11 th NUCL USER USER Work

NUCLEAR MAGNETIC RESONANCE USERS MEETING

Workshop: NMR in South America

MAY 7th - 11th, 2007 Hotel do Frede - Angra dos Reis, RJ, Brezil

Extended Abstracts Book

11th Nuclear Magnetic Resonance Users Meeting / Workshop: NMR in South America

IDENTIFICATION OF PLANTS WITH CITRUS SUDDEN DEATH BY LOW RESOLUTION NMR

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Keywords: MSC; CPMG; CWFP

Citrus Sudden Death (CSD) is a new graft-transmissible disease of sweet orange (*Citrus sinensis* L.) trees grafted on rangpur lime (*C. limonia* Osbeck) or *Citrus volkameriana* rootstocks¹. It was first seen in Brazil in 1999 and has been detected in more than 2 millions trees (infected or dead) and it is a serious threat to the Brazilian citrus industry, as more than 85% of 200 million sweet orange trees are grafted on the rangpur lime rootstock. The causal agent of CSD is unknown and the diagnostic of disease is based on symptoms such as generalized foliar discoloration and is the presence of yellow stain in the bark of rangpur lime rootstock.

In this paper we evaluated the use of low resolution ¹H NMR techniques such as CPMG (Carr-Purcell-Meibbom-Gill) and CWFP (Continuous Wave Free Precession)² to identify the CSD plants. We analyzed lyophilized samples from scion and rootstock barks of valencia sweet orange grafted on rangpur lime, without symptoms (WSY) and with mild symptoms (CSD1) and severe symptoms (CSD2). The analyses were performed in a 2.1 T magnet and a CAT 100 Tecmag console. For CPMG and CWFP measurements we used pulse width = 14 µs, acquisition time = .64µs, 1000 pulses ant recycle delay = 2 s. The time between pulses (τ) values were 100 and 300 µs for CPMG and CWFP respectively. The frequency offset for zero and 5KHz (Ψ =3 π) for CPMG and CWFP respectively. The auto scaled data were analyzed by principal components analysis (PCA) using Pirouette software (Informetrix).

The NMR signals of lyophilized bark were assigned to triacylglycerides (TAG), which are in liquid state, in the samples. The amount of TAG in the WSY, CSD1 and CSD2 rootstocks were about 1.5, 1.7 and 2.5%, respectively. Figure 1 shows the PCA maps (scores data) of factor 1 versus factor 2 of CPMG (1A) and CWFP data (1B) acquired from rootstock barks. The PCA of CPMG decay did not show any distinction between WSY (ξ) and CSD1 (\bullet) and CSD2 (8) plants. On the other hand, the CWFP data (1B) show the CSD1 and CSD2 plants in negative values of factor 1 and WSY plants in the positive side. Only 3 sample of WSY out of 20, were in the CSD side. This is an indication that the CWFP was able to characterize this sample as a CSD plants, before the expression of the phenotype symptoms. The CSD1 and CSD2 plants are not well separated by CWFP data but, the CSD1 is more in the negative side of factor 2 and CSD2 in the positive side.

The classification of WSY and CSD plants by CWLP and not by CPMG may indicates the differences longitudinal relaxation time (T₁) of the TAG in the barks. The CWLP signal contains both T₁ and transverse $(T_2)^2$ while CPMG signal contains only T₂ information.