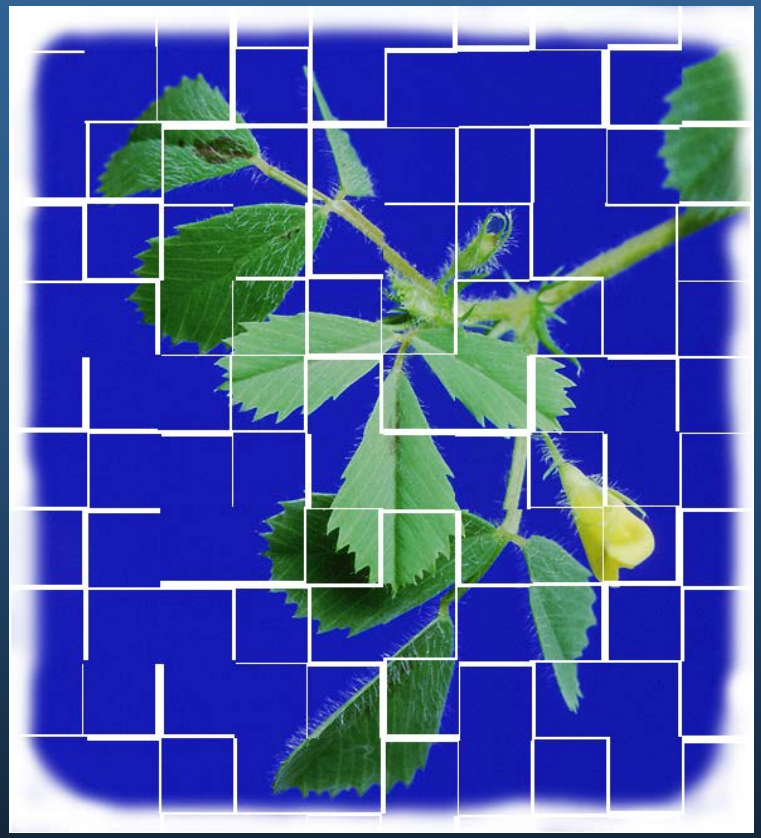
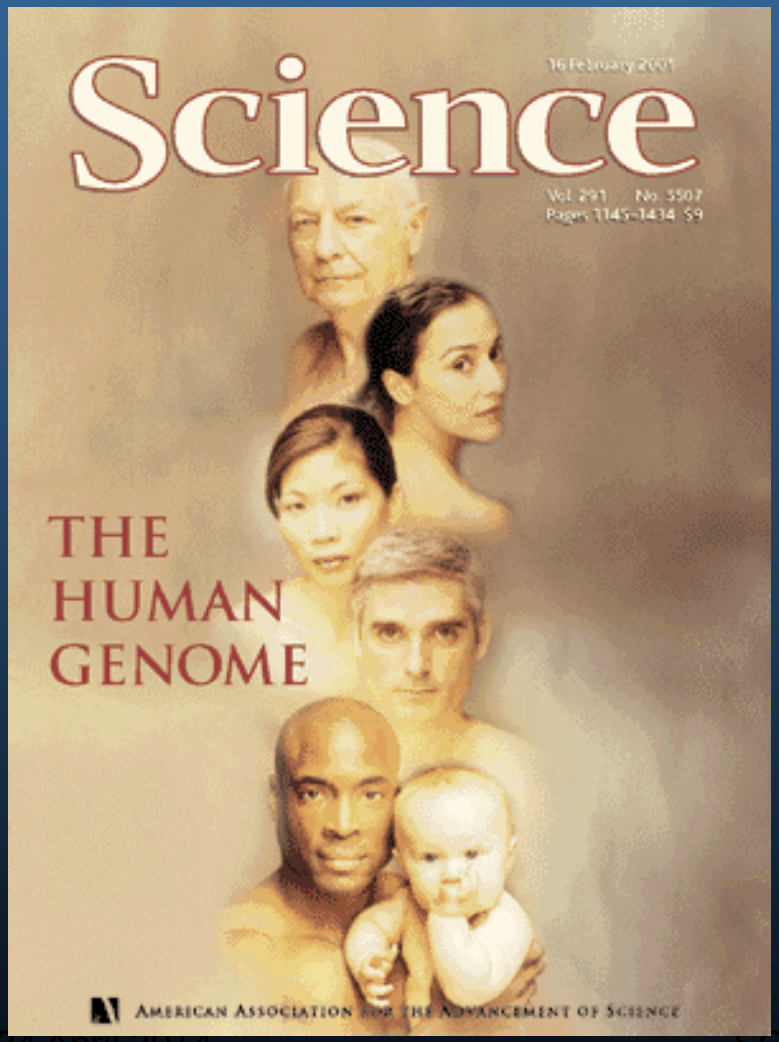
Metabolomics Gateway for Milestone Discoveries in the Post Genomic Era

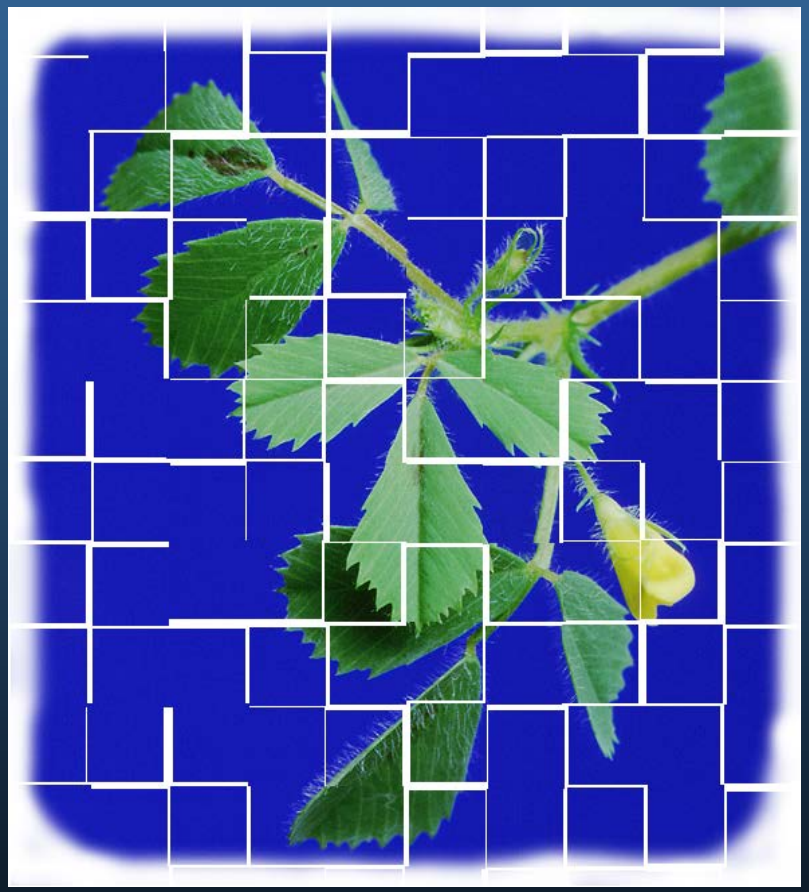


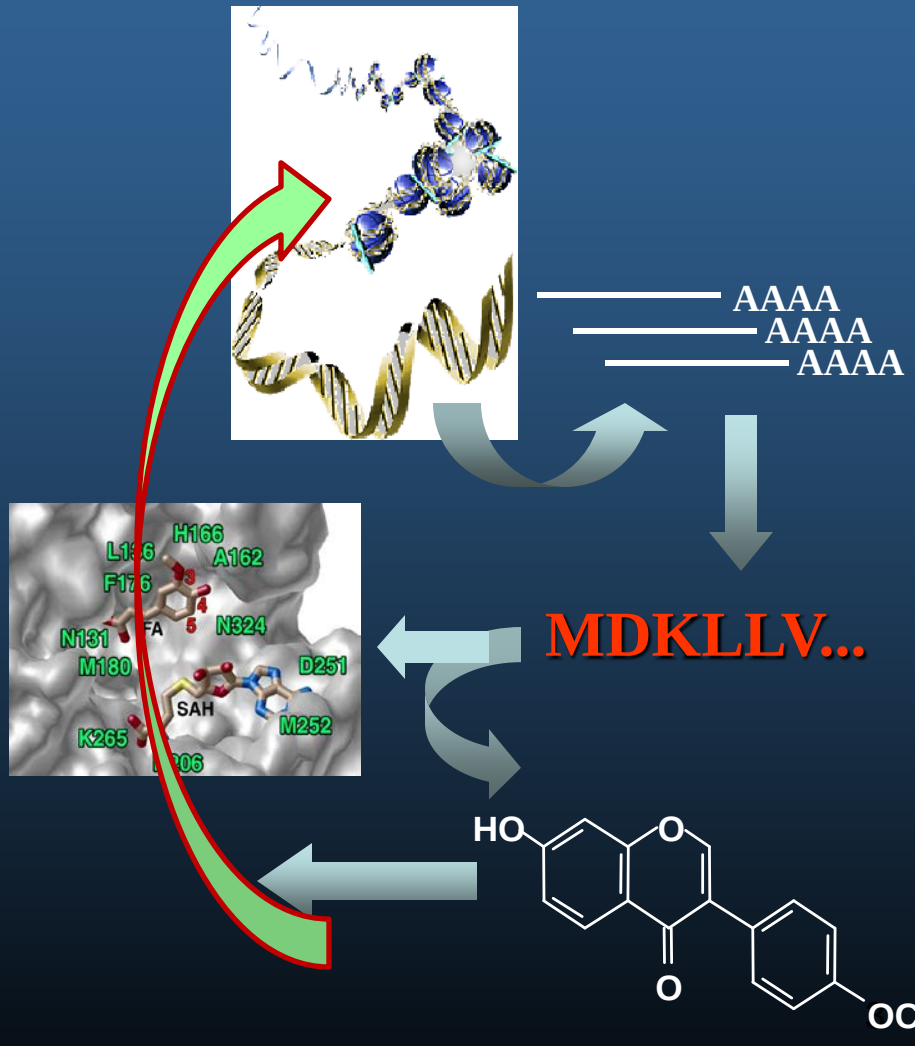
Dr. Mohamed A. Farag
TWAS 25th General Meeting
Associate Professor, Cairo University

2001: The human genome



Metabolomics Gateway for Discoveries in the Post Genomic Era

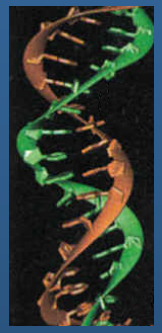




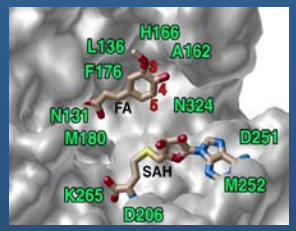
Dr. Mohamed A. Farag
Alexander von Humboldt fellow
Associate Professor, Cairo University

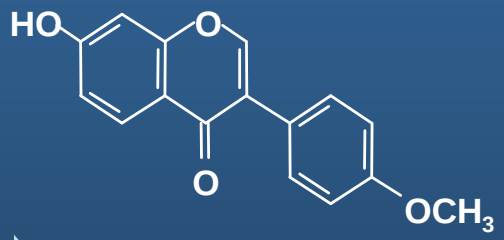
Integrated Functional Genomics

genome



- Integrating several ‘omic’ technologies to understand gene function





AAAA
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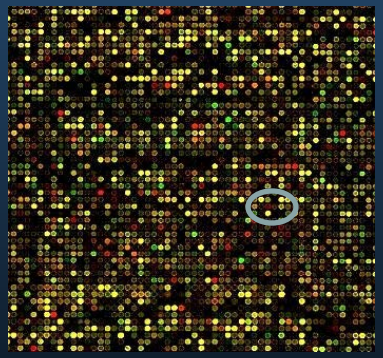
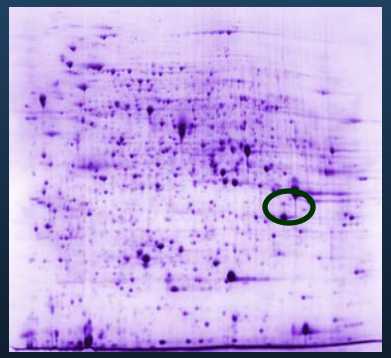
MDKLLV...

