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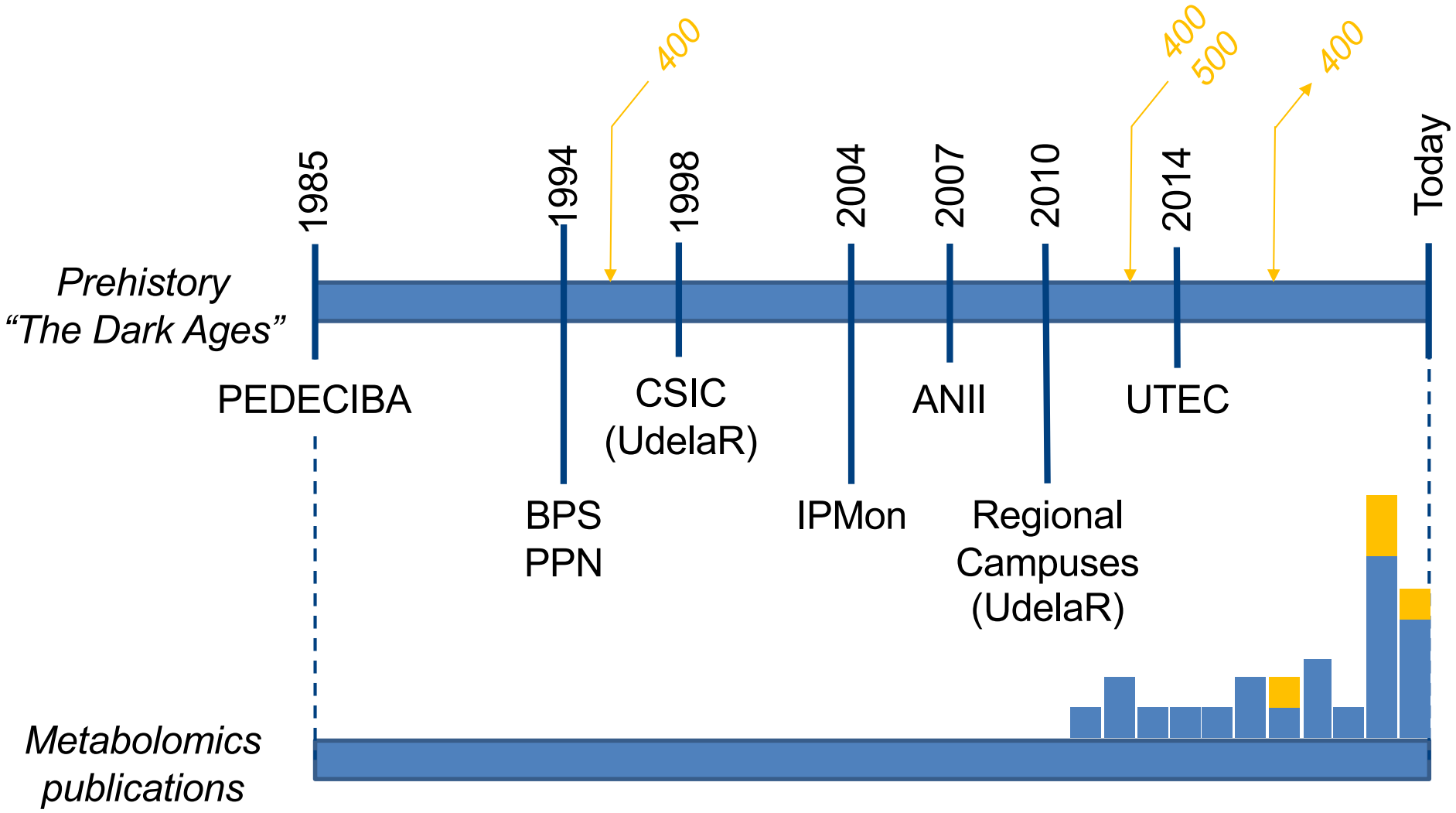
UNIVERSIDAD DE LA REPÚBLICA URUGUAY

IMPS

A Short and Biased Account of Metabolomic Analysis in Uruguay: A Few Experiences from the Healthcare and Productive Sectors

