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Classification of wine grape biotypes according to their variety and sanitary condition by fingerprinting untargeted analysis

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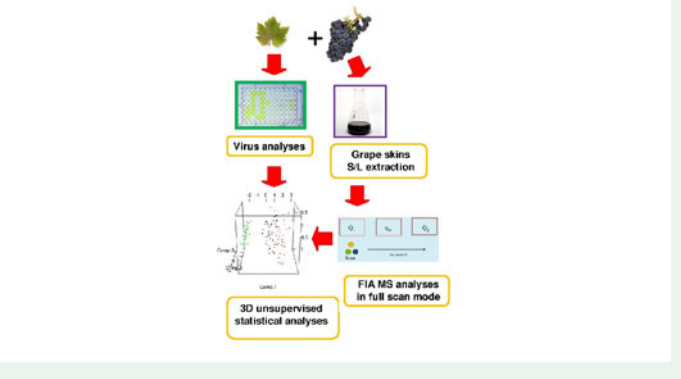
ABSTRACT

Grapes secondary metabolites content mainly depends on variety, but also on climate and cultural conditions, including sanitary status. This study aimed to use a metabolomic fingerprinting approach for grouping 72 wine grape biotypes, Negro amaro n. (N), Malvasia nera di Brindisi/Lecce n. (M), and Uva di Troia n. (U), on the basis of their cultivar and virological conditions. The skins were extracted and analysed by flow injection mass spectrometry; a one-way ANOVA/Principal Component Analysis (PCA) allowed to efficiently cluster the samples, recognizing M from N and U biotypes. Conversely, the clusterisation of the biotypes affected by different virus complexes was really more tough and a clear distinction among infected plants was not always observed. However, very interestingly, by applying ANOVA/PCA to the biotypes of each varieties, singularly, healthy biotypes were sharply separated in all the varieties and a relationship between anthocyanin compounds and Grapevine leafroll associated virus (GLRaV3) slightly appeared.



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1. Introduction

Grapes metabolites, in particular secondary metabolites content mainly depends on variety, but also on climate and cultural conditions, including the sanitary status (Guidoni et al. 1997; Crupi et al. 2012; Coletta et al. 2014; Popovic-Djordjevic et al. 2017; Lakićević et al. 2018). Indeed, Grapevine leafroll associated virus (GLRaV), Grapevine virus A (GVA), Grapevine fanleaf virus (GFLV), and Grapevine fleck virus (GFKV), for instance, reducing the rate of photosynthesis, may influence the overall quality of grapes and musts (Vega et al. 2011; Endeshaw et al. 2014; Alabi et al. 2016); although, they have showed a controversy effect on the biosynthesis of secondary metabolites (i.e. polyphenols) (Brar et al. 2008; Guiă and Buciumeanu 2016; Mannini and Digiaro 2017).

The aim of this study was to employ a metabolomic fingerprinting approach, through flow injection triple quadrupole mass spectrometry (QqQ MS) analyses, in order to group 72 wine grape biotypes, belonging to three Apulian autochthonous varieties, i.e. Negro amaro n. (N), Malvasia nera di Brindisi/Lecce n. (M), and Uva di Troia n. (U), on the basis of cultivar and virus diseases (such as, GLRaV3, GVA, GFLV, and GFKV), by which they were, eventually, infected.

2. Results and discussion

The skins of M (32), N (23), and U (17), whose identity was confirmed by genomic DNA microsatellites profiles, were extracted through a solution of water/ethanol/