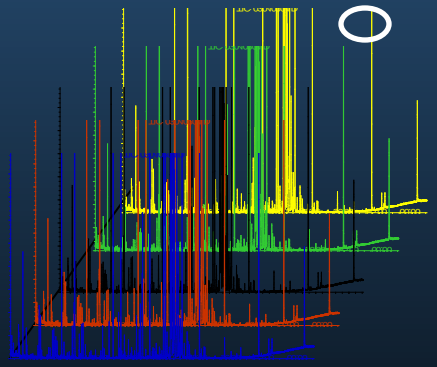
Transcriptome | **Proteome** | **Metabolome**



Microarray technology

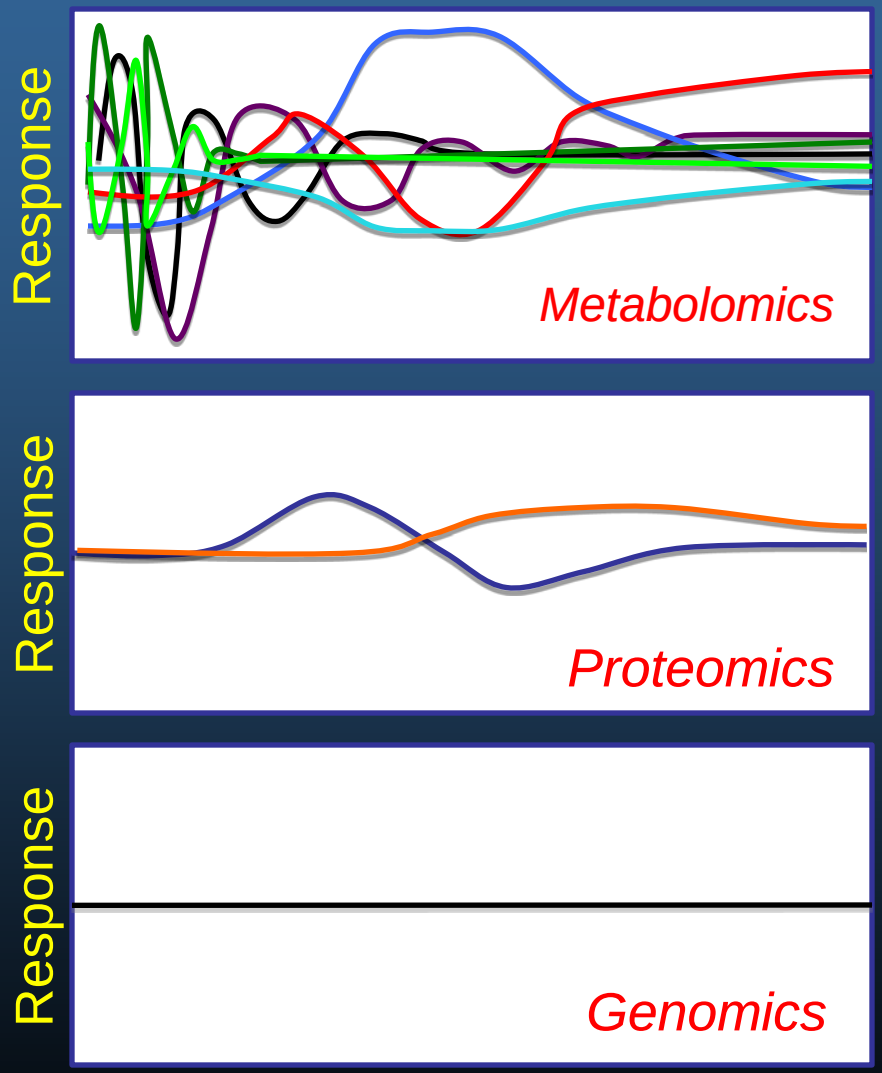


2D gel electrophoresis

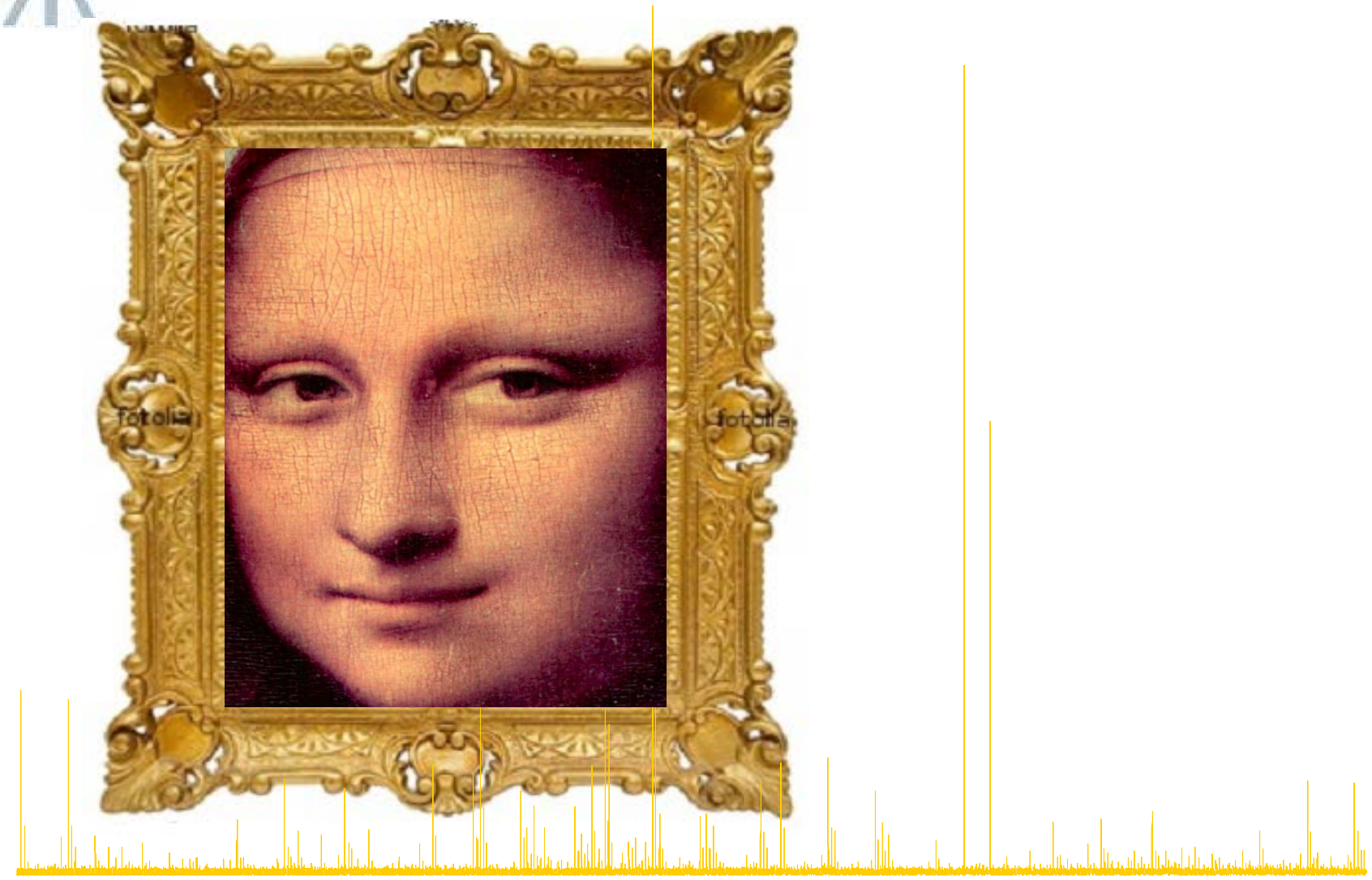


Chromatographic separation coupled to mass spectrometry or NMR

Metabolomics is More Time Sensitive Than Other “Omics”



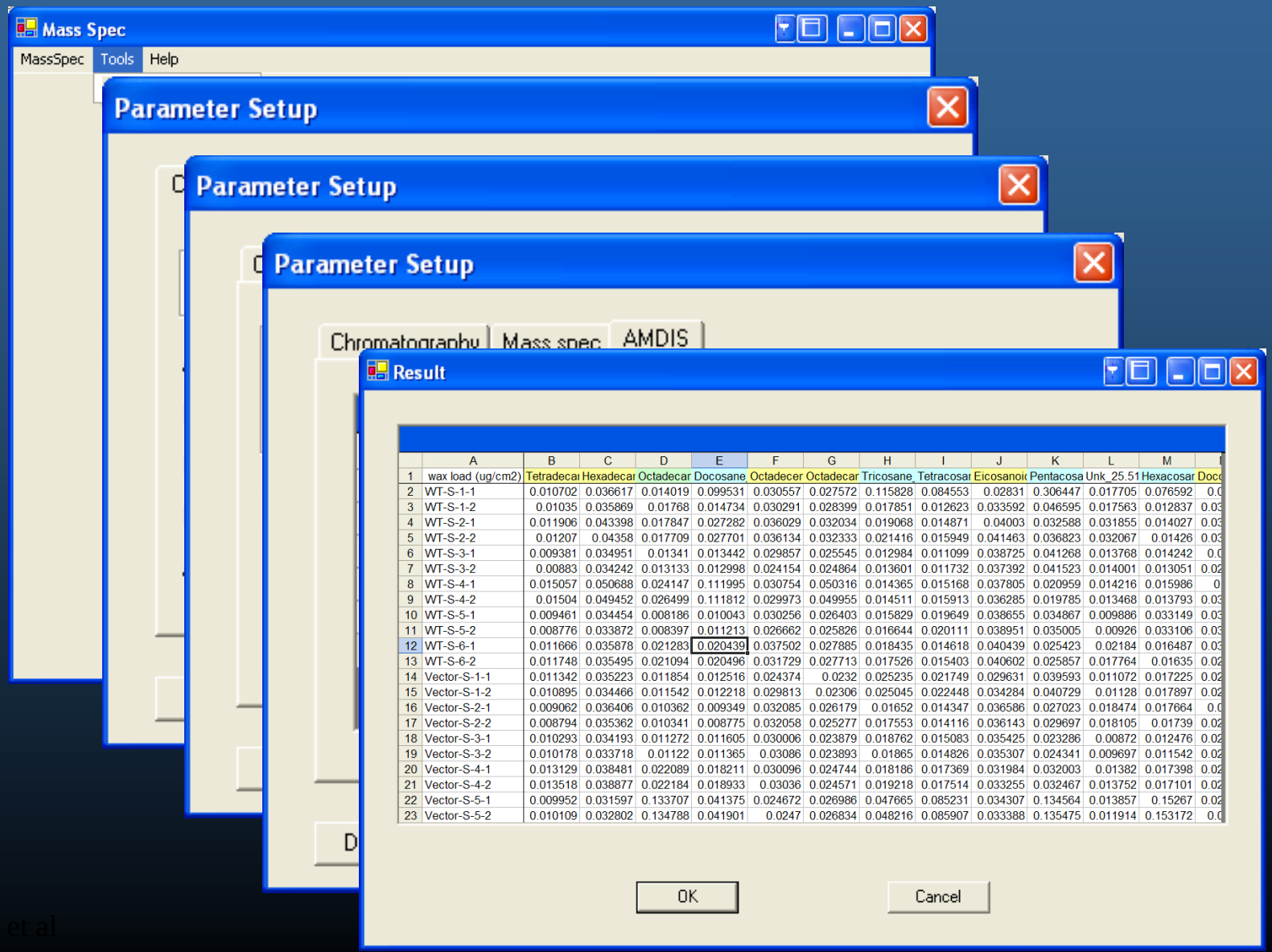
Picture of metabolome using **MS**



Picture of metabolome using **NMR**

Metabolome Picture, yet to be achieved

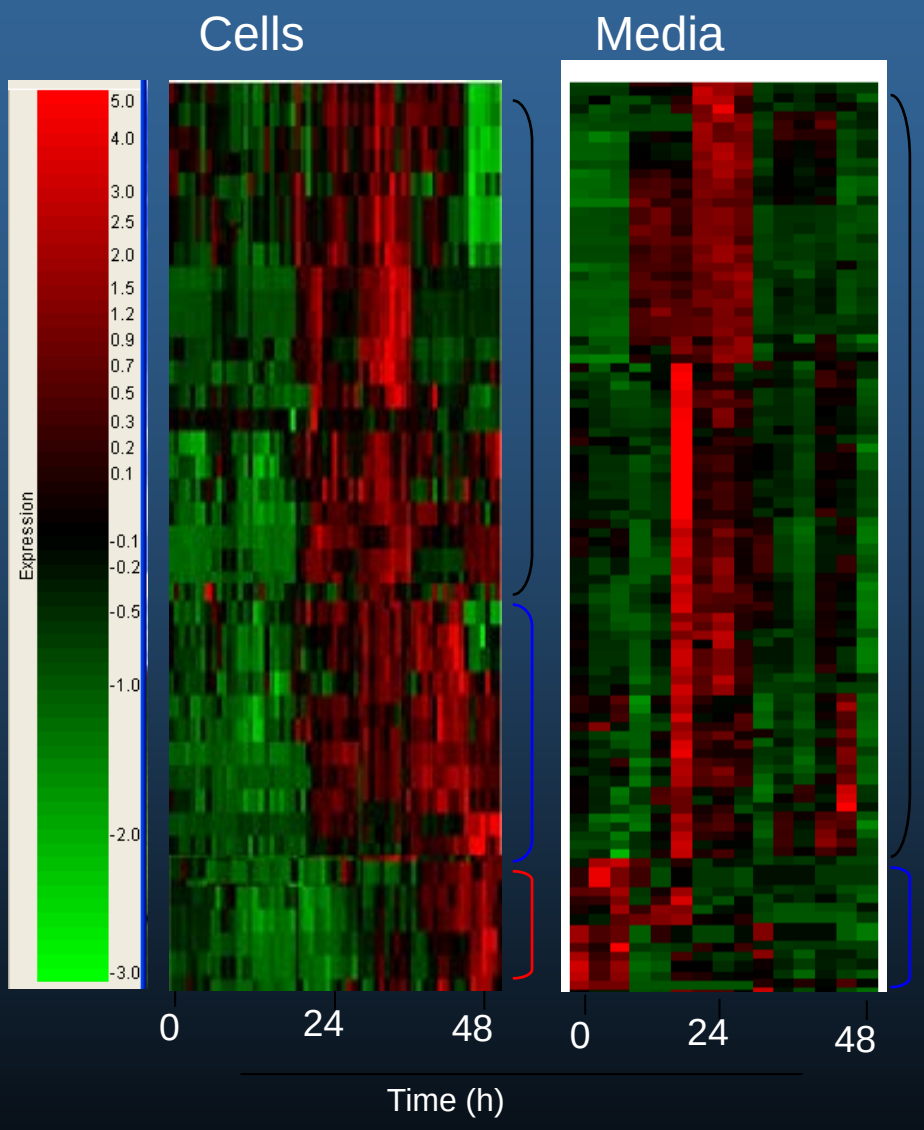
Huge data set !!!!!!

