COMPLEX PROTEOMIC ANALYSIS OF FURMINT GRAPES INFECTED WITH *BOTRYTIS CINEREA*

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Abstract

Under optimal microclimatic and climatic conditions, as well as suitable soil characteristics in the Tokaj-Hegyalja wine region, *Botrytis cinerea* is of unique benefit in the production of Furmint aszú wine. The aim of our research was a complex proteomic study of grapes at different stages of ripening. We analysed berry samples collected from outside in which Furmint proteins were present, as well as intracellular and extracellular proteins of the fungus *B. cinerea*, which colonises the berries. The Kruskal–Wallis test revealed significant differences between samples at different stages of noble rot, both in terms of grape protein intensities and fungal protein intensities. In the case of fungal proteins, a significant difference between the intensity data of the samples was found when comparing fungi grown on grape berries with that of grown on agar medium. In all cases, the type of medium (artificial agar medium or grape berries) had a significant effect on the expression of fungal proteins, which was also true for the majority of grape proteins. The vast majority of the ANOVA and Kruskal–Wallis tests revealed significant differences for each protein, so that the stage of noble rot and the nature of

the medium had a detectable effect on the majority of the proteins. The majority of grape proteins were produced in decreasing amounts as the grapes progressed through the senescence period, while the production of most of the fungal proteins increased over the same period.

Keywords: *Botrytis cinerea*, Furmint, grapevine, Tokaj, noble rot

Összefoglaló

A *Botrytis cinerea* optimális mikroklimatikus és időjárási feltételek, valamint megfelelő talajadottságok mellett a Tokaj-Hegyalja borvidéken egyedülálló hasznot hoz a Furmint szőlőből való aszúbor készítése során. Kutatásunk célja az aszúsodás különböző fázisaiban lévő szőlőbogyók komplex proteomikai vizsgálata volt. Olyan, a természetből gyűjtött bogyómintákat vizsgáltunk, melyekben jelen voltak a Furmint fehérjéi, valamint a bogyókat kolonizáló *B. cinerea* fonalas gomba intra- és extracelluláris fehérjéi. Szignifikáns különbségeket tártunk fel a botritizáció különböző fázisaiban lévő minták között mind a szőlőfehérjék intenzitásait vizsgálva, mind a gombafehérjék intenzitásait vizsgálva. A gombafehérjék esetében szignifikáns különbség mutatkozott a minták intenzitásadatai között, amennyiben szőlőbogyókon nevelt gombákat hasonlítottunk össze agartartalmú táptalajon nevelt gombákkal. A táptalaj jellege (mesterséges agar táptalaj vagy szőlőbogyók) minden esetben szignifikáns hatással volt a gombafehérjék kifejeződésére, ami a szőlőfehérjék többségéről is elmondható. Az egyes fehérjék esetében a botritizáció mértékének és a táptalaj jellegének kimutatható hatása volt. A szőlőfehérjék döntő részét az aszúsodás előrehaladtával csökkenő mennyiségben termelte a szőlő, míg a gombafehérjék többségének termelődése ugyanezen idő alatt növekedett.

Kulcsszavak: *Botrytis cinerea*, Furmint, szőlő, Tokaj, aszúsodás

Introduction

The polyphagous *B. cinerea* can infect more than 1400 plant species (Fillinger and Elad, 2016), and is estimated to cause more than $10 billion in losses to the global agricultural industry each year (Boddy, 2015). However, infection by *B. cinerea* can be not only harmful but may also beneficial for some grape (*Vitis vinifera* L.) varieties, such as "Hárslevelű" and "Furmint" ones, depending on when and under what environmental conditions the grapes are exposed and how the berry's fruit quality parameters are developed (Naár and Szarvas, 2012).