Metabolomics in Uruguay – A Stitch in Time

- Uruguayan life sciences research highlights post-1985…



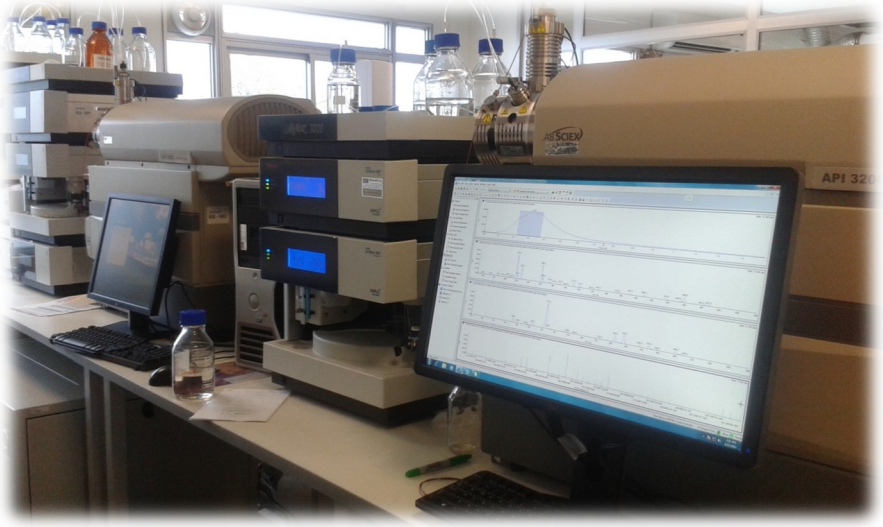
- ~0.5% of the country's annual scientific production (comparable to ARG, BRA…)

Metabolomics in Uruguay – Newborn Screening

- Very nice example of targeted metabolomics that usually goes unnoticed by those of us claiming to do metabolomics in Uruguay…

- The national newborn screening system (PPN) analyzes dried blood samples (DBS) from all kids born in Uruguay. Mandatory. Involves hospitals, the post, and clinical biochemistry lab from the state's social security administration (BPS).

- Initially umbilical cord blood samples from the BPS maternity ward screened for congenital hypertiroidism (FIA).



- First case detected after 2800 samples analyzed. After 10 cases were detected analysis was made mandatory (1994).

- In 2007 tandem MS incorporated (SCIEX 3200 x 2), and screening included markers for congenital adrenal hyiperplasia (FIA) and phenylketonuria (MS/MS).

Metabolomics in Uruguay – Newborn Screening

- Nowadays the lab screens for 25 congenital disorders:

598

Congenital hypothyroidism	CH	Fluoroimmunoassay AutoDELFIA Fluoroimmunoassay AutoDELFIA ECLIA
Congenital adrenal hyperplasia	CAH	Fluoroimmunoassay AutoDELFIA
Cystic Fibrosis	FQ	Fluoroimmunoassay AutoDELFIA ELISA
Phenylketonuria / hyperphenilalaninemia	PKU/ HPA	MS/MS
Medium-chain acyl-CoA dehydrogenase deficiency	MCADD	MS/MS

15

Haemoglobinopathy	Hbpatía	HPLC -CE Variant BioRad
Maple syrup urine disease	MSUD	MS/MS
Citrullinemia	CIT	MS/MS
Tyrosinemia	TYR	MS/MS
Argininemia	ARG	MS/MS
Carnitine uptake defect/carnitine transport defect	CUD	MS/MS
Carnitine palmitoyltransferase type I deficiency	CPT-1	MS/MS
Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency	LCHAD	MS/MS
Very long-chain acyl-CoA dehydrogenase deficiency	VLCAD	MS/MS
Glutaric acidemia type II	GA II	MS/MS
Short-chain acyl-CoA dehydrogenase deficiency	SCAD	MS/MS
Glutaric acidemia type I	GA I	MS/MS
Isovaleric acidemia	IVA	MS/MS
Propionic acidemia	PA	MS/MS
Methylmalonic acidemia	Cbl/Mut	MS/MS
3-Hydroxy-3-methyglutaric aciduria	HMG	MS/MS
ß-ketothiolase deficiency	BKT	MS/MS
3-Methylcrotonyl-CoA carboxylase deficiency	3-MCC	MS/MS
2-methylbutyryl-CoA dehydrogenase deficiency	2-MBG	MS/MS
3-Methylglutaconic aciduria	3MGA	MS/MS

- A total of 974277 UCBS and 532684 DBS have been analyzed.
- Coverage is > 98% of newborns born in the country.
- The PPN has detected 617 cases of congenital disorders in 25 years of activity.