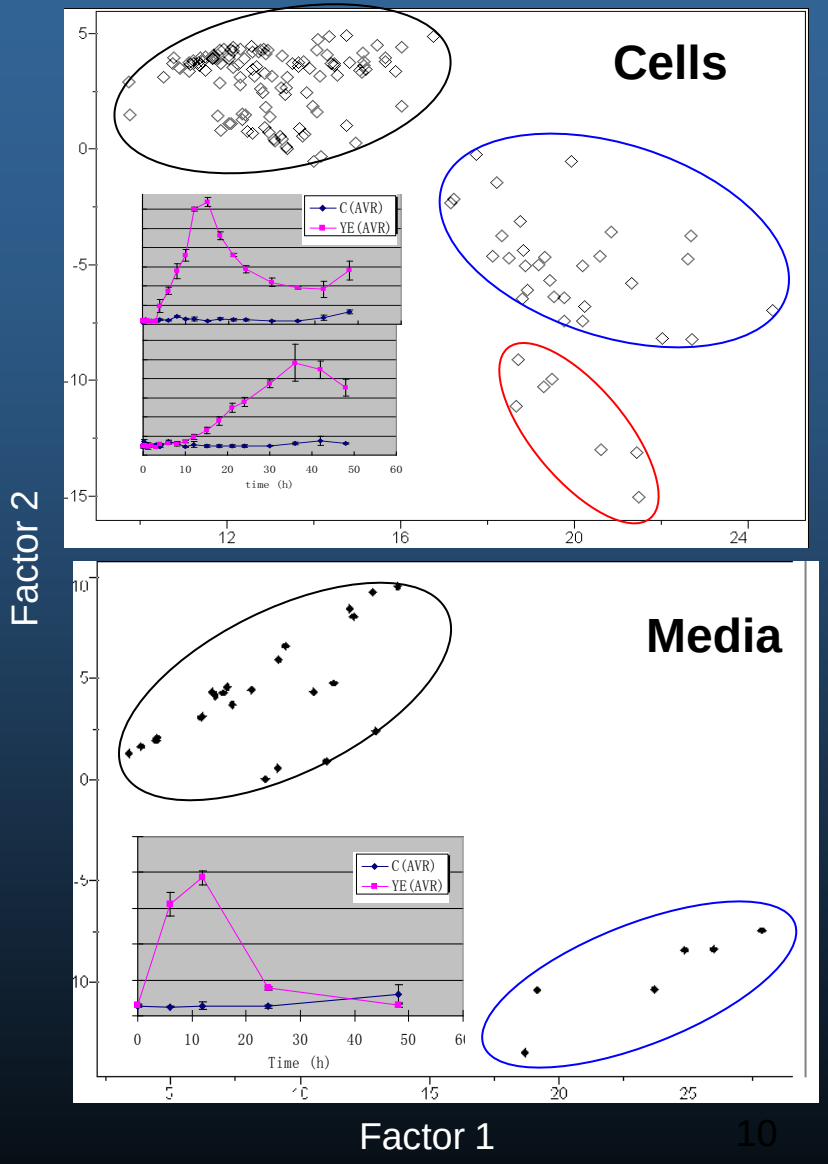


	A	B	C	D	E	F	G	H	I	J	K	L	M
1	wax load (ug/cm2)	Tetradecai	Hexadecar	Octadecar	Docosane	Octadecer	Octadecar	Tricosane_	Tetracosar	Eicosanoic	Pentacosa	Unk_25.51	Hexacosar
2	WT-S-1-1	0.010702	0.036617	0.014019	0.099531	0.030557	0.027572	0.115828	0.084553	0.02831	0.306447	0.017705	0.076592
3	WT-S-1-2	0.01035	0.035869	0.01768	0.014734	0.030291	0.028399	0.017851	0.012623	0.033592	0.046595	0.017563	0.012837
4	WT-S-2-1	0.011906	0.043398	0.017847	0.027282	0.036029	0.032034	0.019068	0.014871	0.04003	0.032588	0.031855	0.014027
5	WT-S-2-2	0.01207	0.04358	0.017709	0.027701	0.036134	0.032333	0.021416	0.015949	0.041463	0.036823	0.032067	0.01426
6	WT-S-3-1	0.009381	0.034951	0.01341	0.013442	0.029857	0.025545	0.012984	0.011099	0.038725	0.041268	0.013768	0.014242
7	WT-S-3-2	0.00883	0.034242	0.013133	0.012998	0.024154	0.024864	0.013601	0.011732	0.037392	0.041523	0.014001	0.013051
8	WT-S-4-1	0.015057	0.050688	0.024147	0.111995	0.030754	0.050316	0.014365	0.015168	0.037805	0.020959	0.014216	0.015986
9	WT-S-4-2	0.01504	0.049452	0.026499	0.111812	0.029973	0.049955	0.014511	0.015913	0.036285	0.019785	0.013468	0.013793
10	WT-S-5-1	0.009461	0.034454	0.008186	0.010043	0.030256	0.026403	0.015829	0.019649	0.038655	0.034867	0.009886	0.033149
11	WT-S-5-2	0.008776	0.033872	0.008397	0.011213	0.026662	0.025826	0.016644	0.020111	0.038951	0.035005	0.00926	0.033106
12	WT-S-6-1	0.011666	0.035878	0.021283	0.020439	0.037502	0.027885	0.018435	0.014618	0.040439	0.025423	0.02184	0.016487
13	WT-S-6-2	0.011748	0.035495	0.021094	0.020496	0.031729	0.027713	0.017526	0.015403	0.040602	0.025857	0.017764	0.01635
14	Vector-S-1-1	0.011342	0.035223	0.011854	0.012516	0.024374	0.0232	0.025235	0.021749	0.029631	0.039593	0.011072	0.017225
15	Vector-S-1-2	0.010895	0.034466	0.011542	0.012218	0.029813	0.02306	0.025045	0.022448	0.034284	0.040729	0.01128	0.017897
16	Vector-S-2-1	0.009062	0.036406	0.010362	0.009349	0.032085	0.026179	0.01652	0.014347	0.036586	0.027023	0.018474	0.017664
17	Vector-S-2-2	0.008794	0.035362	0.010341	0.008775	0.032058	0.025277	0.017553	0.014116	0.036143	0.029697	0.018105	0.01739
18	Vector-S-3-1	0.010293	0.034193	0.011272	0.011605	0.030006	0.023879	0.018762	0.015083	0.035425	0.023286	0.00872	0.012476
19	Vector-S-3-2	0.010178	0.033718	0.01122	0.011365	0.03086	0.023893	0.01865	0.014826	0.035307	0.024341	0.009697	0.011542
20	Vector-S-4-1	0.013129	0.038481	0.022089	0.018211	0.030096	0.024744	0.018186	0.017369	0.031984	0.032003	0.01382	0.017398
21	Vector-S-4-2	0.013518	0.038877	0.022184	0.018933	0.03036	0.024571	0.019218	0.017514	0.033255	0.032467	0.013752	0.017101
22	Vector-S-5-1	0.009952	0.031597	0.133707	0.041375	0.024672	0.026986	0.047665	0.085231	0.034307	0.134564	0.013857	0.15267
23	Vector-S-5-2	0.010109	0.032802	0.134788	0.041901	0.0247	0.026834	0.048216	0.085907	0.033388	0.135475	0.011914	0.153172

Broeckling et al

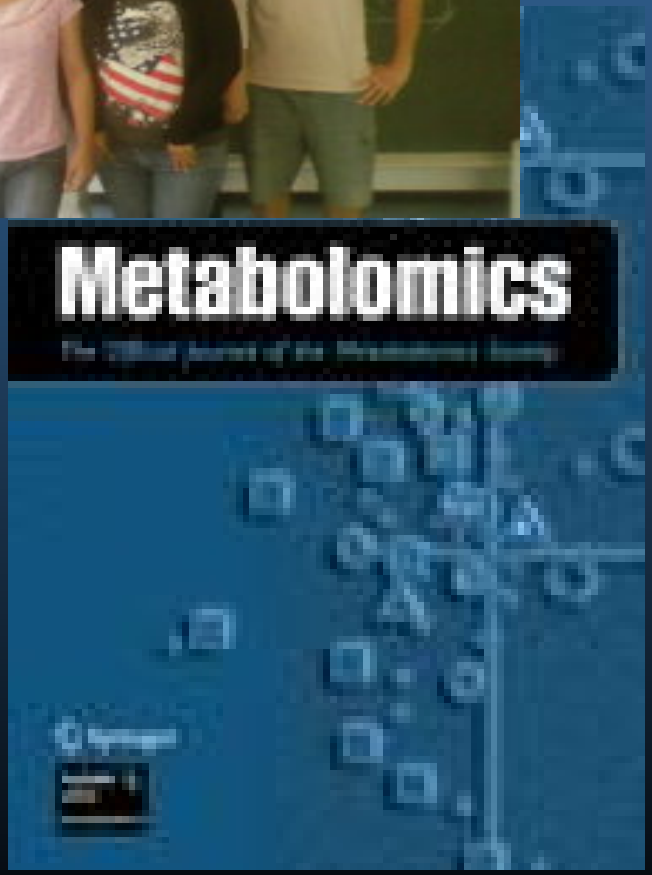
Key aspects in metabolomics: multivariate data analysis





