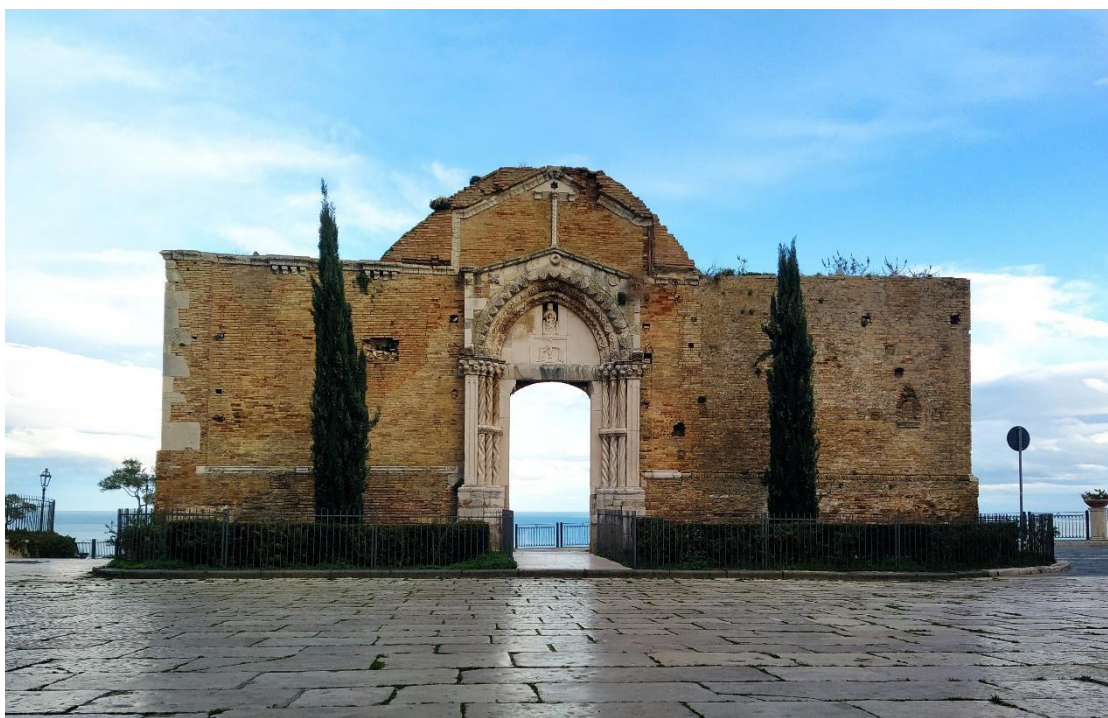


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A WORKFLOW FOR METABOLOMIC ANALYSIS OF HEAT-STRESSED LEAVES

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Grapevine (*Vitis* spp.) is the most extensively cultivated fruit crop in the world and its economic importance is mainly related to wine production¹. In recent years, the increased frequency of extreme phenomena such as heat waves has been recognized as one of the most significant climate factors negatively affecting grape yield and berry composition, with tremendous consequences on wine quality². Therefore, studying the metabolic and genetic factors that are involved in grapevine response to high temperatures is essential to gain knowledge on thermotolerance mechanisms, with the final aim of improving vineyard management strategies and plant breeding programs. However, literature on plants resilience to heat stress is scarce and usually focuses on selected metabolites, mainly related to oxidative stress, with a targeted approach³. Here, we report the development of a metabolomic workflow, from sample collection and extraction to sample analysis and data interpretation, for a preliminary evaluation of leaf metabolome alterations due to stress factors. In particular, the behaviour of selected grapevine plants during hot days was studied by analysing their volatile organic compounds (VOCs) profile by means of headspace-solid phase microextraction (SPME) GC-MS technique. Together with this well-known protocol, the metabolite modulation under high temperatures was investigated with a HPLC-HR-ESI-MS method under different chromatographic approaches, using conventional C18 and weak anionic/cationic mixed exchange stationary phases. This evaluation provided a first wide screening of both primary and secondary metabolites, suggesting the weak cation-exchange mixed mode column as the best compromise for this metabolic fingerprint. A comparison between full-scan, data dependent (DDA) and data independent (DIA) acquisition modes was also performed to select the best approach for our metabolomic application. To this purpose, data processing was carried out with two different software: MS-DIAL 4.9 and an R package “xcms”. Preliminary results obtained from the first two years of study will be presented.

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