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Volatile organic compounds in disease-resistant grapevine hybrids

Flüchtige organische Verbindungen von krankheitsresistenten Weinreben-Hybriden

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Introduction

The constant challenge of modern agriculture is the combination of sustainability and yield. Unfortunately, the use of vast amounts of pesticides is necessary in grapevine production to cope with pathogens like downy mildew (*Plasmopara viticola*) as well as powdery mildew (*Erysiphe necator*). Thus, the high economic costs and negative ecological impact lead to the search of alternative strategies (FERREIRA et al. 2004). One promising approach is the generation of *Vitis* hybrids by crossing *Vitis vinifera* species with disease-resistant American or Asian species. However, besides of resistance traits also negative quality compounds derive from these wild species and can be present in the obtained hybrids. Concerning the grape quality, volatile organic compounds (VOCs) are of great importance. In this study, two complementary techniques were used to characterize the VOCs of 74 different grapevine hybrids. The use of disease-resistant grapevine hybrids in breeding programs leads towards a more sustainable cultivation, since the use of pesticides can be dramatically decreased.

Material and Methods

Grape material: All grape berries were harvested in 2017 at the same location in Marlengo, Italy. Grape berries were randomly picked from at least five clusters, frozen with liquid nitrogen and stored at -80°C until analysis.
HS-SPME-GC-MS: Headspace Solid-Phase Microextraction - Gas Chromatography with Mass Spectrometry was applied to measure the different grapevine accessions. The sample preparation protocol is based on CANUTI et al. 2009. Shortly, around 30 g of unfrozen grape material was homogenized and centrifuged for 10 min at 10.000 rpm. 8 ml of the supernatant were transferred into 20 ml amber vials containing 3 g of NaCl. Internal standard solution and antioxidant solution were added. The incubation time of the fiber (DVB/CAR/PDMS material) in the headspace was 30 min at 60°C. The compounds were eluted from the fiber into the GC-MS (Bruker Scion). The carrier gas flow was 1 ml/min. The data were acquired in full scan mode.
PTR-MS: As a second method, Proton Transfer Reaction - Mass Spectrometry was applied. The grape material was ground and 1 g of the obtained powder was weighted into 20 ml glass vials. 1 ml of antioxidant solution was added. The vials were incubated at 40°C and the headspace was then transferred into the PTR-MS device (Ionicon GmbH). The data were acquired in normal sensitivity mode. The masses were selected based on results of a performed ANOVA (blank vs. grapes), correlation with isotopes and visual comparison. In total, 150 masses were selected for the analysis performed in this work.
Statistical calculations: The data were imported into Simca (version 13.0) and a PCA was performed.

Results and Discussion

The two complementary methods deliver different results. In case of HS-SPME-GC-MS, the VOCs are separated by the GC column and reference mass spectra are available in public databases. Thus, the identification of compounds is possible. In case of the PTR-MS analysis the outcome is an exact molecular mass which allows only a tentative assignment of the signals of interest.
HS-SPME-GC-MS results: This analysis allows the establishing of aroma profiles and the determination of volatile compounds. The most abundant compounds were identified as: hexanal, β-linalool, linalool oxide, α-terpineol, β-citronellol, nerol, β-damascene and geraniol. As can be seen in Figure 1, the aroma profiles of different accessions show a certain level of variation.