Metabolomics: New Applications, New Science

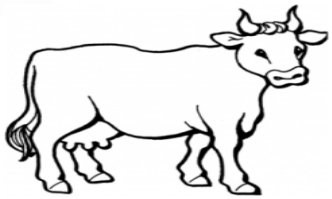
Metabolomics 2007 meeting, Boston



Metabolomics , a multidisciplinary science to bridge different fields

Metabolomics and Food Security Problems

Food comes from plants: leaves, fruits, shoots, roots and seeds



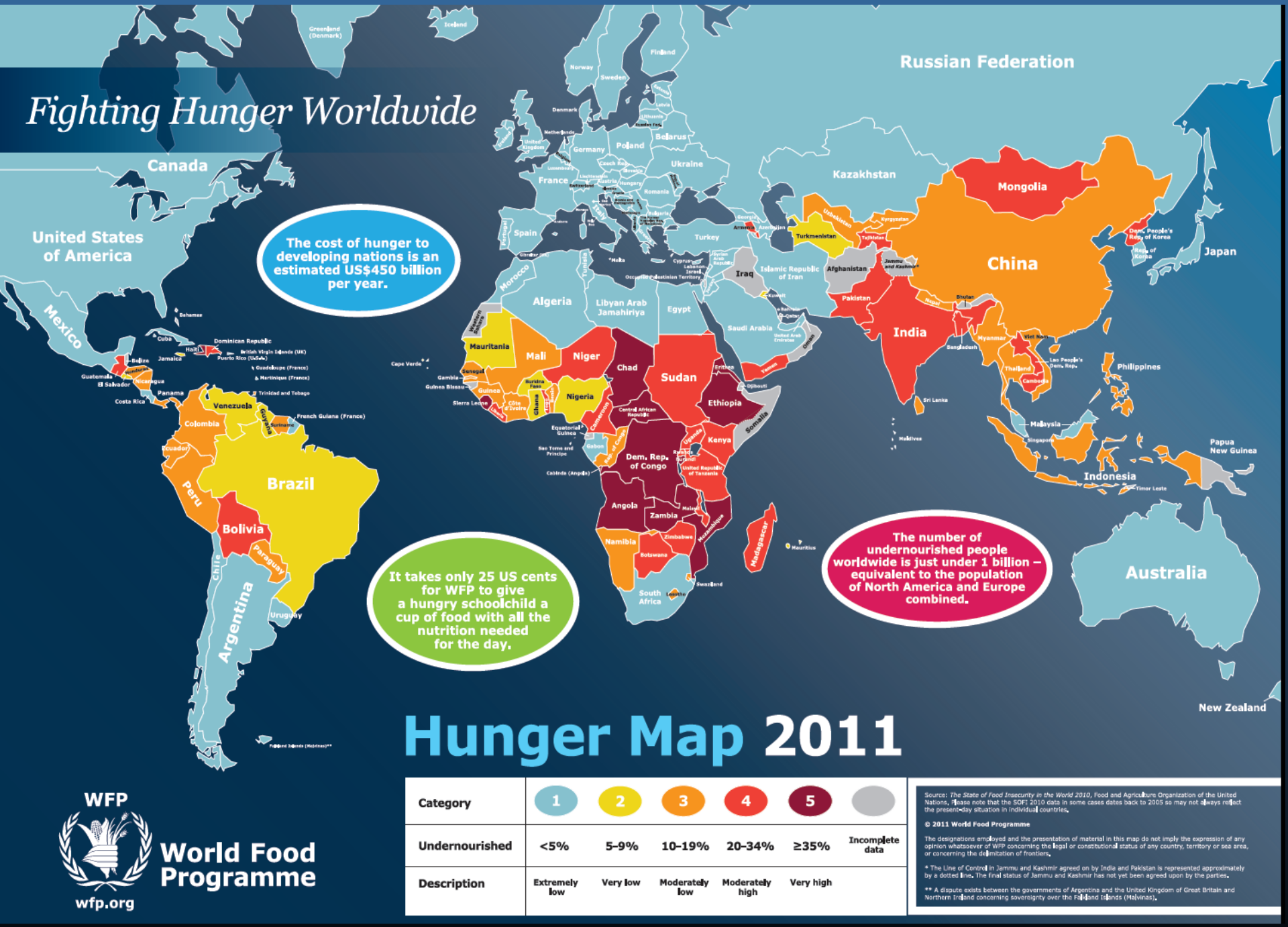
- Macronutrients – contain energy (calories)
  - Carbohydrates, fats and proteins
- Micronutrients Vitamins, trace minerals
- Phytonutrients Protective functions

Plants are surrounded with many enemies with a common goal

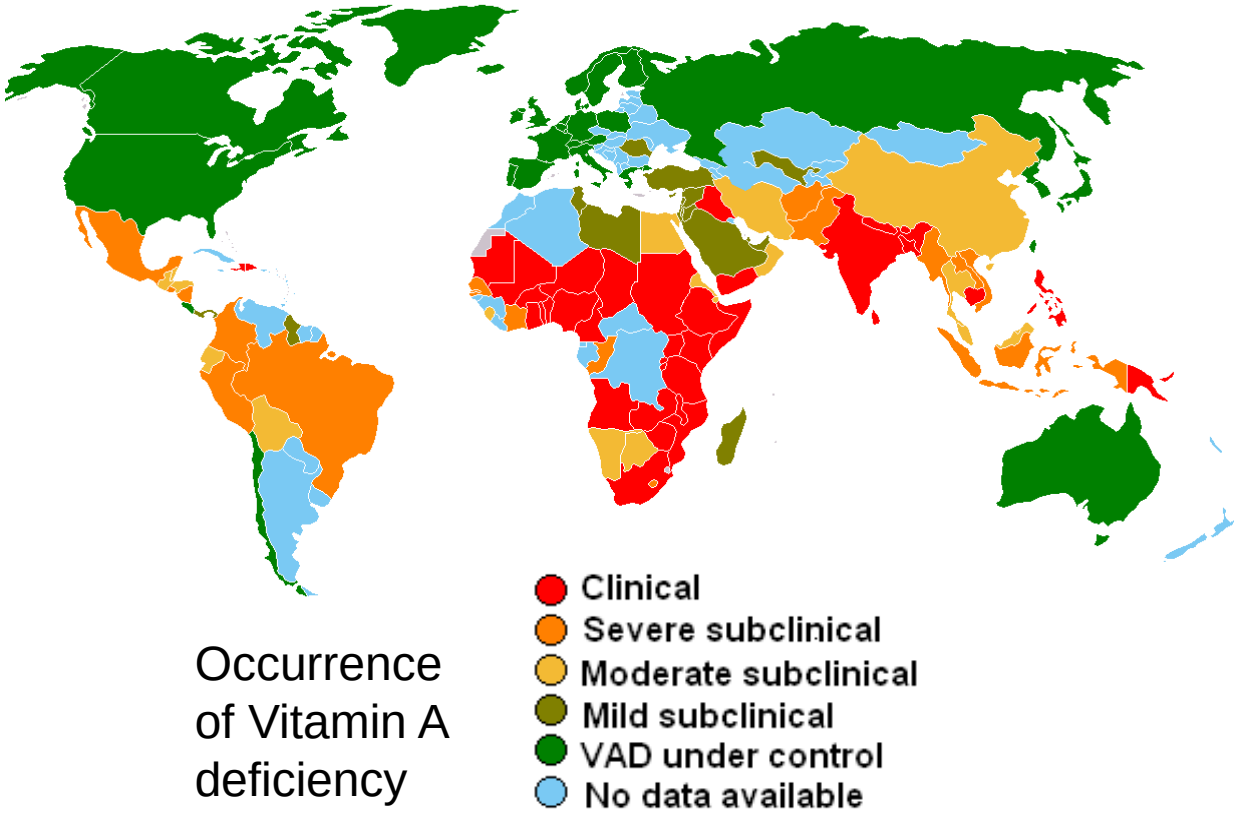
... to “eat” them!

Jim Plaskowitz, USDA, and Stan Diffie, University of Georgia

We as humans are the biggest threat to plants


Fighting Hunger Worldwide
The cost of hunger to developing nations is an estimated US$450 billion per year.
It takes only 25 US cents for WFP to give a hungry schoolchild a cup of food with all the nutrition needed for the day.
The number of undernourished people worldwide is just under 1 billion – equivalent to the population of North America and Europe combined.
Hunger Map 2011
Category
1
2
3
4
5
Undernourished
<5%
5-9%
10-19%
20-34%
≥35%
Incomplete data
Description
Extremely low
Very low
Moderately low
Moderately high
Very high
WFP
World Food Programme
wfp.org
Source: The State of Food Insecurity in the World 2010, Food and Agriculture Organization of the United Nations, Please note that the SOFI 2010 data in some cases dates back to 2005 so may not always reflect the present-day situation in individual countries.
© 2011 World Food Programme
The designations employed and the presentation of material in this map do not imply the expression of any opinion whatsoever of WFP concerning the legal or constitutional status of any country, territory or sea area, or concerning the delimitation of frontiers.
* The Line of Control in Jammu and Kashmir agreed on by India and Pakistan is represented approximately by a dotted line. The final status of Jammu and Kashmir has not yet been agreed upon by the parties.
** A dispute exists between the governments of Argentina and the United Kingdom of Great Britain and Northern Ireland concerning sovereignty over the Falkland Islands (Malvinas).
www.wfp.org

Not Only are we Limited in Food Supply



Occurrence of Vitamin A deficiency (VAD)

100 million children are Vitamin A deficient

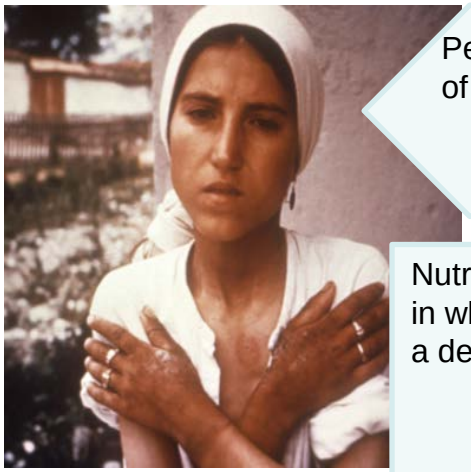
Up to 500,000 children become blind every year and half of these die within 12 months of losing their sight

Improving dietary intake of carotenes can reduce child mortality by 25 %

WHO data

Vitamin A deficiency is a leading cause of blindness

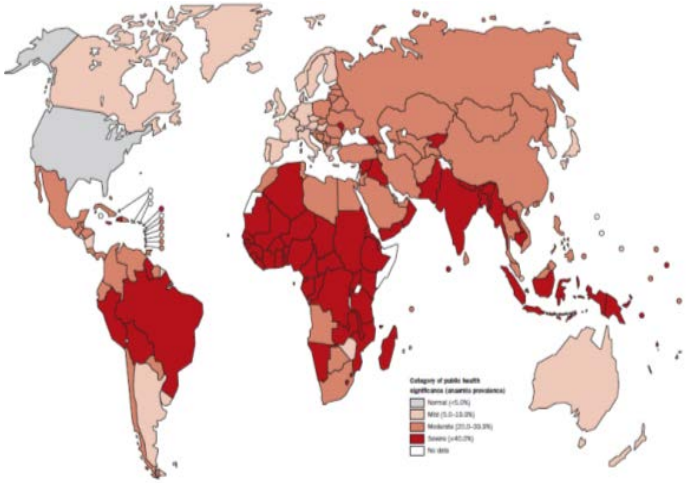
Many people suffer deficiencies of other vitamins and minerals



Pellagra is a deficiency of niacin (vitamin B3)

Nutritional Rickets is a condition in which bones are too soft due to a deficiency of vitamin D

Inflamed, scorbutic gums caused by vitamin C deficiency



Anemia caused by iron deficiency affects half of all pre-school aged children

How can Plant scientists contribute to the alleviation of hunger & malnutrition???

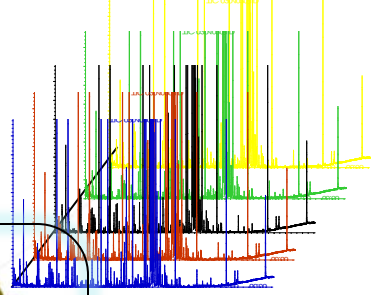
Genetic Engineering of Plants for increased nutrient content

Iron-biofortified rice

Photo credit: Neil Palmer (CIAT)

Today, biotechnology is being used as a tool to give plants new traits that benefit agricultural production, the environment, and human nutrition and health.

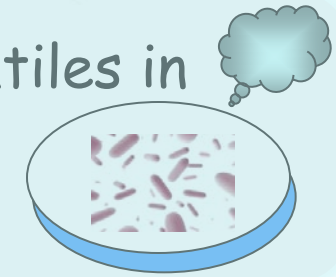
Metabolomics: New Applications, New Science



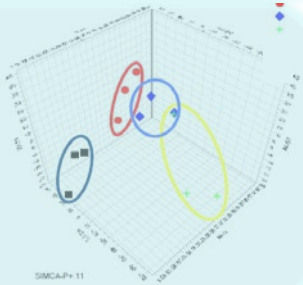
1-Functional genomics of phytonutrients in legumes

How do legumes produce isoflavones?