Metabolomics in Uruguay – Health Sciences

- Next example is very personal.
- Mid-2018 I was admitted to the hospital with a severe case of cholestatic hepatitis with unknown etiology.
- Early-2019, docs were still puzzled with my liver enzymes, so ended up in the Liver Disease Unit / National Liver Transplant Program at the Army Hospital (SEH/PNTH).
- The word "Transplant" made me an itsy bitsy nervous…
- I met wonderful people there, particularly Dr. Solange Gerona and Dr. Ofelia Noceti. I mentioned "metabolomics" to break the ice, and we ended up working together…

Metabolomics in Uruguay – Health Sciences

- Project with SEH/PNTH broadly aims at applying metabolomics for diagnosis and monitoring of liver diseases, including autoimmune disorders and viral infections.

- Bookeeping as important as science: Started in early 2020 with ~7000 SEH/PNTH case files…

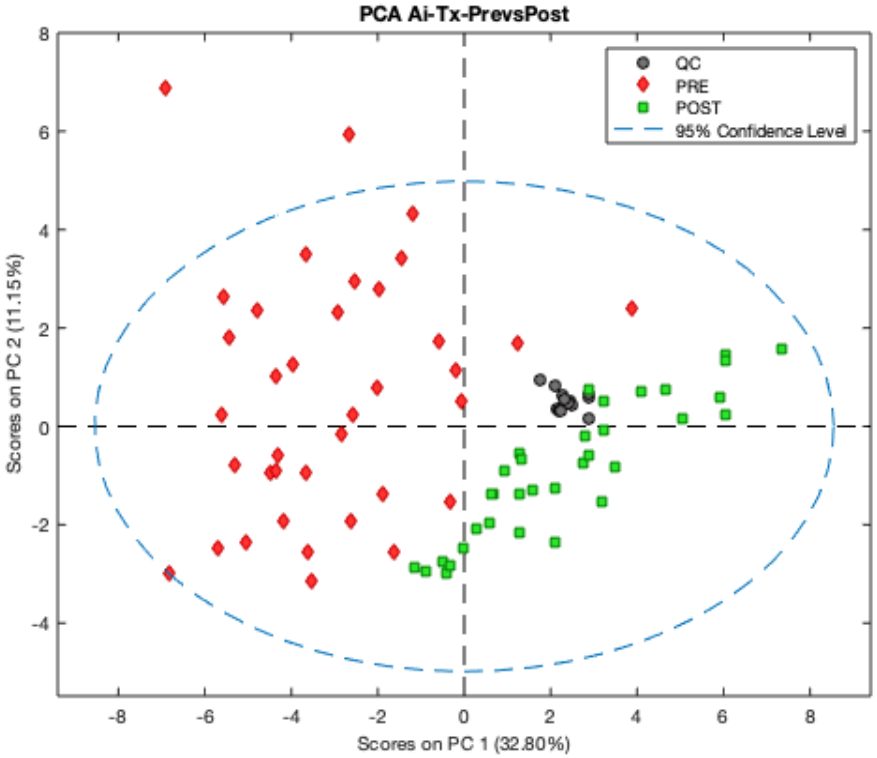
- A total of 199 SEH/PNTH patients and 99 healthy volunteers, pandemic an all:
  - 112 AI disorders, many with overlaps
  - 61 hepatitis C (HVC)
  - 26 hepatocellular carcinoma (HCC)