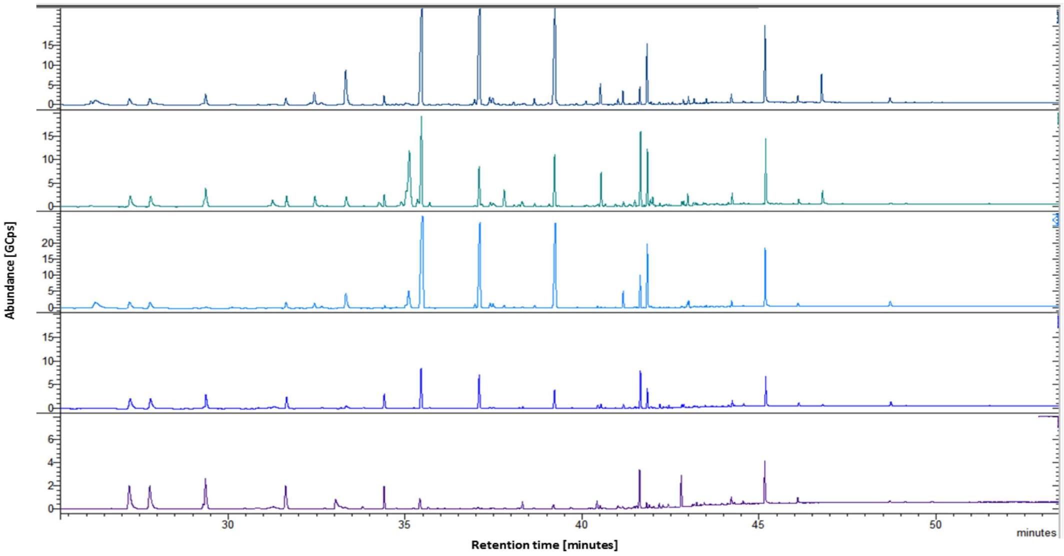

Figure 1: **GC-MS chromatograms (aroma profiles) of five different grapevine accessions**

PTR-MS results: The score scatter plot (Figure 2) shows the distribution of white grapevine accessions (grey dots) as well as red accessions (red dots). The white-skinned hybrids are more diverse, whereas the red ones are more clustered. One red accession and three white ones were found to be outlier. Many *m/z*-values contribute to the extreme red outlier (e.g. *m/z* 107.0685, *m/z* 135.0733, *m/z* 165.0308). The tentative peak assignment is still ongoing. Interestingly, the red and white accessions show a different trend based on their VOCs.

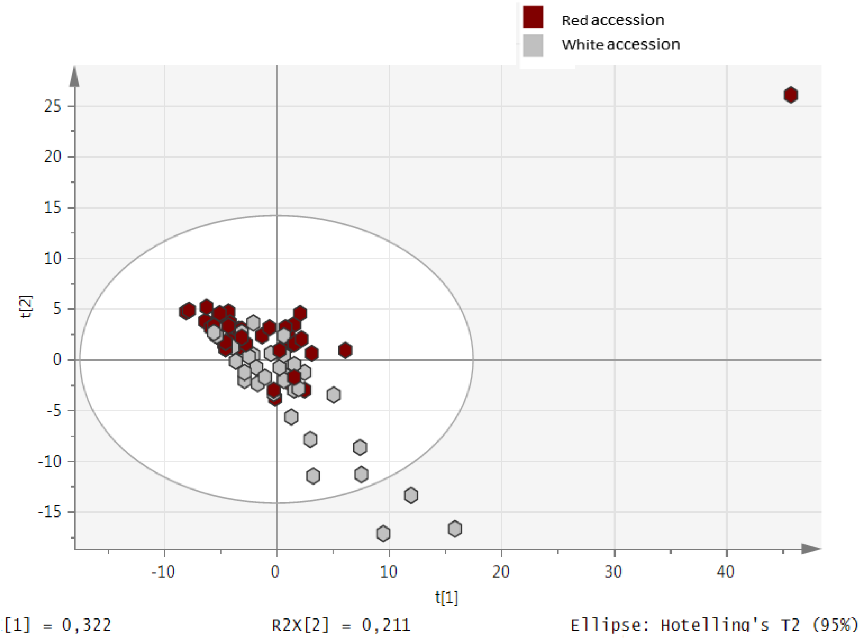

Figure 2: **Score Scatter Plot of PCA of 74 grapevine accessions according to the measured values of the PTR-MS analysis**

Discussion: The conducted hybrids vary strongly in their aroma profiles. White and red accessions show different patterns. For grapevine breeding, it is important to learn about quality compounds of hybrids. In this way, positive and negative selection can be applied to choose crossing parents. Both techniques, which have been used in this study, aim to detect volatile organic compounds. GC-MS allows an efficient separation and identification of those compounds, but it is more time-consuming than PTR-MS. On the other hand, PTR-MS allows only a tentative peak assignment. Both techniques have their strengths and are complementary, thus the use of both is desirable.