2-Metabolic Profiling of Volatiles in Soil Bacteria PGPR



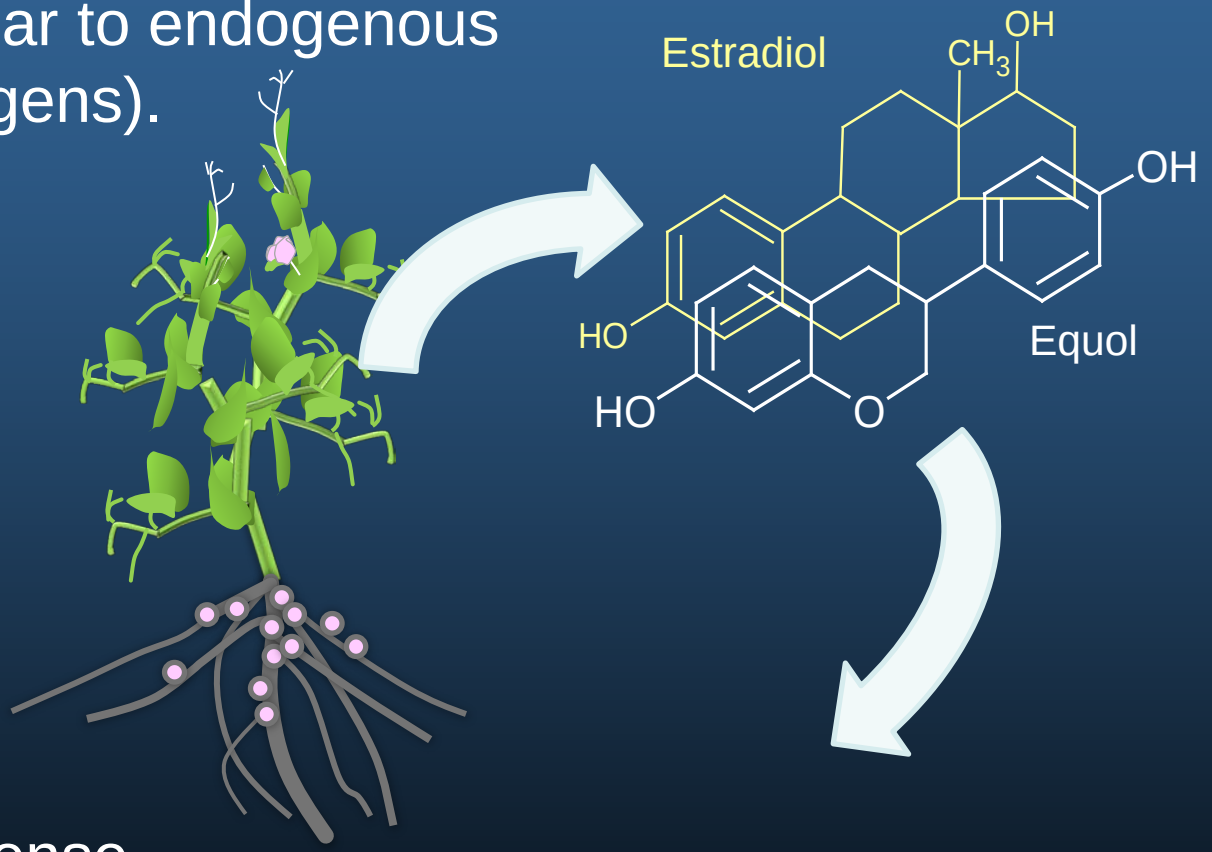
3-Metabolomics in quality control analysis of phytomedicines



4-Metabolomics in biomarker discovery and disease diagnosis

What Are Isoflavones

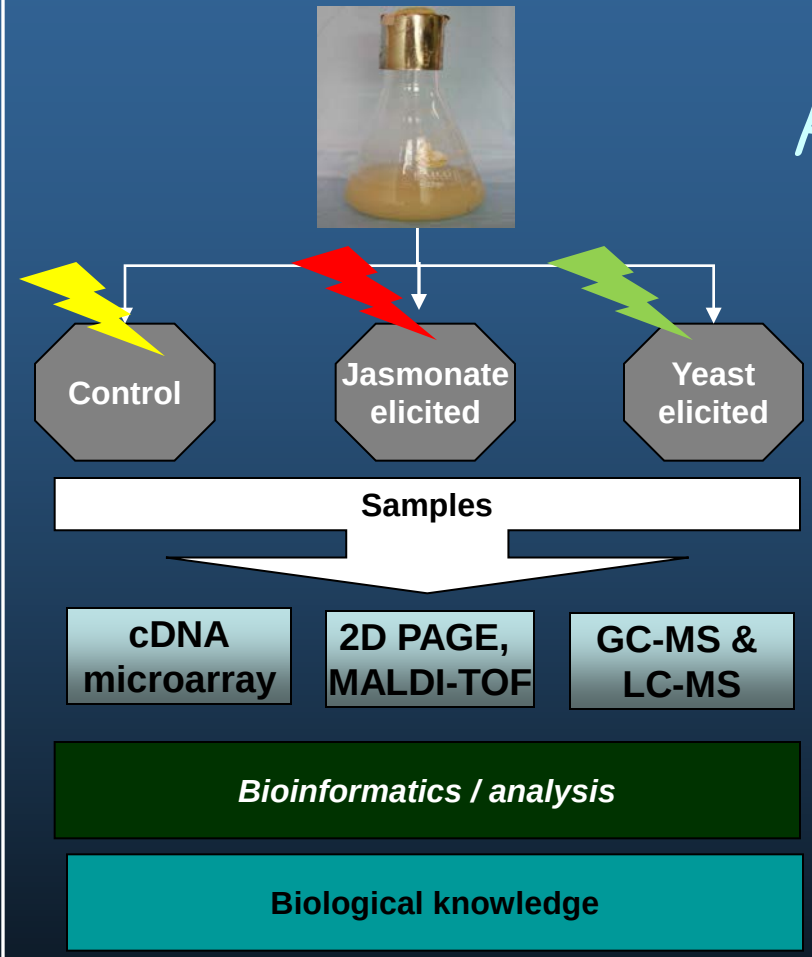
Bioactive phytochemicals found in legumes, have a structure similar to endogenous estrogen (phytoestrogens).



Function in plant defense responses & in legumes symbiosis relationship (nod gene inducer).

Farag et al., PNAS 2007

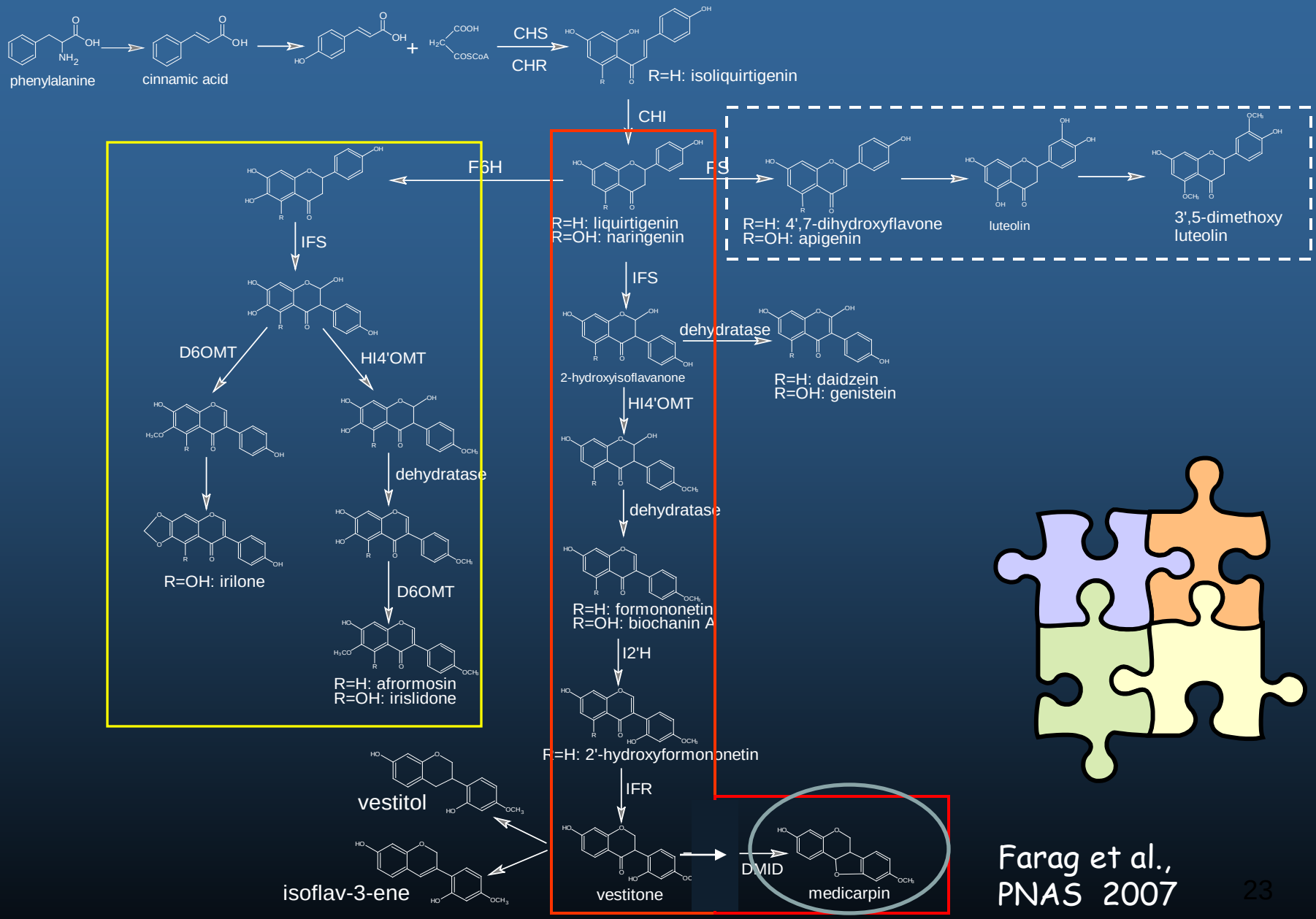
*An integrated approach to functional genomics in a model legume (**NSF-PGRP #0109732**)*



Profiling of phenolics using LC/MS

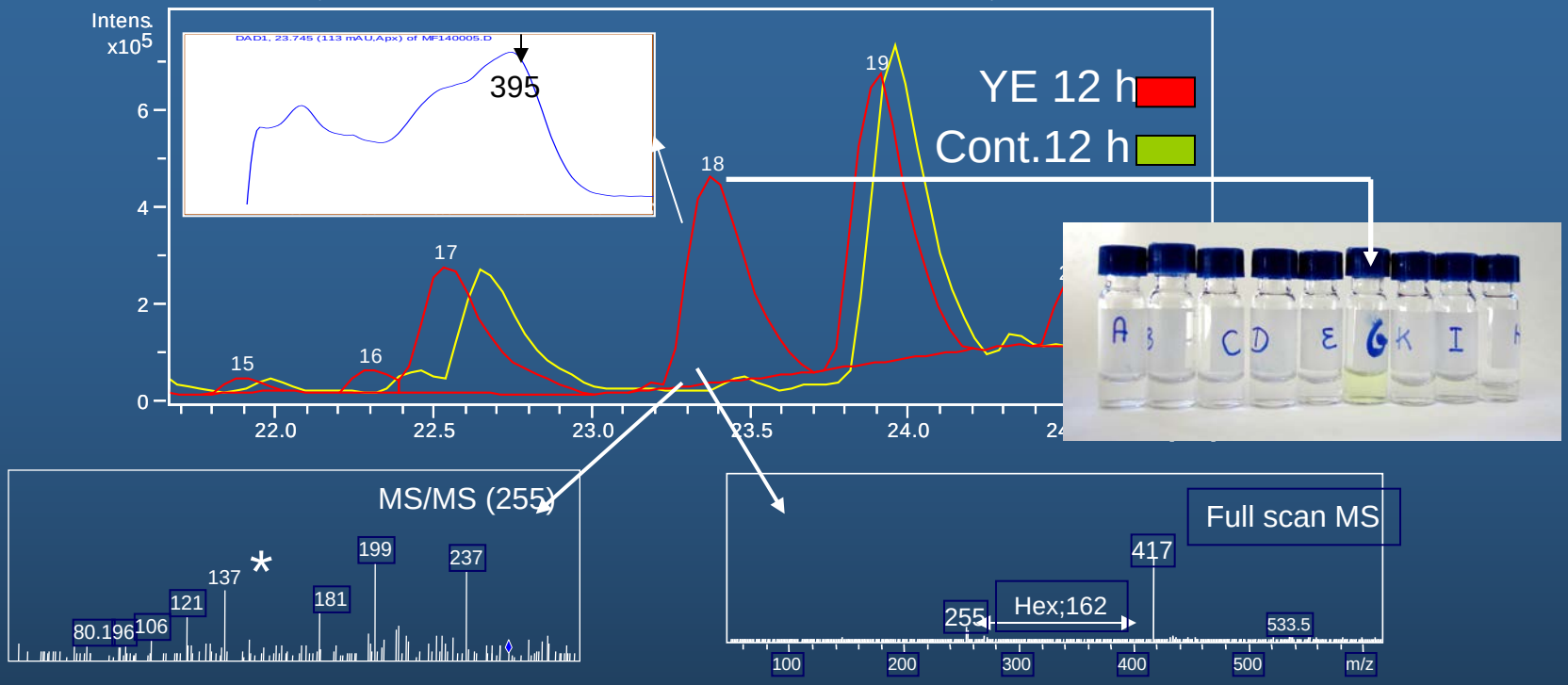
- ✓ Construct a biosynthetic pathway for isoflavonoids
- ✓ Novel Natural product discovery

