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Analysis of phytoanticipins in *Citrus sinensis* grafted on *C. limonia* after *Xylella fastidiosa* infection by HPLC-UV and MALDI imaging mass spectrometry

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A high performance liquid chromatography-ultraviolet (HPLC-UV) method was developed for quantifying hesperidin and rutin levels in leaves and stems of *Citrus limonia* with a good linearity over a range of 1.0-80.0 and 1.0-100.0 $\mu\text{g mL}^{-1}$ respectively with $r^2 > 0.999$ for all curves. The limits of detection (LOD) for both flavonoids were 0.6 and 0.5 $\mu\text{g mL}^{-1}$, respectively with quantification (LOQ) being 2.0 and 1.0 $\mu\text{g mL}^{-1}$, respectively. The quantification method was applied to *C. sinensis* grafted on to *C. limonia* with and without CVC (*Citrus* variegated chlorosis) symptoms after *Xylella fastidiosa* infection. The total content of rutin was low and practically constant in all analyses in comparison with hesperidin, which showed a significant increase in its amount in symptomatic leaves. Scanning Electron Microscopy studies on leaves with CVC symptoms showed vessel occlusion by biofilm and a crystallized material was noted. Considering the difficulty in isolating these crystals for analysis, tissue sections were analyzed by matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) to confirm the presence of hesperidin at the site of infection. The images constructed from MS/MS data with a specific diagnostic fragment ion (m/z 483) also showed higher ion intensities for it in infected plants than in healthy ones, mainly in the vessel regions. These data suggest that hesperidin plays a role in the plant-pathogen interaction, probably as a phytoanticipin. In similar stem inoculating experiments we determined the population of *X. fastidiosa* in symptomatic plant, which showed the highest bacterial population in the leaves in stem and roots it was present but lower than in leaves. The chemical profile of scion and rootstock differed notably for absence in the second of flavonoid glycosides and low content of coumarins in the first. Thus, we also developed a rapid and sensitive HPLC method for quantitative determination of the coumarins xanthyletin and seselin in roots of *C. sinensis* grafted on *C. limonia*. The method showed a good linearity and was also applied to *Citrus sinensis* grafted onto *C. limonia* with and without CVC symptoms. The total content of seselin was practically constant in all analyses in comparison with xanthyletin, which showed a significant increase in its amount in symptomatic plants. These data suggest that xanthyletin plays a role in the plant-pathogen interaction, probably as a phytoanticipin in roots. The HPLC-UV method developed here and applied to citrus plants showed an increase in hesperidin and xanthyletin contents in asymptomatic plants. Between the samples analyzed the plants without CVC symptoms are the plants with a positive PCR test for *X. fastidiosa*, whereas the negative control were the plants that were not inoculated with bacteria. Thus these increase may indicate the presence of the bacteria. Hence the HPLC-UV methods have become a powerful tool for detecting CVC in *Citrus* before the symptoms appear. Thereby, informing the *Citrus* producers in advance when the plant should be removed from the orchard. This could prevent the disease from being transmitted to other plants by insects and also represent significant savings in pesticide application costs.

Biography

Maria Fatima Das Graças Fernandes Da Silva got her degree from University of Sao Paulo, Brazil in 1973. After her doctorate degree in Sciences - Emphasis in organic chemistry in 1978 at University of São Paulo, Institute of Chemistry, Sao Paulo, under the supervision of Dr. Otto R. Gottlieb, she carried out Postdoctoral research as a visiting scholar at University of Strathclyde Scotland-U.K, under the guidance of Professor Peter G. Waterman. Her academic career started at São Carlos Federal University, Department of Chemistry, as an Assistant Professor in 1976; in 1978 she was promoted to Associate Professor and in 2005 to Full Professor. The central theme of her research is isolation and structure elucidation of plant secondary metabolites and the application of findings on numerous biological screening and in phylogenetic studies. She is the director of Natural product division of Brazilian Chemical Society (SBQ) 2006-2008 and was honor with Fellow of the International Union of Pure and Applied Chemistry (IUPAC) 2008.

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