Abstract

Grapevine is one of the most important crop plants worldwide. Among many existing grapevine species, *Vitis vinifera* subsp. *sativa* is by far the most commonly used one in winemaking. However, this species is susceptible to many diseases such as downy or powdery mildew. For this reason, the use of pesticides has become essential in the grape production. One approach to overcome this problem was the generation of *Vitis* hybrids by crossing *Vitis vinifera* species with disease-resistant American or Asian species. Unfortunately, besides of resistance traits also negative quality compounds derive from these wild species and can be present in the obtained hybrids. Since volatile organic compounds (VOCs) play a major role in terms of grape quality, this work gives an inside into the aroma profile of various grapevine hybrids. Two different techniques were used: Headspace Solid-Phase Microextraction - Gas Chromatography with Mass Spectrometry (HS-SPME-GC-MS) and Proton Transfer Reaction - Mass Spectrometry (PTR-MS). These two complementary techniques allowed a differentiation of the 74 studied grapevine accessions according to their aromatic profile. These results are important for grapevine breeding as they can be employed both for positive and negative selection.

Zusammenfassung

Die Weinrebe ist eine der wichtigsten Kulturpflanzen weltweit. Neben vielen verschiedenen Weinrebensorten wird hauptsächlich die Unterart Edle Weinrebe (*Vitis vinifera* subsp. *vinifera*) zur Produktion von Wein genutzt. Diese ist jedoch anfällig für verschiedene Krankheitserreger wie dem Falschen Mehltau sowie dem Echten Mehltau. Aus diesem Grund ist der Einsatz von Pflanzenschutzmitteln von hoher Wichtigkeit im Weinbau. Zur Lösung dieses Problems bietet sich die Kreuzung von *Vitis vinifera*-Spezies mit amerikanischen oder asiatischen, krankheitsresistenten Spezies an. Die Nachkommen dieser Kreuzungen, sogenannte Hybride, beinhalten nun Resistenzen gegenüber Krankheitserregern aber nunmehr auch negative Qualitätsstoffe, welche von den asiatischen oder amerikanischen Spezies übertragen werden. Für die Qualität von Weinreben spielen flüchtige organische Verbindungen eine große Rolle. Diese Arbeit gibt einen Einblick in die Aromaprofile von verschiedenen Weinreben-Hybriden. Zwei Methoden kamen zum Einsatz: Headspace-Festphasenmikroextraktion (HS-SPME) gefolgt von der Gaschromatographie-Massenspektrometrie (GC-MS) sowie Protonentransfer-Reaktions-Massenspektrometrie (PTR-MS). Diese Methoden erlauben eine Differenzierung von insgesamt 74 verschiedenen Weinreben-Hybriden basierend auf deren Aromaprofilen. Die erzielten Ergebnisse können in die Entscheidung für positive sowie negative Selektion in der Weinrebenzüchtung einfließen und sind damit von hoher Relevanz.

References

CANUTI V, CONVERSANO M, CALZI ML, HEYMANN H, MATTHEWS M A, EBELER S E, 2009: Headspace solid-phase microextraction-gas chromatography-mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. J. Chrom. A, 1216(15), 3012-3022.

FERREIRA R B, MONTEIRO S S, PICARRA-PEREIRA M A, TEIXERA A R, 2004: Engineering grapevine for increased resistance to fungal pathogens without comprising wine stability. Trends Biotechnol, 22(4), 168-173.

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