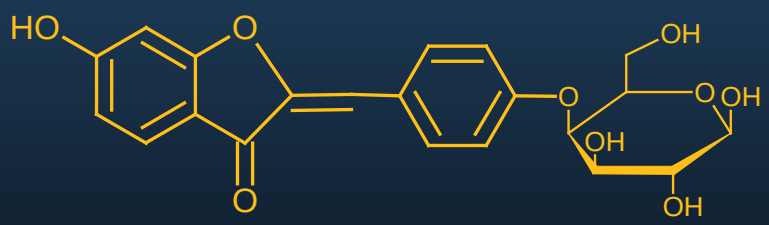
Constructing an Isoflavonoid Biosynthesis Pathway



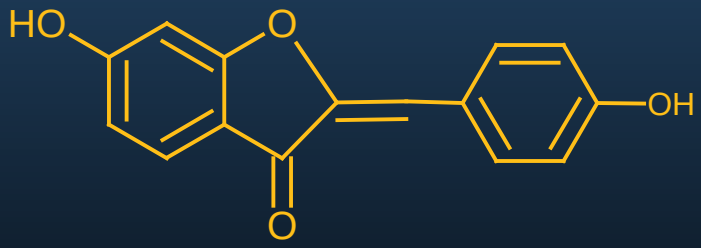
Farag et al., PNAS 2007

A Novel Phytoalexin induced in Response to Yeast





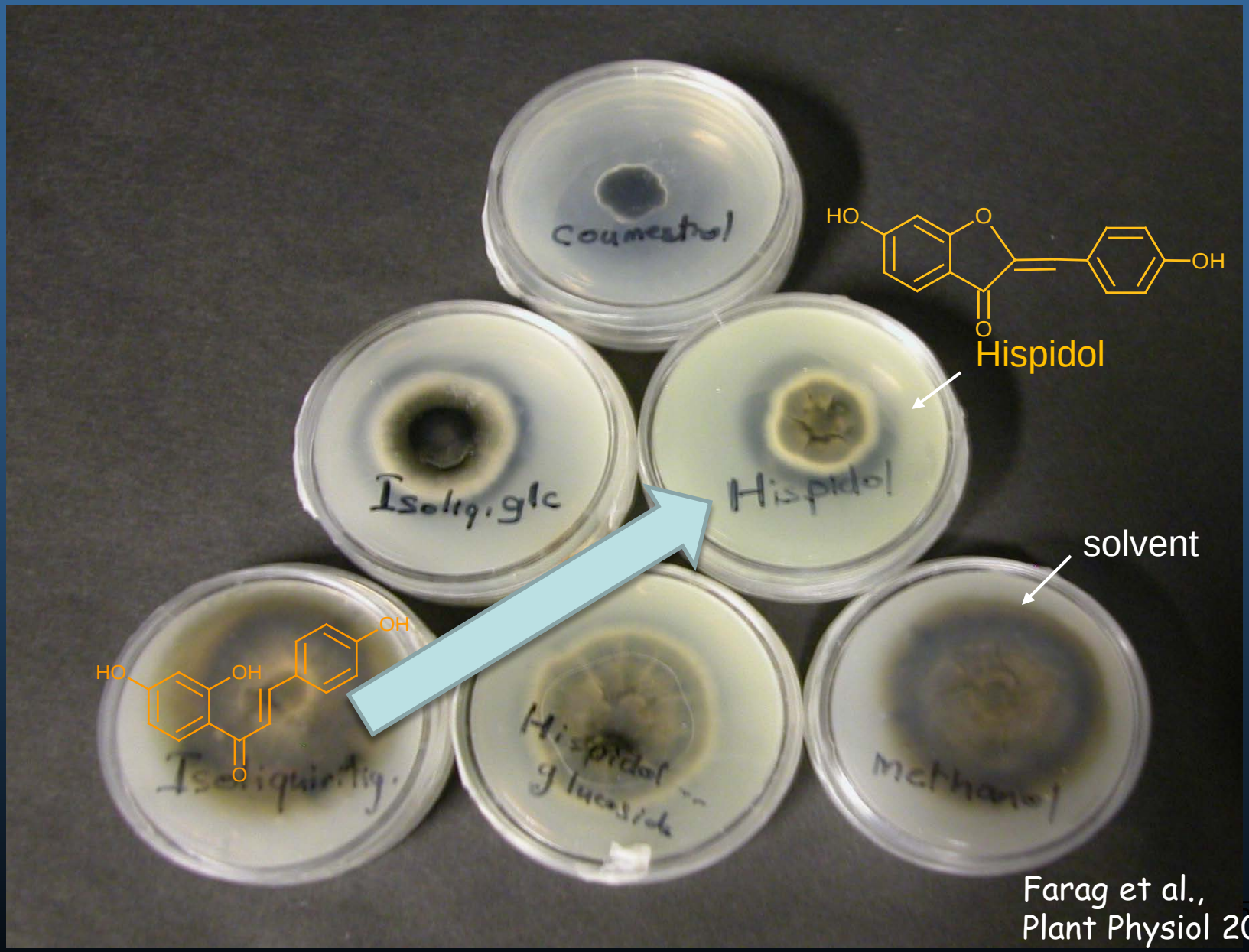
4', 6-Dihydroxy aurone (Hispidol glucoside)



4', 6-Dihydroxy aurone (Hispidol)

Farag et al., Plant Physiol 2009

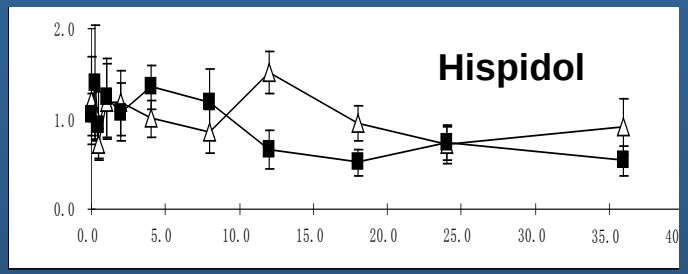
Antifungal Effect of Isoflavonoids in Cell culture

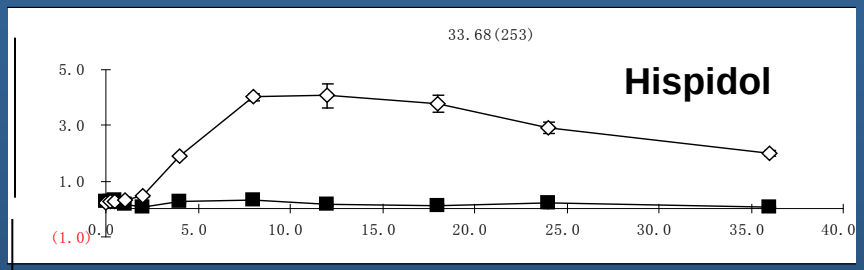


Peroxidase genes expression coincides with hispidol biosynthesis

MeJA (Inhibition)

YE (induction)



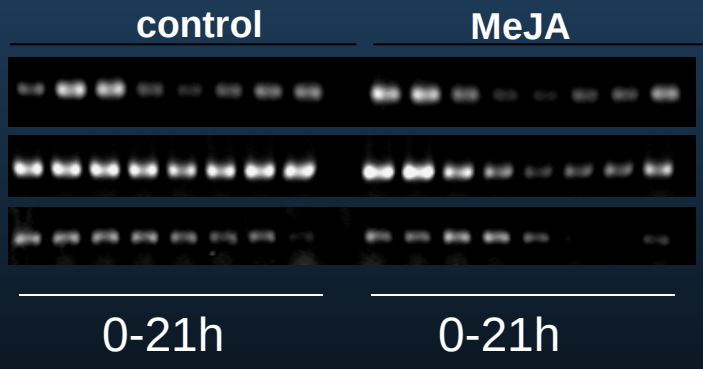


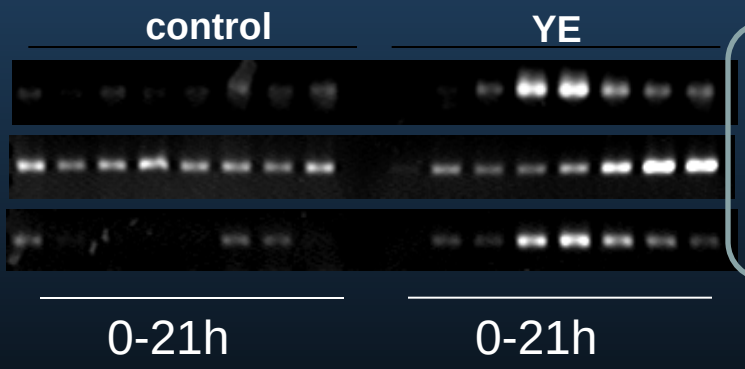
Time (h)

Guideline for fishing candidate genes?

Look for peroxidase(s) induced with YE but not with MeJA

AAAA
AAAA
AAAA

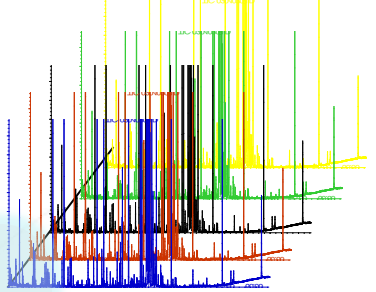




TC56738
TC50994
TC50982

Farag et al.,
Plant Physiol 2009

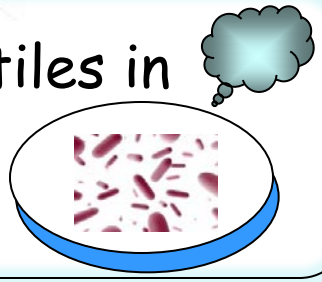
Metabolomics: New Applications, New Science



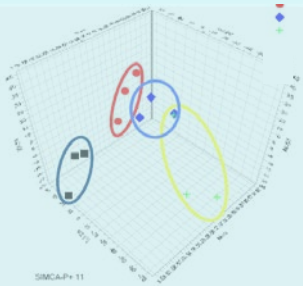
1-Functional genomics of natural products in legumes (*Medicago truncatula*)

How and Why?