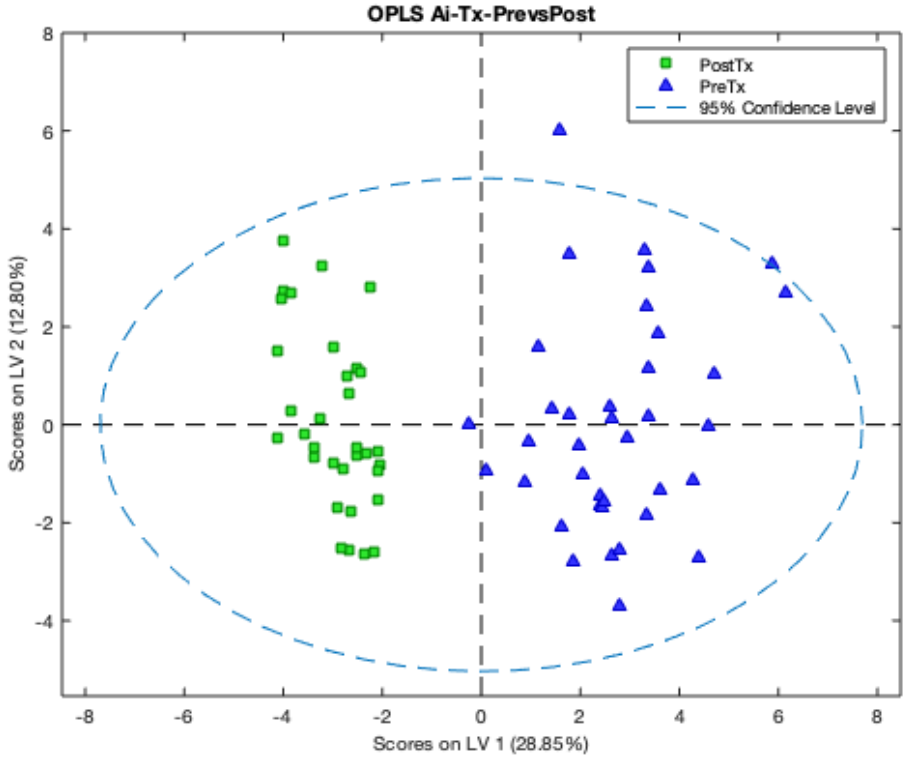
- Mostly a “one-woman” show…

Metabolomics in Uruguay – Health Sciences

- Lots of data to analyze and comparisons to consider. Our first study involved AI disease transplant recipients.

- NMR-based serum metabolomics from 48 patients paired pre- and post-surgery.

