Plant Physiology and Biochemistry 118 (2017) 64–70

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

# Proteomic profiling of German Dornfelder grape berries using data-independent acquisition

Matthias Riebel [a], Petra Fronk [a, **], Ute Distler [b], Stefan Tenzer [b], Heinz Decker [a, *]

[a] Institute for Molecular Physiology, Molecular Biophysics, Johannes Gutenberg University Mainz, Jakob-Welder-Weg, 26, D-55128 Mainz, Germany
[b] Institute for Immunology, University Medical Center of the Johannes Gutenberg University Mainz, Langenbeckstr. 1, D-55131 Mainz, Germany

ARTICLE INFO

*Article history:*
Received 14 April 2017
Received in revised form
29 May 2017
Accepted 1 June 2017
Available online 7 June 2017

*Keywords:*
Protein
Mass spectrometry
Grapevine
Proteome
Dornfelder

ABSTRACT

Grapevine is one of the most important fruit plants throughout the world. Sequencing of the grape genome in 2007 enabled in-depth analyses of the grape proteome. Whereas many studies addressed changes in proteomic composition of grapes during ripening, we focused on the proteome of mature grape berries from Dornfelder, a characteristic red wine grape for Germany. Current data-independent acquisition proteomics technology enables the analysis of proteomic compositions in a degree of accuracy that was unreachable only a few years ago. Using a label-free proteomics approach, we quantified 712 proteins in mature Dornfelder grape berries, of which 650 could be annotated by the Blast2GO software. Besides identification of proteins, our analysis provides protein amounts using the TOP3 absolute quantification approach. Most of the proteins (200) in mature Dornfelder grape berries are involved in stress response. In addition, all glycolytic key enzymes were detected in mature grape berries suggesting that glycolysis is still active, whereas sugar accumulation through gluconeogenesis utilizing malate as substrate seems to play a minor role.



## 1. Introduction

Grapevine (*Vitis vinifera* L.) is an important fruit crop worldwide with a vineyard area of 7.4 million ha. Germany accounts for about 102.000 ha subdivided into 13 wine growing regions with Rheinehessen located in Rhineland-Palatinate being the largest. In recent years, red grape varieties gained more importance and currently account for about one third of the vineyard area. Among them, Dornfelder is the most frequently cultivated red grapevine in Rhinehessen (German Wine Statistics 2015/2016, German Wine Institute). It is a characteristic German grape variety, which was bred 1955 in Weinsberg, Germany. This dark-skinned grape variety is rich on pigments and phenols and shows high resistance towards pathogens.

Pathogen-related (PR) proteins such as thaumatin-like proteins (TLPs), chitinases and lipid transfer proteins (LTPs) constitute the most abundant proteins in ripe grape berries (Pocock et al., 2000; Sarry et al., 2004). During wine making, the total protein concentration decreases significantly. However, especially PR-proteins are resistant to acidic and proteolytic conditions during wine making and remain detectable in wine (Kwon, 2004; Wigand et al., 2009). Despite a low protein concentration of about 15–230 mg/l (Ferreira et al., 2001) proteins have a considerable effect on wine quality. Especially PR proteins may cause economic problems due to haze formation in white wines (Marangon et al., 2011).

In 2007, the grapevine genome of Pinot noir was published (Jaillon et al., 2007). About 487 million base pairs code for 30.434 genes (Jaillon et al., 2007). However, not all genes have already been annotated to particular proteins. In addition, not all genes are expressed in all stages of ripening (Newton et al., 2004). Knowledge of the genetic code made it possible to predict the existence of grape proteins that have not yet been characterized directly. Due to advances in genomics and also in mass spectrometry (MS)-based applications the use of proteomics approaches to characterize grape proteins increased rapidly during the last years.

The first proteomic analysis of wine grapes was presented 2004 by Sarry et al. (2004) who identified 67 proteins in the mesocarp of Gamay grapes using MALDI-MS out of 270 spots found in 2D-gel. In the following years, several reports were published on protein composition of different grape components using 2D-

* Corresponding author.
** Corresponding author.
*E-mail addresses:* riebelm@uni-mainz.de (M. Riebel), petra.fronk@uni-mainz.de (P. Fronk), ute.distler@uni-mainz.de (U. Distler), tenzer@uni-mainz.de (S. Tenzer), hdecker@uni-mainz.de (H. Decker).

http://dx.doi.org/10.1016/j.plaphy.2017.06.003
0981-9428/

gelelectrophoresis coupled with MS analysis. However, many of them focus on the change of the protein profile either during ripening or protein response to stress such as water deficit, salinity, herbicide or salicylic acid treatment (Deytieux et al., 2007; Vincent et al., 2007; Negri et al., 2008; Grimplet et al., 2009; Martinez-Esteso et al., 2011; Cramer et al., 2013; Cai et al., 2014). Geographical origin and grape variety were also investigated with respect to protein composition (Fraige et al., 2015). Lately, a quantitative proteomic isobaric tag for relative and absolute quantification (iTRAQ) approach have been applied for identifying grape protein profile during different ripening phases (Martinez-Esteso et al., 2013; Kambiranda et al., 2014). To date, no attempt has been made to profile the complete proteome of mature grape berries by directly extracting the proteins and identifying them by mass spectrometry.

In the present study, we analyzed the proteome profile of mature Dornfelder grapes from the wine region Rhinehessen. The proteomic composition was quantitatively analyzed using a data-independent acquisition based high-resolution liquid chromatography-mass spectrometry approach. Our results provide a highly detailed quantitative overview of the Dornfelder grape proteome. Finally, we discuss the protein composition of mature wine grapes with respect to the localization and possible function of the proteins expressed in this commercially important red wine grape berry.