2-Metabolic Profiling of Volatiles in Soil Bacteria **PGPR**

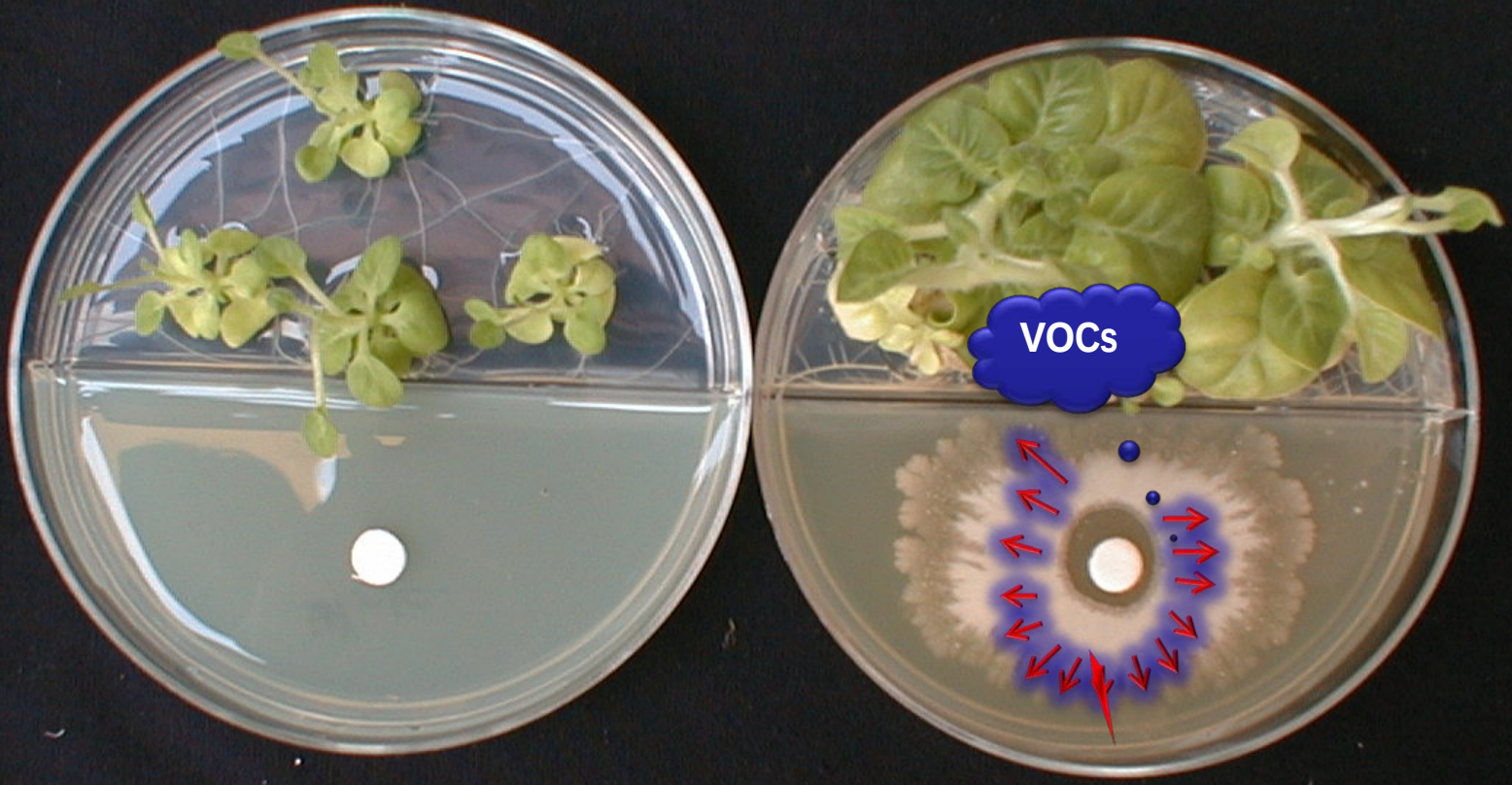


3-Metabolomics in quality control analysis of phytomedicines



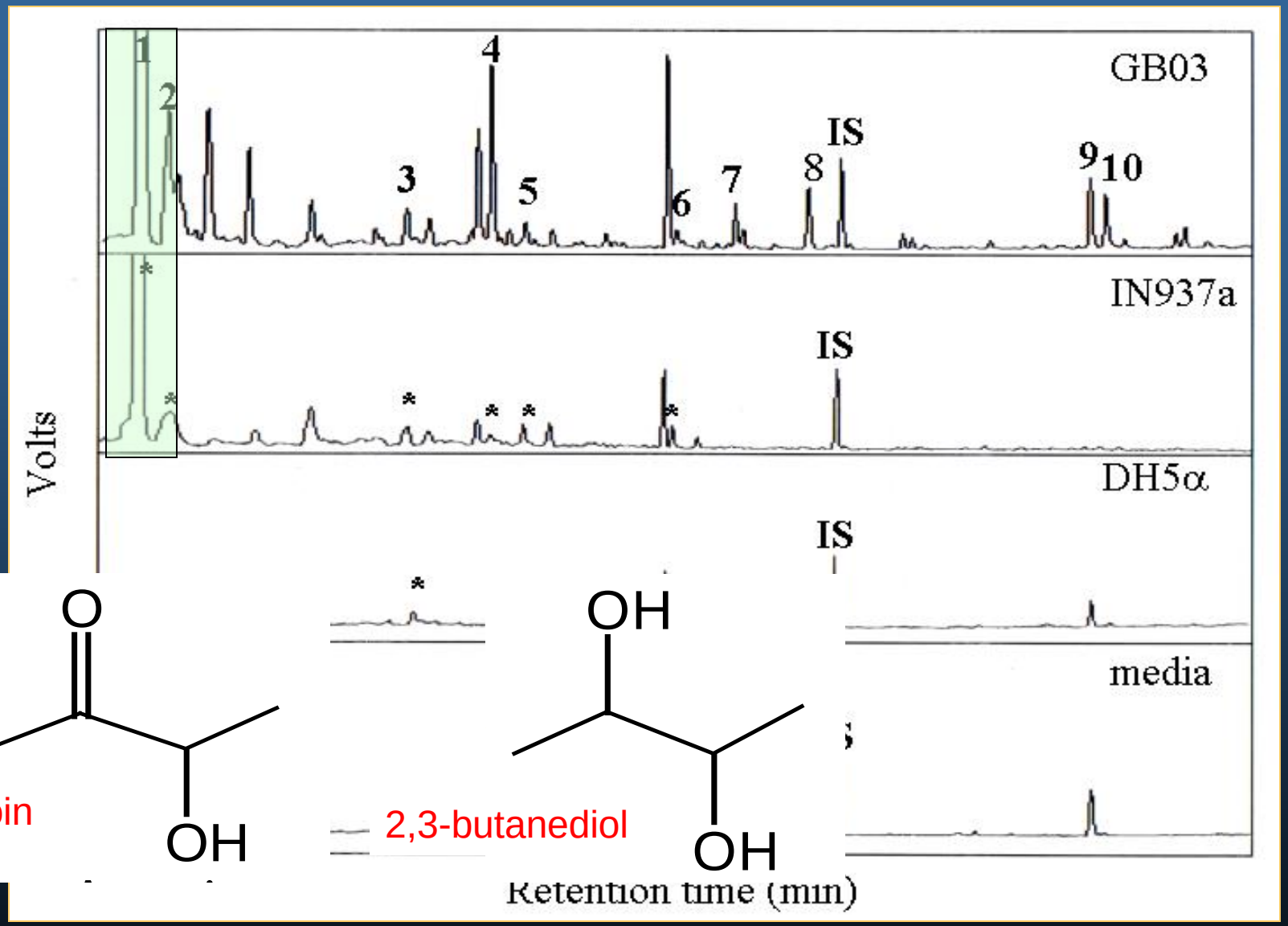
4-Metabolomics in biomarker discovery and disease diagnosis

2. Metabolic Profiling of Volatiles in Soil Bacteria



Growth promotion by volatiles produced by PGPR

Chromatographic profiles of head space volatiles from GB03, IN937a and DH5a

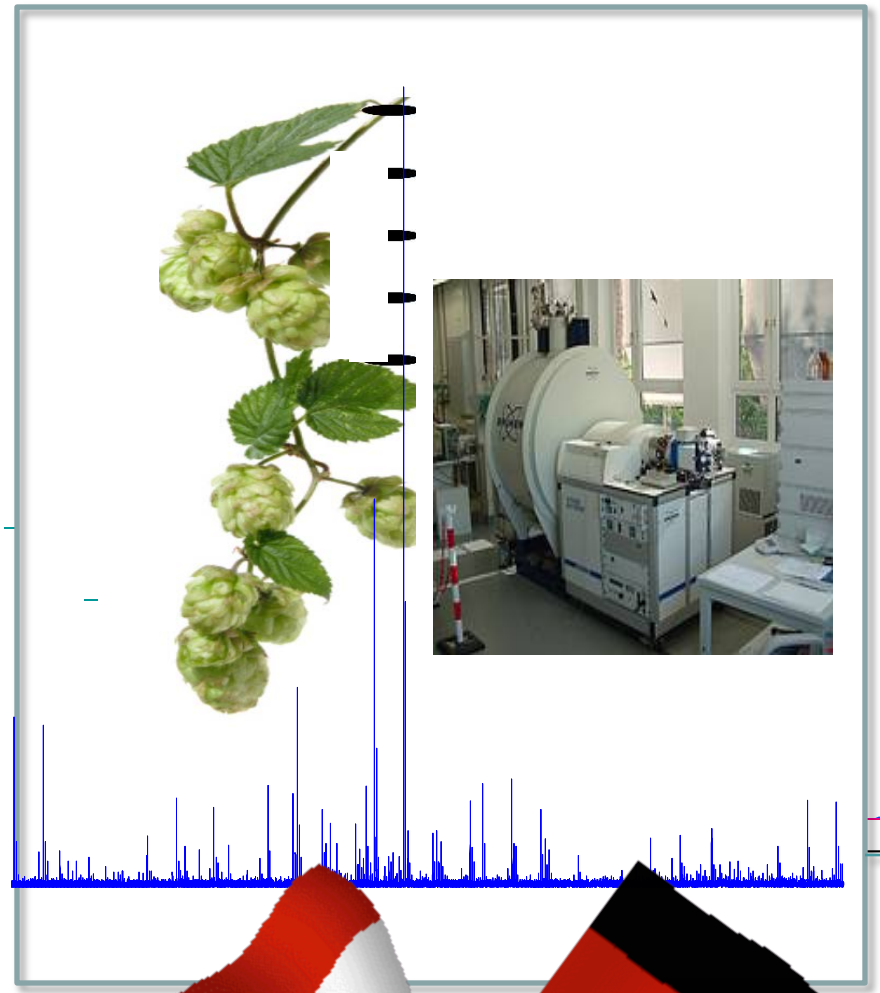


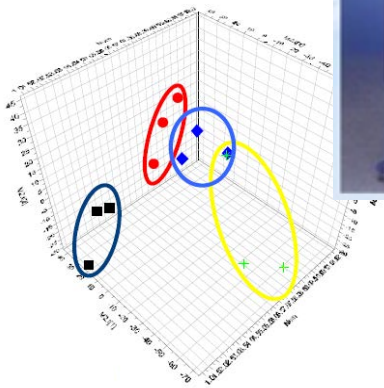
acetoin

2,3-butanediol

Brain Drainage case !!!!

4-Metabolomics as a tool for the quality control of phytomedicines



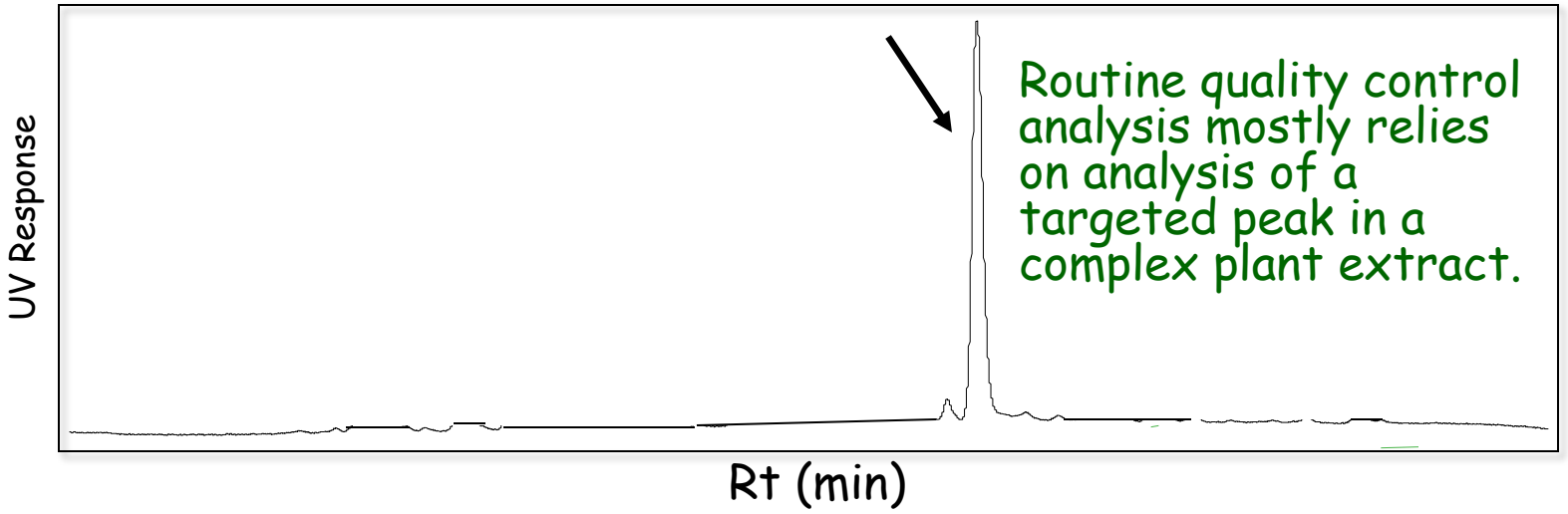


Alexander von Humboldt
Stiftung/Foundation

Quality control in phytomedicines

The quality and phytochemical composition of herbs are highly variable depending on:

- Species, varieties, cultivars
- Geographical origins
- Cultivation and harvesting methods
- Post-harvesting formulation processes