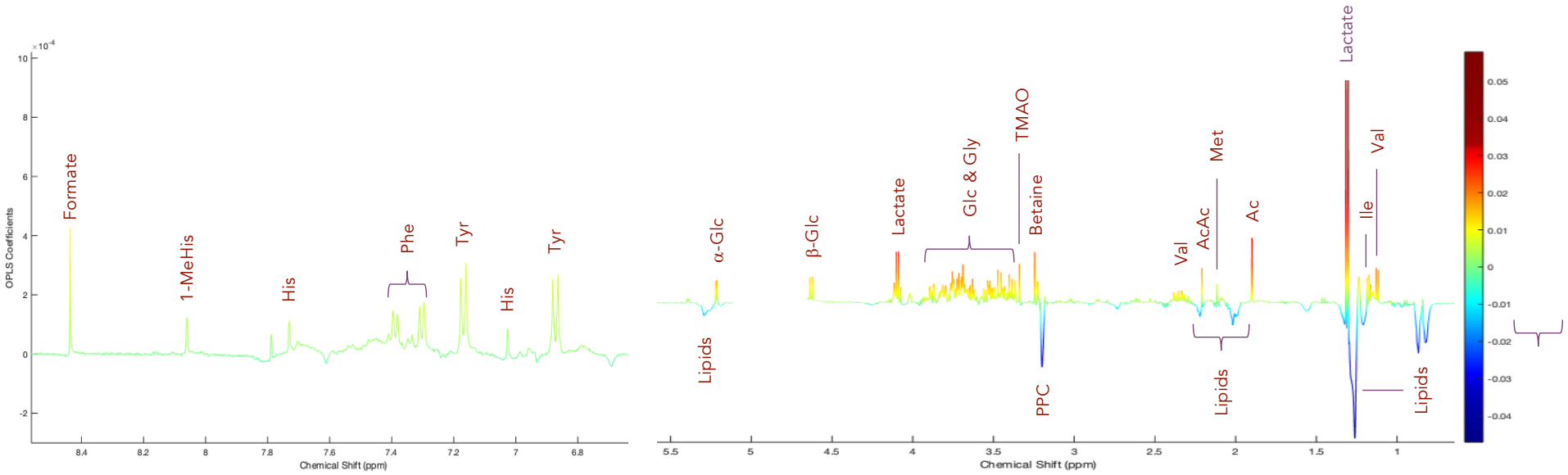




- Nice clustering in PCA score plot…

- Great OPLS-DA discriminating model…

Metabolomics in Uruguay – Health Sciences



- Pre-TX lots of lactate, AAs (protein catabolism), and glycolitic/glycogenic pathway intermediates. Post-TX phosphocholine and lipids…
- Consistent with pre-TX state (ICU patients, pronounced metabolic stress). Lipids in post-TX patients linked to resuming normal (bad) diets.
- Good to learn, but not that useful for clinicians. How do TX survivors compare to TX non-survivors? Working on it…
- By the way, what I had was Hepatitis E…

Metabolomics in Uruguay – Plant Sciences

- On to less altruistic topics, but by no means less important: Barley (and yes, beer!)

- Very important winter crop in Uruguay, mostly destined to beer-making.

- Aphids affect barley through direct and indirect damage. Pretty oblivious to pesticides. This leads to excessive applications, and a myriad of problems.

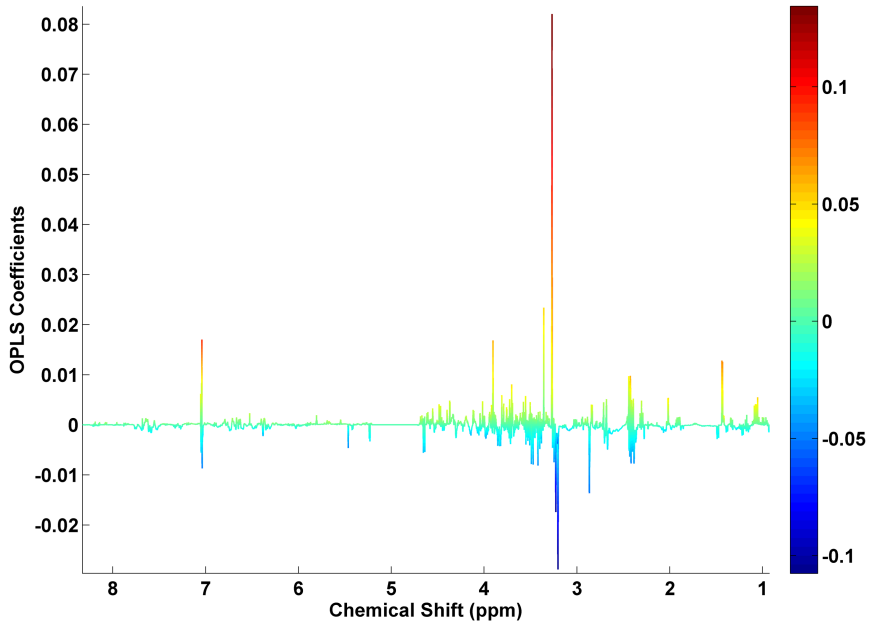
- An alternative is the selection and development of resistant varieties: integrated pest management (IPM).

- Resistance can be due to antibiosis (aphids do not like it), or antixenosis (aphids would rather eat something else…).