Material and method

Samples of Furmint grapes infested with *B. cinerea* were collected in the Betsek vineyard near the south-eastern administrative boundary of the municipality of Mád. During collection, we separated four different groups – based on morphological features –, each containing 10 samples. The first group of 10 samples consisted of intact grapes without visible damage. The second, third and fourth groups of samples were obtained from the collection of grapes in successive stages of senescence. The collected samples were frozen in liquid nitrogen and transported to the Institute of Plant Protection Institute of the Centre for Agricultural Research for further experiments. Here, protein extraction was carried out by phenolic extraction following the method of Vincent et al. (2006), and the resulting samples were subjected to mass spectrometry using the Maxis II ETD Q-TOF basic instrument. Protein identification was followed by label-free quantification using MaxQuant software. Intensity data obtained from mass spectrometry measurements were analysed by comparative statistical methods (ANOVA, Kruskal–Wallis test, paired Kolmogorov–Smirnov test) using SPSS 25.0 at p=0.05 significance level, unless otherwise indicated.

Results

Based on the measurements, the grape protein intensity values of the grape samples are similar, but their median and range decrease with increasing *B. cinerea* (Figure 1a). The biological explanation for this is to be found in the process of noble rotting, i.e. the process by which *B. cinerea* proteins take place in the grape berry and colonise the flesh, thereby reducing the intensity of the proteins in the grape. The control sample is only contained grape proteins as impurities.

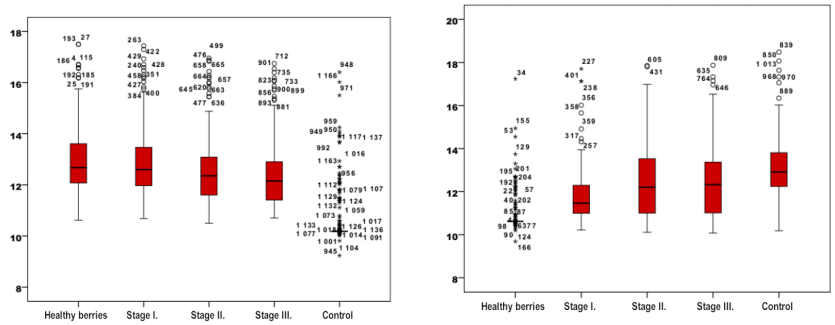

Figure 1. Grape protein intensity (Figure 1a, left) and B. cinerea protein intensity (Figure 1b, right) data for samples in four consecutive stage of noble rot and control. The vertical axis shows the natural logarithm of the intensity.

The most significant increase in intensity is between samples I containing intact grapes and samples II. containing grapes already infected with *B. cinerea*, which have undergone a slight colour change and lost firmness (Figure 1b). During the process of noble rot, the amount of *B. cinerea* fungal proteins increased in parallel with the decrease in grape protein production. It was also observed that the increase was true for most, but not for all of the proteins, the type of proteins be a determining factor. The median and range of the control sample data are larger compared to the grape samples. The Kruskal–Wallis test results show that the medians of the intensity data for both the grape protein samples and the fungal protein samples are not equal, with significant ($P<0.001$) differences between them. A two-sample Kolmogorov–Smirnov test

was used to compare sample pairs. The results show that only the distribution of grape protein sample pairs I−II and III−IV are not significantly different. In other words, noble rot could have affected the different evolution of the proteins in the majority of cases.

We assumed that the intensity of the proteins might not only depend on the progress of noble rot and the medium, but also on the type of protein, so we analysed the intensity data series for each protein separately. The hypothesis was confirmed: 189 out of 236 grape proteins showed significantly different expression levels at four different stages of noble rot. As noble rot, the production of all but two of the grapevine proteins decreased, the exceptions being the RVX13051.1 (*Vitis vinifera*) and XP_010654674.1 (*Vitis vinifera*) proteins, whose role requires further proteomic studies. The production of *B. cinerea* proteins was more influenced by the quality of the medium. All but two of the 204 *B. cinerea* proteins showed significantly different expression in this comparison, in contrast to the protein pattern changes in intact and unripe grapes, where 186 of the 204 differentially expressed extracellular proteins were detected.

Discussion

In our research, 440 *B. cinerea* and Furmint grape proteins were detected. Significant differences between samples at different stages of noble rot for grape proteins and fungal proteins were found. The type of medium (artificial agar-containing plate or grape berries) had a significant effect on the expression of fungal proteins in all cases. In most cases, the noble rot had a significant effect on the expression of unique *B. cinerea* proteins, which was also the case for most of the unique grape proteins.

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