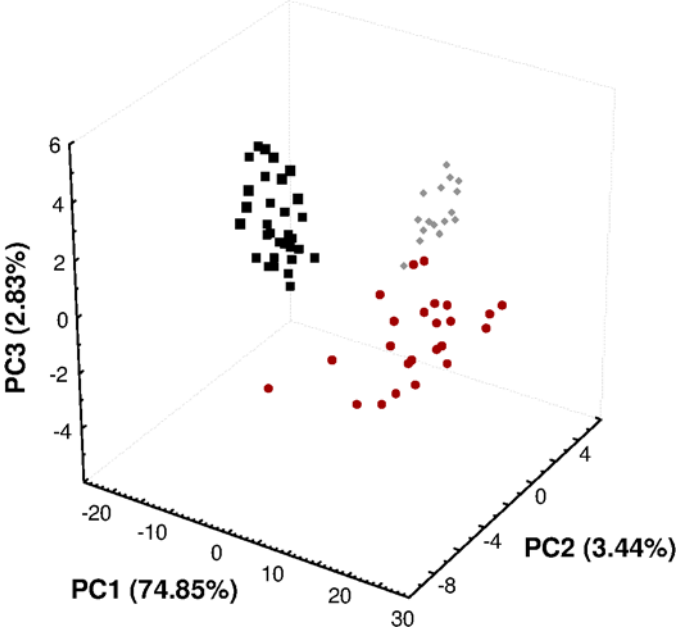

Figure 1. Principal component score of the investigated grape biotypes (black squares: Malvasia nera di Brindisi/Lecce; dark red circles: Negro amaro; dark gray diamonds: Uva di Troia) according to PC1, PC2, and PC3 obtained by autoscaled $[M+H]^+$ abundances, and accounting for 81.12% of the total variance.

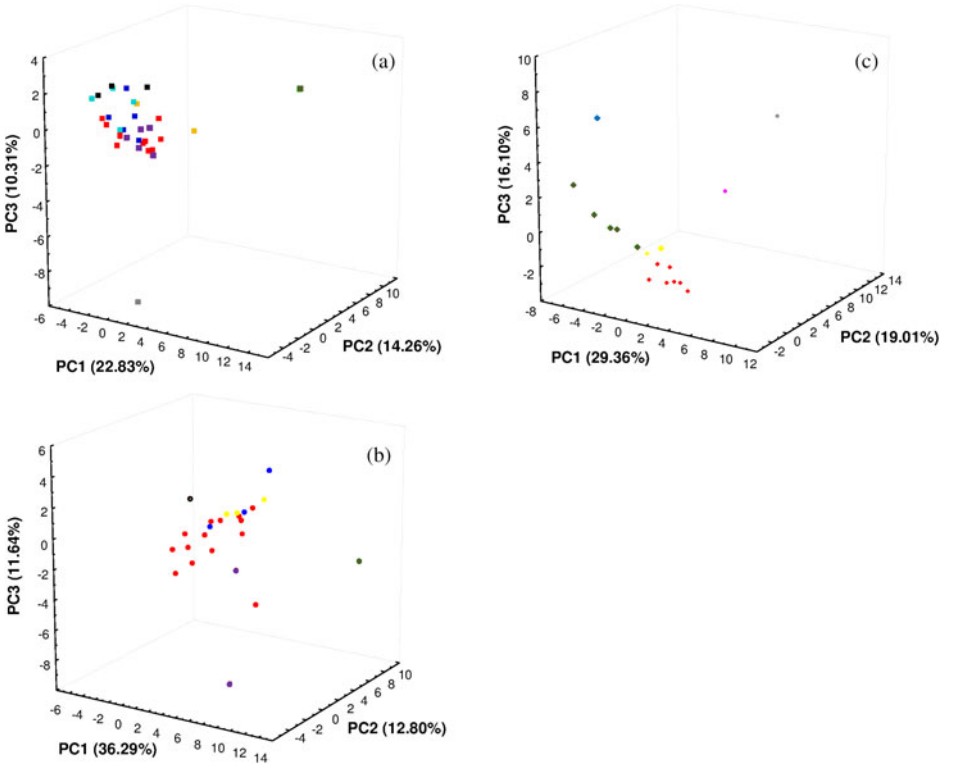

Figure 2. Principal component score of a) Malvasia nera di Brindisi/Lecce (squares), b) Negro amaro (circles), and c) Uva di Troia (diamonds) grape biotypes affected by different complex viruses, according to PC1, PC2, and PC3 obtained by autoscaled $[M+H]^+$ abundances, and accounting for 81.12% of the total variance. Biotypes infected by GLRaV3 (red), GLRaV3 + GFLV (purple), GLRaV3 + GFLV + GVA (black), GLRaV3 + GVA (blue), GLRaV3 + GVA + GFKV (teal), GLRaV2 + GLRaV3 + GVA (dark gray), GLRaV3 + GFKV (dark yellow), GLRaV3 + GFLV + ArMV (yellow), GLRaV1 + GLRaV3 + GFLV (black and white), GVA (light blue), GVA + ArMV (pink), and not infected (dark green).