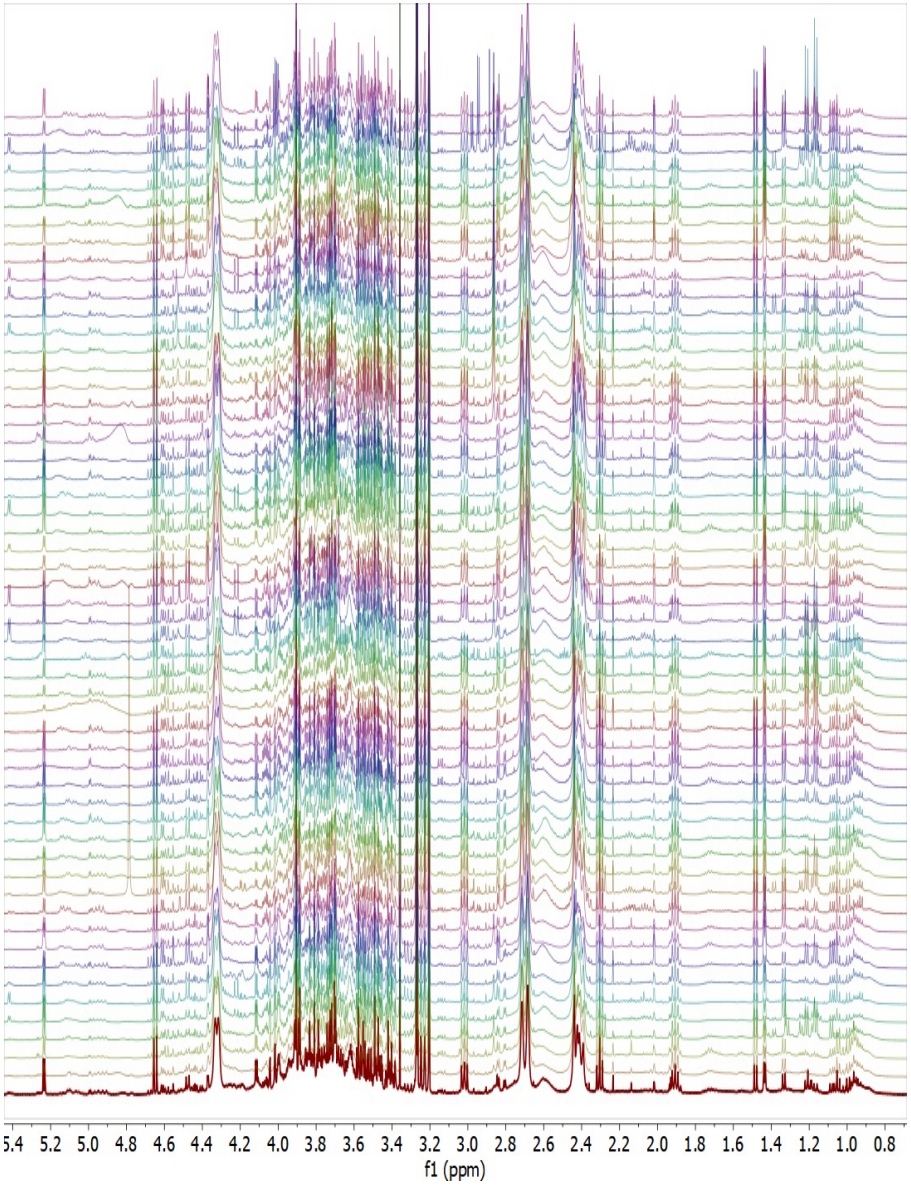
- A pilot study with 5 barley varieties studied at the UdelaR experimental station in Paysandú (EEMAC). Three more (“susceptible”) and two less (“resistant”) preferred.

Metabolomics in Uruguay – Plant Sciences

- Ten plants / variety, processed 15 days after sowing.

