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Untargeted metabolomic LC-MS fingerprinting of apple cultivars for the identification of biomarkers related to resistance to rosy apple aphid

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MAIN CONCLUSION

The isomerisations of hydroxycinnamic acids are the key metabolic reactions of the phenylpropanoid pathway that establish the tolerance of apple cultivars to rosy apple aphid (RAA), the isomers of caffeoylquinic and p-coumaroylquinic acids being the particular phenolic compounds involved.

Untargeted metabolomic LC-MS fingerprinting of apple cultivars is proved to be a useful less time consuming approach to identify biomarkers than the profiling approaches.

INTRODUCTION

Phenolic compounds may play important roles in plant responses to biotic-stress and in resistance mechanisms. In a previous work, the correlation between the phenolic composition of apple cultivars and their tolerance to RAA, *Dysaphis plantaginea*, a key insect pest of *Malus domestica* (Borkh.) was demonstrated [1]. The study was performed on a full-sib family of apple cultivars derived from a crossing between the local cider apple cultivar ‘Meana’ and the resistant cultivar ‘Florina’, using a profiling approach. Concentrations of forty individual phenolic compounds (flavan-3-ols, hydroxycinnamic acids, dihydrochalcones and flavonols) were determined by UHPLC-DAD in the parent cultivars and 155 ‘Meana’ x ‘Florina’ descendants; as well as their resistance to RAA by aphid inoculation. Pattern recognition techniques disclosed that the caffeoylquinic acid isomers, i.e. 4-CQA, 1-CQA and 3-CQA, and 4-p-coumaroylquinic acid (4-pCoQA) were related to the resistance of apple cultivars to RAA through the phenylpropanoid pathway; the isomerisations being the key metabolic reactions for a cultivar to be resistant or susceptible to RAA. This profiling approach was time consuming since it involved the identification and quantitation (including peak integration and external standard calibration) of the forty phenolics studied. Moreover, only the determined compounds could be considered as possible biomarkers; indeed the presence of other compounds related to RAA resistance could not be disclosed. In the present work, an untargeted metabolomic approach is proposed to identify biomarkers related to the resistance of apple cultivars to RAA. This is a fingerprinting approach, which is less time consuming since the identification and quantitation steps required in target analysis for profiling are not performed, and compound identification is only carried out for the actual biomarkers disclosed by the multivariate data analysis. Furthermore, unknown biomarkers can be discovered.

MATERIALS & METHODS

Apple juice samples were analysed by UHPLC-DAD-ESI(+)-QToF/MS using an Waters ACQUITY UPLC™ system, equipped with binary solvent delivery pump, autosampler, column compartment,

DAD detector and Waters SYNAPTTM G2 HDMS spectrometer. A Phenomenex Kinetex™ C18 column (2.1×100mm, 1.7µm) was used at 40 °C. Mobile phases: 0.1%(v/v) acetic acid(aq.) (A) and 0.1%(v/v) acetic acid in methanol (B). Elution conditions: 0–1.6min, 2%B isocratic; 1.6–2.11min, 0–8%Bgradient; 2.11–8.8min, 8%B isocratic; 8.8–9.8min, 8–10%B gradient; 9.8–17min, 10%B isocratic; 17–22min, 10–20%B gradient; 22–23.4min, 20–23%B gradient; 23.4–28.4min, 23–35%B gradient; 28.4–30.4min, 35–51%B gradient; 30.4–31.4min, 51–100%B gradient; 31.4–32.4min, 100 % B isocratic; and system re-equilibration. Flow rate was 0.35mL/min; injection volume, 5µL; and autosampler temperature, 4°C. UV-visible spectra were recorded from 210–500nm. The ESI(+)-QToF-MS^E conditions were previously reported [2].

RESULTS & DISCUSSION

UHPLC-ESI(+)-QTOF/MS fingerprinting of the same ‘Meana’x‘Florina’ descendants was carried out. The proposed untargeted metabolic approach consisted in: *(i)* data acquisition (retention time (RT), m/z, MS signal intensity); *(ii)* conversion of MassLynx raw files into CDF files, grouping them according to sample categories (resistant (R) and susceptible (S)); *(iii)* processing of NetCDF files by Metlin R-package XCMS, in order to convert the 3D LC-MS data (RT, m/z, intensity) into a table of time-aligned detected features for each sample (LC-MS artefacts were filtered by R-package CAMERA); and *(iv)* univariate and multivariate data analysis.

Apple fingerprints and data of their resistance to RAA were submitted to pattern recognition techniques: Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), Partial Least Square-Discriminant Analysis (PLS-DA) and Orthogonal PLS-DA (OPLS-DA). Dataset containing 1983 variables were pareto-scaled and logarithmic transformed. No outliers or extreme samples were detected by PCA. The bidimensional plot of the sample scores in the spaces defined by the two first principal components, as well as HCA, showed two clusters of the samples according to the established classes (R and S). PLS-DA achieved a binary classification model with recognition and prediction abilities in cross-validation of 100% of hits for both classes. The S-plots of the cross-validated OPLS-DA model obtained was used to select the relevant features for the differentiation of the cultivars according to their resistance to RAA. All these techniques revealed that the main features were due to 4-CQA, present in higher concentrations in resistant cultivars; and to 5-pCoQA, in higher concentrations in susceptible cultivars, in accordance with previous study [1], and confirmed that the isomerisations of hydroxycinnamic acids are the key metabolic reactions of the phenylpropanoid pathway for a cultivar to be resistant or susceptible to RAA.

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