

B047 A global metabolomic approach involving LC/MS and GC/MS for a detailed study of the changes occurring in *Arabidopsis thaliana* under stress conditions*A. Thiocone, K. Ndjoko and J.-L. Wolfender*

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Metabolite profiling provides a deeper insight into the ultimate functions of gene expression and is the key to understanding how changes at the level of the genome and proteome affect cellular function. Unlike genomics and proteomics, a single analytical technique does not exist that is capable of profiling all metabolites of a given organism. In order to study in depth the metabolic changes that occur upon stress induction in plants, *Arabidopsis thaliana* (ecotype Columbia) has been chosen as a model. Recent advances in molecular biology and total genome sequencing make this plant a key target for such type of analysis. The plant was submitted to various general stresses including wounding by forceps or superoxide exposure by paraquat spraying in order to obtain a general view of all changes that might occur at the level of secondary metabolite production. Frozen fresh plant material was extracted at low temperature (-40°C) by solvents of increasing polarities by miniaturised pressurised extraction in order to minimize interferences by enzymatic reactions. This novel microextraction procedure was applicable already to few mg of plant material with a satisfactory reproducibility. The lipophilic extracts (CH₂Cl₂ and 2-propanol) were analysed by LC/MS on a long phenyl column (50 cm), while the more polar extracts (MeOH) were analysed on a C-18 column (50 cm). Various MeCN-water gradients were applied in order to obtain a high LC resolution of most of the metabolites present. Complementary GC/MS analyses were also performed in order to assess changes that occur in the more volatile constituents and especially the oxylipins. Comparison of all the MS chromatograms obtained were performed using a comprehensive chemometric method which enabled to reconstruct automatically single ion traces characteristic for changes in metabolite production. The results demonstrated that noticeable changes occurred upon elicitation of *A. thaliana* in all extracts qualitatively and quantitatively. A characterisation of the most interesting induced constituents is underway. Based on the metabolite profiling methods presented, studies related to the production of defence compounds, molecules involved in signalling and relation between metabolite induction and defence gene expression are foreseen.

Acknowledgement: Swiss National Science Foundation (grant number 21-63637.00 to J.-L. Wolfender).

B048 Capillary GC-MS analysis of tropane alkaloids from the roots and stem-bark of *Schizanthus grahamii**S. Bieri^a, O. Muñoz^b and P. Christen^a*

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The genus *Schizanthus* contains a wide range of tropane alkaloids. The formation of ester derivatives from mainly angelic, tiglic, senecioic, itaconic or mesaconic acids, as well as the formation of dimers, constitutes the peculiar characteristics of this genus (1). The potential of direct identification of tropane alkaloids in crude extracts from roots and stem-bark of endemic Chilean *Schizanthus grahamii* (Solanaceae) by GC-MS is presented and discussed.

Notwithstanding the success of some combination of chromatography with spectrometric techniques, GC-EIMS remains the prominent technique for performing qualitative analysis. However, some drawbacks may arise in the differentiation of structural isomers showing superimposable mass spectra. In order to detect eventual artefacts or thermodegradation in the hot and surface-active injection port, leading to erroneous peak identification, different injection techniques are evaluated.

In this study, crude alkaloid extracts and some purified fractions were analysed by GC-EIMS using split, splitless and on-column injection. The latter was used as the reference method because it avoids artefact formation in the injection port. Thus, the different series of isomeric compounds in the extracts were confirmed. More than twenty alkaloids including four series of isomers were detected in the stem-bark, while only ten minor alkaloids were identified in the roots. Hygrine derivatives, tropine, pseudotropine, tropinone, 3 α ,7 β -dihydroxytropane together with hydroxytropanes esterified with isomeric C₅ acids are present in both plant parts. However monomeric and dimeric tropanol diesters of mesaconic and itaconic acids were solely detected in the stem-bark. The difficulty to assign these isomeric compounds is pointed out.

Reference: 1. Muñoz, O. (1992) Química de la flora de Chile, Departamento Técnico de Investigación Universidad de Chile, Santiago.