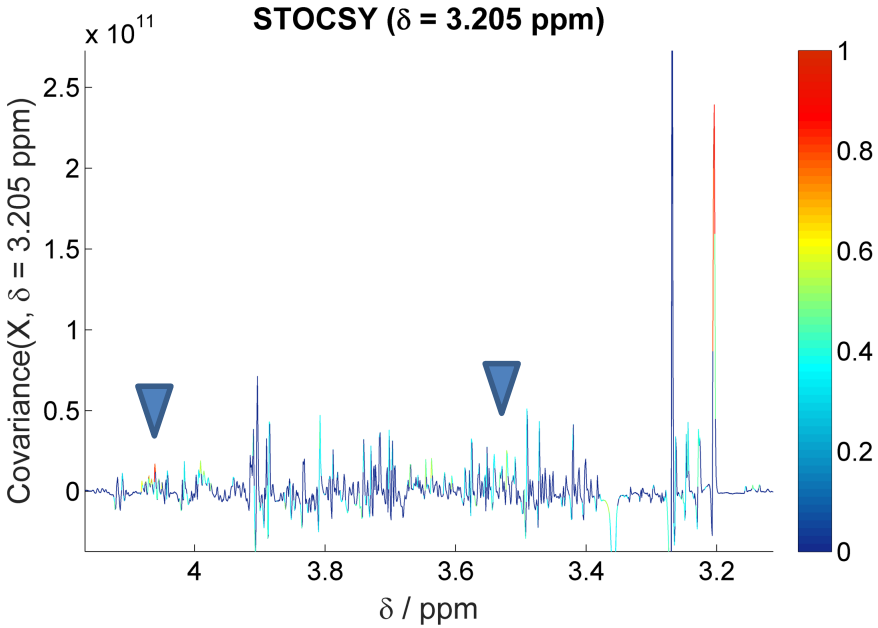


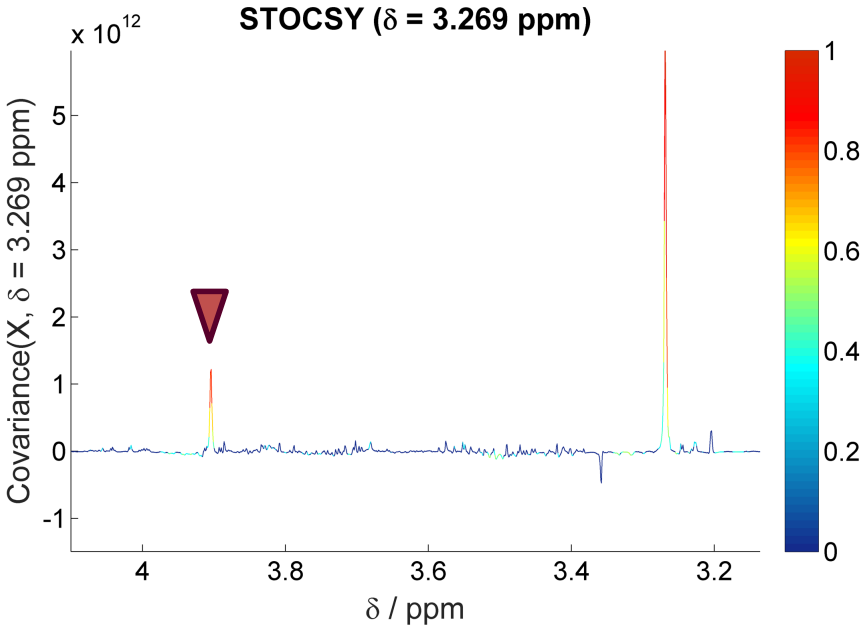
- The "NMR-like" loading plot points to discriminating signals at 3.21 (susceptible) and 3.28 ppm (resistant).



Metabolomics in Uruguay – Plant Sciences

- Using these signals as *drivers* for STOCSYs we identified other resonances from each discriminating metabolite.