hydrochloric acid (30:70:1, v/v/v) and analysed by means of a G6430 QqQ MS device (Agilent Technologies) equipped with an electro spray ionization source (ESI) in positive full scan mode and in 100-700 *m/z* range. The obtained $[M+H]^+$ abundances were processed by logarithmic transformation and autoscaling, thereby they were subjected to one-way ANOVA/Principal Component Analysis (PCA). As reported in Figure 1, PCA allowed to efficiently cluster the 72 samples, distinguishing M (black squares) biotypes from N (dark red circles) and U (dark gray diamonds), especially thanks to ions at *m/z* 115, 143, 261, 391, 409, 445, 561, 579, 625, and 639 which have the highest loadings (>0.95) onto PC1. Conversely, N and U were only slightly separated by the less determinant PC2 and PC3.

This information can be useful for obtaining chemical response about the metabolites responsible for the differentiation among the three varieties of wine grape biotypes; indeed, for instance, the protonated molecular ions at *m/z* 115, 143, and 261 would be characteristic for phenolic acids, as well as $[M+H]^+$ at *m/z* 391, 579, 625, and 639 could be assigned to piceid, procyanidin, petunidin-3O-*trans-p*-

coumaroylglucoside, and malvidin-3O-*trans*-*p*-coumaroylglucoside, respectively, typically identified in grape skins (Crupi et al. 2012; Crupi et al. 2013; Vujovic et al. 2016; Pejin et al. 2016); even though, other metabolites isobars belonging to the same mass range cannot be excluded. Then, evidently, M biotypes resulted richer than N and U in these compounds.

The clusterisation of the biotypes affected by different virus complexes was really more tough and a clear distinction among infected plants was not always observed (Figure 2); this behaviour was really expected because we analysed biotypes already infected by different virus complexes, also contemporarily, which were picked as such from various part of South Italy, re-planted in the same experimental vineyard of CREA-VE in Turi (Bari, Italy) and grafted on the same rootstock (1103 Paulsen - *Vitis Berlandieri* x *Vitis Rupestris*), in order to preserve the viticulture biodiversity. However, very interestingly, by applying ANOVA/PCA to the biotypes of each varieties, singularly, healthy biotypes (dark green dots) were sharply separated in all the varieties, but also GLRaV3 infected samples (red dots) were clearly clustered, especially in the case of U (Figure 2c). Indeed, from the gathered analyses, GLRaV3 infected U biotypes were characterized by higher abundance of ions at *m/z* 479, 609, and 465 (factor loadings $>$ 0.9 onto PC1), maybe imputable to petunidin-3O-glucoside, peonidin-3O-*p*-coumaroyl-glucoside, and delphinidin-3O-glucoside, respectively (as confirmed by the aglycones product ions at *m/z* 303 and 301 obtained by MS/MS and depicted in Figure S1), than the healthy ones.

3. Conclusions

In this pilot study, fingerprinting MS analyses were applied to skin extracts of 72 biotypes of three Apulian varieties eventually infected by virus diseases, in order to tentatively discriminate between cultivar and virological situation. Gathered findings showed that the proposed metabolomic approach could actually be used as a rapid method for screening varietal and, less evidently, sanitary difference between grape biotypes. Moreover, a slight relationship between anthocyanins content and virological status (especially on GLRaV3 infected biotypes) seemed to emerge. Further studies are in progress to extend and validate this method to an higher number of varieties in order to confirm the results of this research.

Author contributions

A.R.C. conceived and designed the experiments. M.G. and P.C. performed the experiments and wrote the manuscript. All the authors reviewed the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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