Metabolomic profiling with ^{1}H NMR spectroscopy of Sterile Male of Mediterranean fruit fly *Ceratitis capitata*

PO-17

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INTRODUCTION

The Sterile Insect Technique (SIT) is among the most environment-friendly insect pest control method ever developed for the suppression or eradication of a number of insect pests such as the Mediterranean fruit fly *Ceratitis capitata* (Fig.1). Irradiation, such as with gamma rays and X-rays, is used to sterilize mass-reared insects so that, while they remain sexually competitive. Although the basic mechanisms of Radiation-induced sterile insect technique is that radiation damages genetic material, and the resulting chromosome breaks induce dominant lethal mutations in reproductive cells. Characterization of the metabolic shift associated with gamma radiation exposure in sterile insects would be helpful for understanding the detailed mechanism underlying this technique and promote its practical application. Through the mining of such descriptive data, metabonomics can potentially aid in the identification of as many potential metabolites as possible in biological samples that relate to radiation process. In addition, NMR is a very useful technique for structure elucidation due to various two-dimensional NMR measurement without further fractionation. In this study, the NMR based metabolic fingerprint coupled with multivariate analysis was applied to analyze the metabolic trajectory, in order to determine the metabolic profile changes caused by irradiation.

METHODS

The Mediterranean fruit flies VIENNA 8 genetic sexing strain (GSS) were maintained in the Tunisian Medfly rearing facility situated in the National Center for Nuclear Science and Technology (CNSTN). Male pupae 2 d before emergence were Cobalt-60 irradiated at the following radiation doses: 0, 70, 90, 110 and 145 Gy. All the experiments used virgin sexually mature males (control and irradiated) five days after emergence. Acer anesthesia with CO2, we instantly froze the samples (flies) in liquid nitrogen, placed them in a -80°C freezer and subsequently lyophilized for 2 days. 3 mg of lyophilized flies was weighed into 2 ml centrifuge tube. Metabolites were extracted choosing the previously optimized methanol-chloroform protocol (Fig.2).

NMR spectra were recorded at 305K on a Bruker Avance III 800 MHz NMR equipped with a 1H-13C-15N TCI cryo-probe. 1D-1HNMR spectra were acquired using a water suppressed CPMG spin-echo pulse sequence with a spin-echo delay of 300 µs and a total spin-spin relaxation delay of 240 ms, with 256 scans, a relaxation delay of 5 s and 32 k time domain points. NMR spectra were processed in TopSpin, Icoshic aligned and analyzed in MetaboAnalyst 4.0 using principal component analysis (PCA).

RESULTS

Unsupervised analysis indicates that irradiated males samples could be separated by PCA on 3 components. Four groups are observed, first 0 Gy and 110 Gy are well defined by combining PC1/PC3. The high dose 145 Gy irradiated males are segregated by PC1/PC2 (Fig.3). Intermediate irradiation doses, 70 and 90 Gy could not be discriminated, and the model is not a linear dose-response. Chemical components are identified as Lactate, Alanine and Acetate using 1H and 13C chemical shifts (Fig.4).

CONCLUSION

Supervised analysis (OPLS-DA in Fig.5) roughly gives the same four clusters than unsupervised analysis. Juggling between various NMR Spectra and loading plots allows to identify 3 metabolites, but many of the others could not be assigned mainly due to crowding in the 3.0 – 4.5 ppm spectral region. An alternative to avoid this, is sexual organs extraction. Population sampling for realistic statistics should also be increased at least by a factor of 3, to reach reasonable FDR.

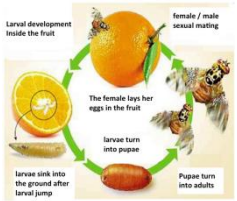

***Figure 1**. Life cycle of GSS (Vienne 8) of the Medfly Ceratitis capitata. Male and female Pupae have Different Colours and are easily separated …*

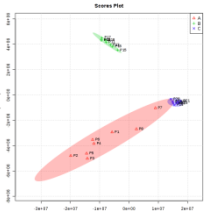

***Figure 2.** Choice of extraction PCA analysis (*) of 3 protocoles ((A) 200 mM PBS, (B) Methanol/ Chloroform and (C) Acetonitrile*

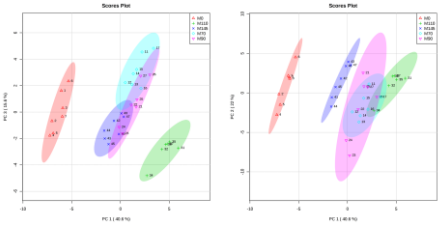

***Figure 3**. PCA*

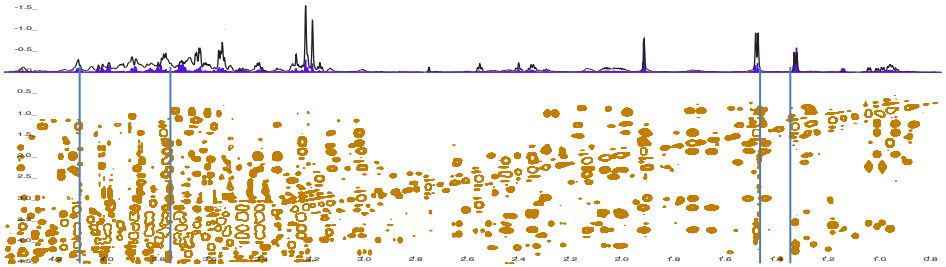

***Figure 4**. Juggling between 1D NMR spectrum (black) , principal components (loading plots in purple) And various 2D NMR experiments, as TOCSY (brown) and 13C--1H HSQC to obtain statistically relevant 1H and 13C chemical shifts.*

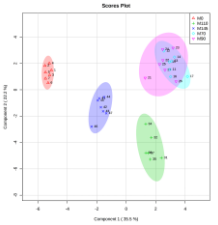

***Figure 5**. OPLS-DA*

Références: Msaad Guerfali et al.,2013,Journal of Economic Entomology 106(2):641-7

Qu, L J et al., 2014, Journal of insect science vol. 14-166

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