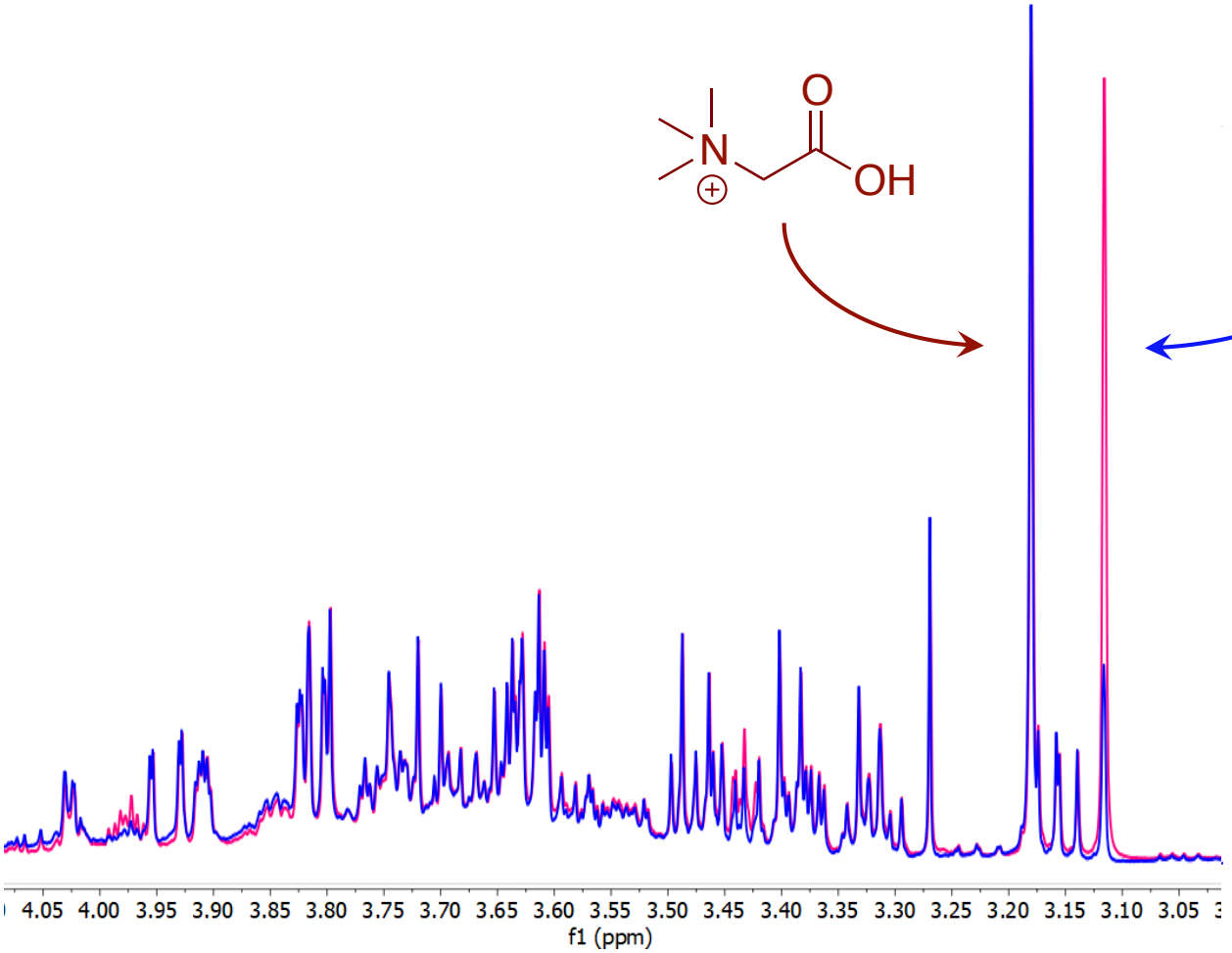


- Singlet at 3.21 ppm correlates with signals at 4.07 and (weakly) 3.43 ppm. Singlet at 3.28 ppm correlates with one at 3.91 ppm.

- Using HSQC ^{1}H-^{13}C correlations the corresponding ^{13}C resonances were identified. Database search suggests **choline** and **betaine**…

Metabolomics in Uruguay – Plant Sciences

- To confirm this one of the samples was spiked with both metabolites.



- Betaine is a known osmoprotectant associated with abiotic stress (drought). Similar results on cannabis affected by powdery mildew. Mostly a "one-man" show…

Metabolomics in Uruguay – Current Project / Colaborations

- Human health:
    - Minimally-invasive diagnosis of endometriosis
    - Differences between severe COVID / influenza / TB infections
    - Macrophage metabolism in TB infections
    - Thermogenic metabolism in adipocites
- Animal health:
    - Effects of biotic and abiotic stress in sturgeon fish farms
    - Obessity and cancer in pets and other small animals
    - Diet management on diary animal production/reproduction
- Agro/Phyto:
    - Selection of resistant varieties (citrus/strawberries/peanuts…)
    - Fingerprinting of *C. sativa* varieties with medical potential
    - Markers for the assessment of aging strategies on meat quality
    - Effects of environmental conditions on *P. patens* metabolism

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