 1422-1427.

PASTORELLO E. A., FARIOLI L., PRAVETTONI V., ORTOLANI C., FORTUNATO D., GIUFFRIDA M. G., GAROFFO L. P., CALAMARI A. M., BRENNA O., CONTI A., 2003.- Identification of grape and wine allergens as an endochitinase 4, a lipid-transfer protein, and a thaumatin.- *Journal of Allergy and Clinical Immunology*, 111(2): 350-359.

Corresponding author: Sabrina PALMANO (e-mail: s.palmano@ivv.cnr.it), Istituto di Virologia Vegetale, CNR, Strada delle Cacce 73, 10135 Torino, Italy.