

RESEARCH PAPER

NMR metabolomics of esca disease-affected *Vitis vinifera* cv. Alvarinho leaves

Marta R. M. Lima¹, Mafalda L. Felgueiras¹, Gonçalo Graça², João E. A. Rodrigues², António Barros³, Ana M. Gil² and Alberto C. P. Dias^{1,*}

¹ University of Minho, Department of Biology, CITAB-Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas, Campus de Gualtar, 4710-057 Braga, Portugal

² CICECO, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

³ QOPNAA, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

* To whom correspondence should be addressed. E-mail: acpdias@bio.uminho.pt

Received 13 March 2010; Revised 21 June 2010; Accepted 22 June 2010

Abstract

Esca is a destructive disease that affects vineyards leading to important losses in wine production. Information about the response of *Vitis vinifera* plants to this disease is scarce, particularly concerning changes in plant metabolism. In order to study the metabolic changes in *Vitis* plants affected by esca, leaves from both infected and non-affected cordons of *V. vinifera* cv. Alvarinho (collected in the Vinho Verde region, Portugal) were analysed. The metabolite composition of leaves from infected cordons with visible symptoms [diseased leaves (dl)] and from asymptomatic cordons [healthy leaves (hl)] was evaluated by 1D and 2D ¹H-nuclear magnetic resonance (NMR) spectroscopy. Principal component analysis (PCA) of the NMR spectra showed a clear separation between dl and hl leaves, indicating differential compound production due to the esca disease. NMR/PCA analysis allowed the identification of specific compounds characterizing each group, and the corresponding metabolic pathways are discussed. Altogether, the study revealed a significant increase of phenolic compounds in dl, compared with hl, accompanied by a decrease in carbohydrates, suggesting that dl are rerouting carbon and energy from primary to secondary metabolism. Other metabolic alterations detected comprised increased levels of methanol, alanine, and γ -aminobutyric acid in dl, which might be the result of the activation of other defence mechanisms.

Key words: Esca, metabolomics, NMR, PCA, *Vitis vinifera*.

Introduction

Esca is a destructive disease that affects grapevines worldwide. The aetiology of the disease is complex and is not fully understood; however, several fungi are known to be associated with this disease: *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum*, and *Fomitiporia mediterranea* or *Fomitiporia punctata*, are the most frequently isolated from diseased plants. However, because not all the attempts to prove the pathogenicity of these fungi were successful and considering that esca-associated deuteromycetes have been isolated from asymptomatic plants, it is thought that other factors are required for the development of esca. These factors are still unclear but may include vine age, variety susceptibility, site and time of infection, the host's

defence response, and environmental factors (Graniti *et al.*, 1999). Symptoms of the disease occur both internally, in the trunk and branches, and externally, in the leaves and berries. Frequently the disease is detected only when external symptoms appear, particularly in leaves, but the plants may be infected for long periods of time before external symptoms become visible (Surico *et al.*, 2000). Typical leaf symptoms appear as small chlorotic spots expanding between the veins or along leaf margins, merging to fill the entire leaf, except a small line of healthy tissue along the main veins (Mugnai *et al.*, 1999). Today, esca affects grapevines worldwide and the incidence of the disease has been increasing on a global scale. In Portugal