## 2. Materials and methods

### 2.1. Plant material

Grape berries were harvested from Dornfelder grapevines grown at Harxheim, Rhinehessen, Germany (Winery Fleischer). Ripe berry clusters were collected on September 23, 2011, washed and immediately frozen at −20 °C. According to J. W. Fleischer the climate of this year can be considered to be an average year with respect to environmental stress.

### 2.2. Protein extraction

Fifteen grams of frozen berries were powdered in liquid nitrogen using a porcelain mortar and pestle. This finely ground powder was solubilized in 15 ml grape-berry-extraction buffer (100 mM sodium phosphate pH 6.5, 20 mM EDTA, 3 mM $NaN_3$, 0.5% Tween-20 and 0.1% w/v of the Calbiochem “Protease Inhibitor Cocktail Set VI” from EMD Bioscience, La Jolla, USA).

Protein precipitation was performed using the “ProteoExtract® Protein Precipitation Kit” from Calbiochem according to the manufacturer's manual (Cat. No. 539180). The kit contains four different precipitants which are not further characterized. These are mixed to yield the precipitation agent. Proteins in 200 µl raw extract were precipitated with 800 µl precipitation agent for 1 h at −20 °C. After centrifugation for 5 min and 2800 g at room temperature to pellet the proteins, the precipitate was washed two times with 1 ml cold wash solution provided with the ProteoExtract® Protein Precipitation Kit (150 ml of ethanol was added to 65 ml of the wash solution and stored at −20 °C). Afterwards, the pellet with the precipitated proteins was dried at room temperature in the open tube.

### 2.3. Protein digest preparation

Precipitated proteins (20 µg) were solubilized in 25 mM ammonium bicarbonate containing 0.1% RapiGest (Waters, Eschborn, Germany) for 15 min at 80 °C. Proteins were reduced by adding 5 mM DTT (45 min, 56 °C) and free cysteins alkylated with iodoacetamide (Sigma, Taufkirchen, Germany). A 0.2 µg aliquot of porcine sequencing grade trypsin (Promega, Mannheim, Germany) was added, and the samples were incubated overnight at 37 °C. After digestion, RapiGest was hydrolyzed by adding 10 mM HCl, the resulting precipitate was removed by centrifugation, and the supernatant was transferred into an autosampler vial.

### 2.4. LC-MS analysis

Nanoscale liquid chromatography (LC) of tryptic peptides was performed with a *Waters NanoAcquity UPLC* system (Waters, Eschborn, Germany) equipped with a Waters 180 µm × 20 mm Symmetry C18 trapping column and a Waters 75 µm × 150 mm HSS-T3 reversed phase separation column. The aqueous mobile phase (mobile phase A) was $H_2O$ with 0.1% formic acid. The organic mobile phase (mobile phase B) was 0.1% formic acid in acetonitril (both LC-MS grade, Roth, Germany). Samples with 2.6 µl volume were injected onto the trapping column in with 1% mobile phase B. Peptides were separated with a gradient from 3% to 35% mobile phase B over 90 min at 300 nl/min. After a rinse with 80% mobile phase B, the column was re-equilibrated at initial conditions for 20 min [Glu1]-fibrinopeptide was used as lock mass at 500 fmol/µl. Samples were analyzed in three technical replicates. Mass spectrometry (MS) analysis of tryptic peptides was performed using a Waters Q-TOF Premier API system. All analyses were performed by using positive mode ESI using a NanoLockSpray source. The lock mass channel was sampled every 30 s. Accurate mass LC-MS data were collected in an alternating, low-energy (MS) and elevated energy ($MS^E$) mode of acquisition. The spectral acquisition time in each mode was 0.7 s with a 0.05 s interscan delay. In low-energy MS mode, data were collected at a constant collision energy of 3 eV. In $MS^E$ mode, collision energy was ramped from 16 to 36 eV during each 0.7 s data collection cycle. One cycle of MS and $MS^E$ data was acquired every 1.5 s.

### 2.5. Database searches

The continuum LC-$MS^E$ data were processed and searched using the IDENTITYE-Algorithm of ProteinLynx Global Server (PLGS) version 2.5.2. Protein identifications were assigned by searching an in-house compiled database containing all *Vitis vinifera* proteins supplemented with known possible contaminants and standard proteins (porcine trypsin, yeast enolase) using the precursor and fragmentation data afforded by the LC-MS acquisition method. The search parameter values for each precursor and associated fragment ions were set by the software using the measured mass error obtained from processing the raw continuum data. Peptide identifications were restricted to tryptic peptides with no more than one missed cleavage. Carbamidomethyl cysteine was set as fixed modification, and oxidized methionine, protein N-acetylation, and deamidation of asparagine and glutamine were searched as variable modifications. A database search was performed allowing a maximal mass deviation of 15 ppm for precursor ions and 30 ppm for fragment ions. For valid protein identification, at least two peptides had to be detected with a total of at least seven fragments. The false positive rate for protein identification was set to 1% based on search of a reversed database, which was generated automatically using PLGS 2.5.2. Retention time alignment, normalization, and label-free quantification were performed using the ISOQuant software (Distler et al., 2014).

### 2.6. Bioinformatic functional analysis

Dornfelder grape proteins identified by mass spectrometry were annotated using the Blast2GO v3.0.4 application (Conesa et al., 2005). This bioinformatic tool enables a functional annotation of