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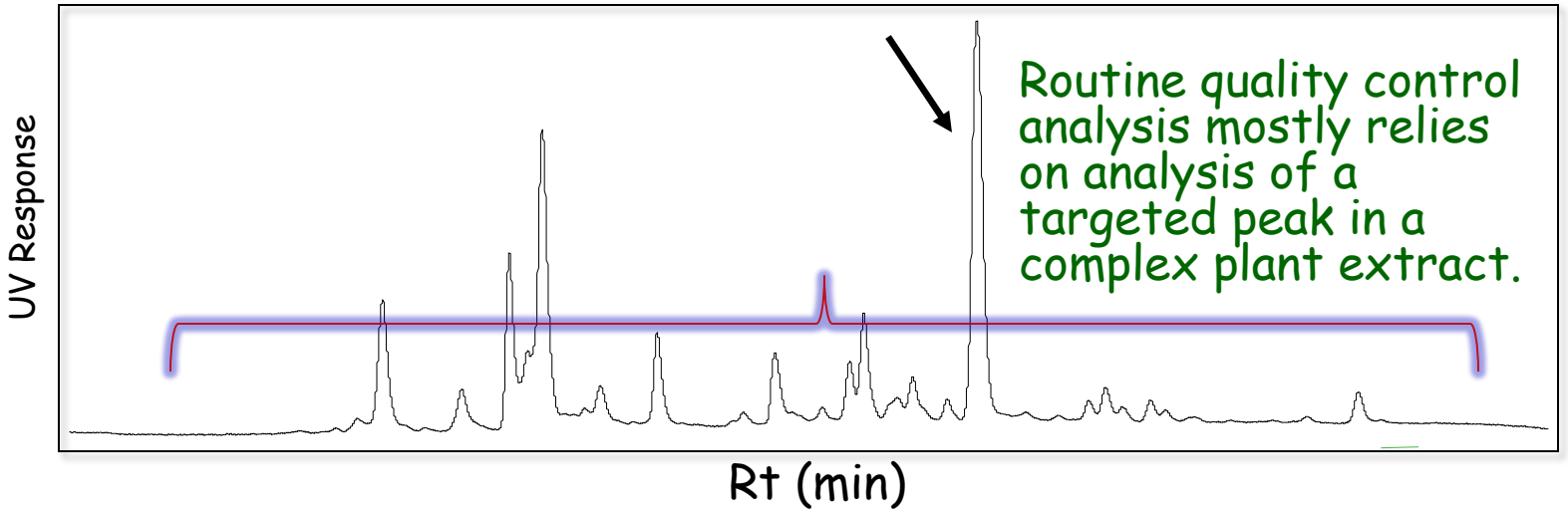
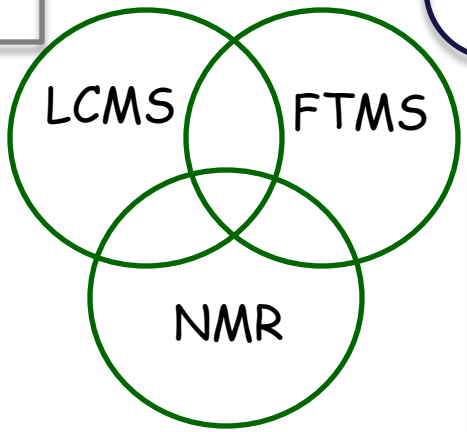
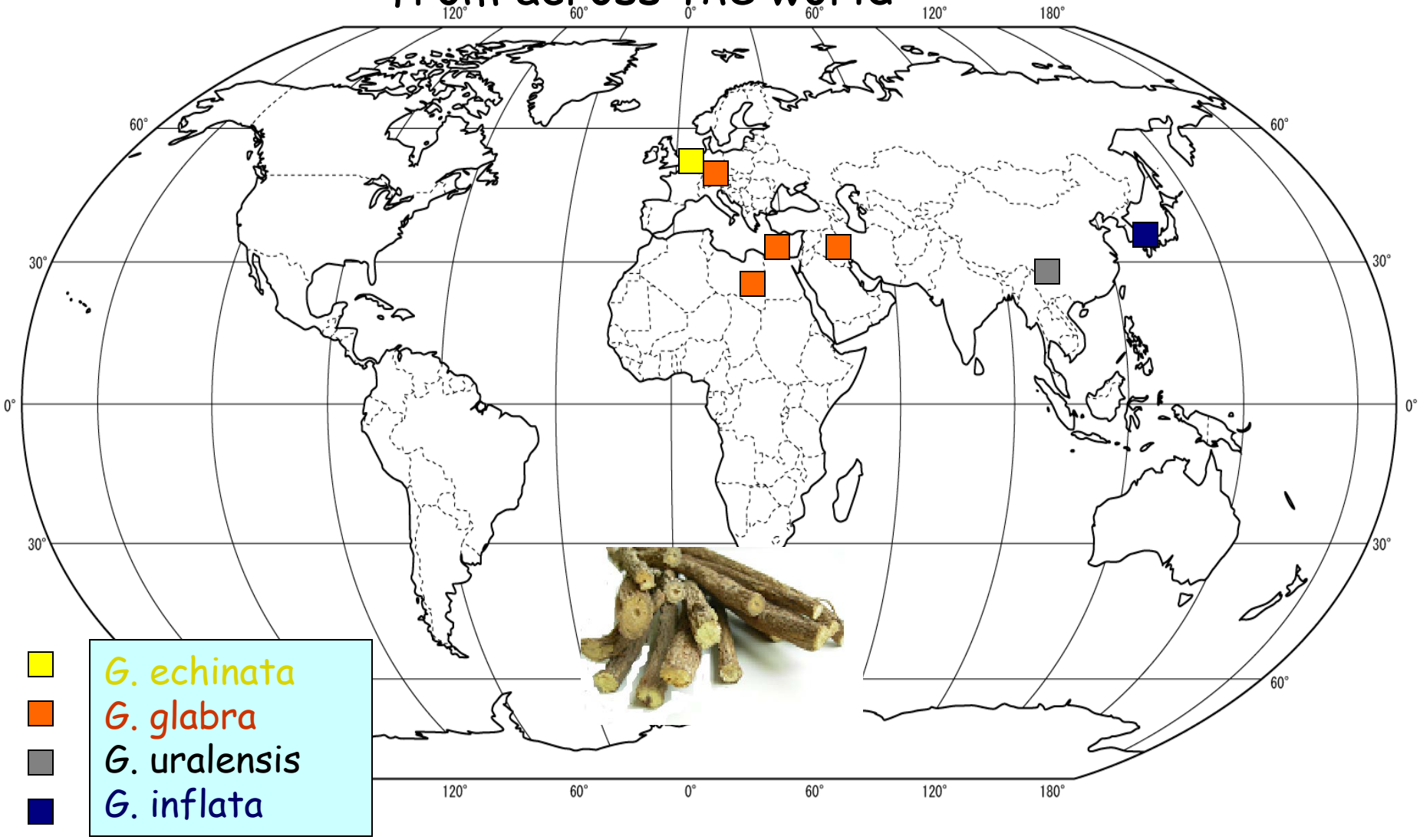


Drugs and functional foods invesitgated include

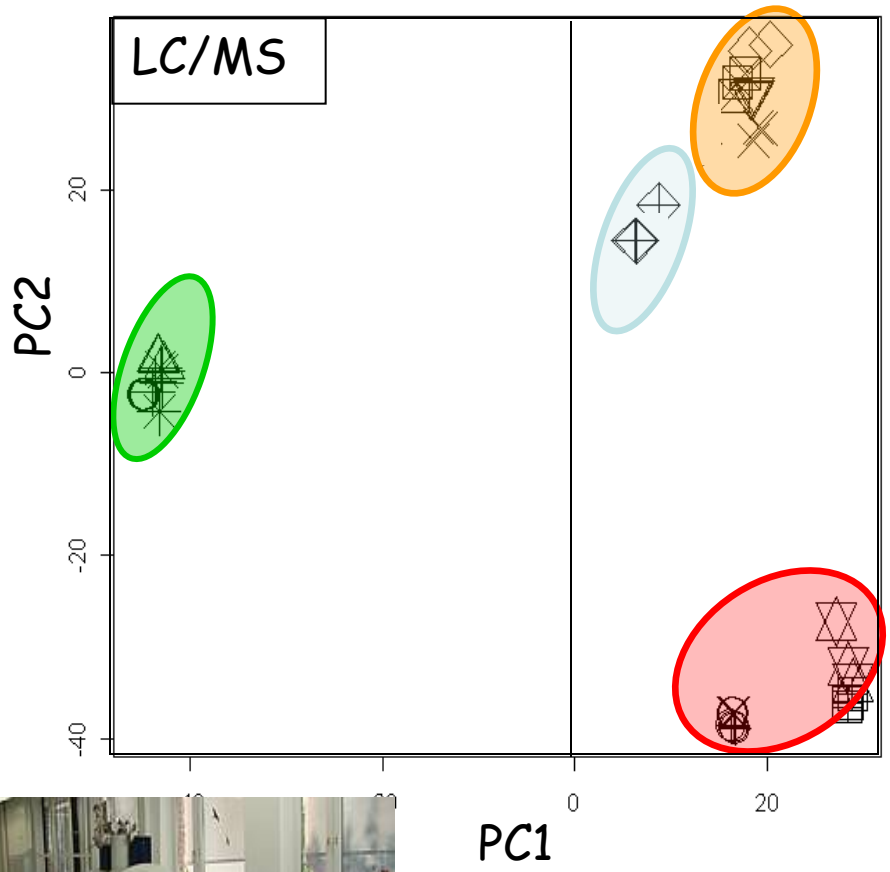


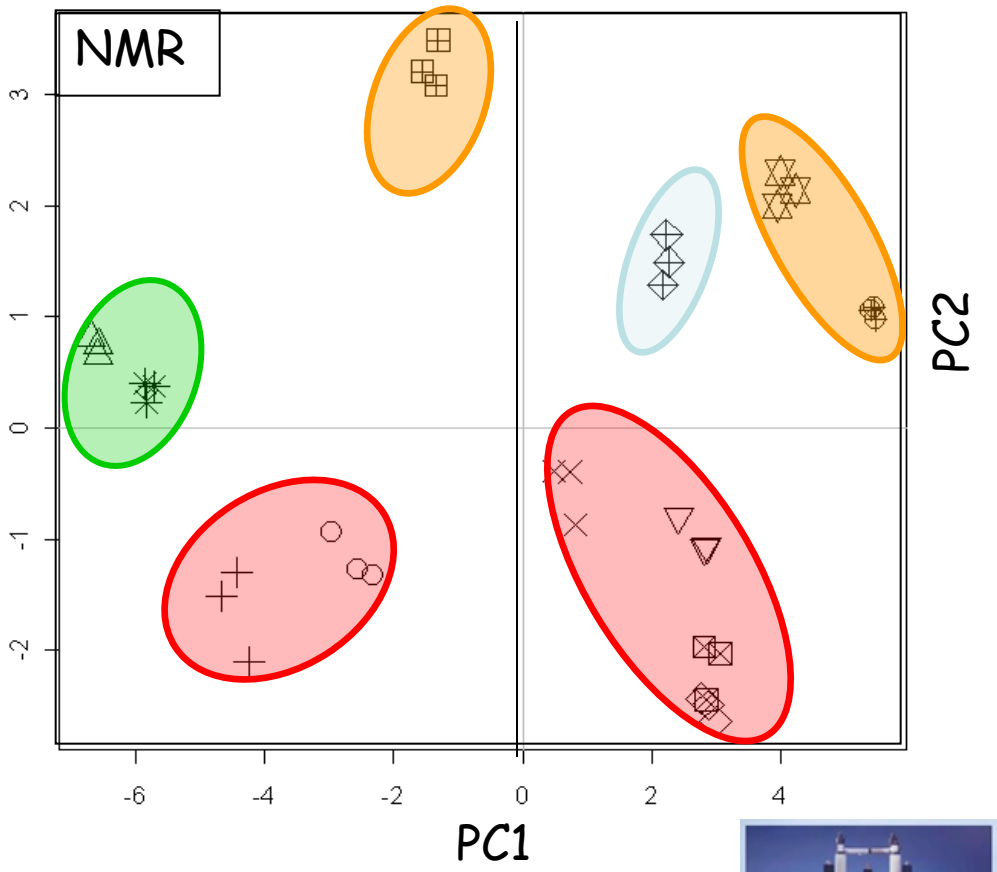
Metabolomics of *Glycyrrhiza* species (LICORICE) from across the world



G. echinata | G. glabra | G. inflata | G. uralensis

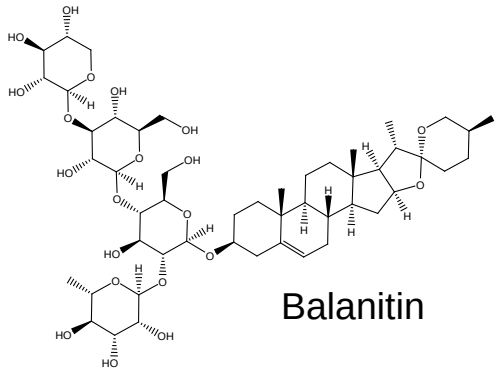
PCA scores for *Glycyrrhiza* sp. (LICORICE)



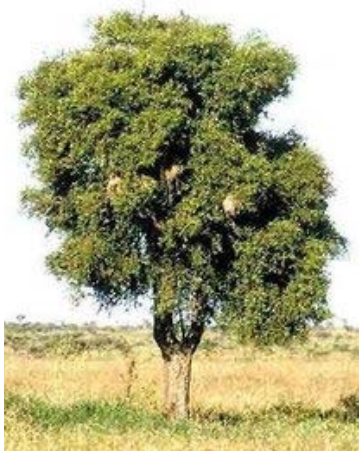


Unravelling *Balanites* date fruit antidiabetic effect.

Date shop in Aswan, Egypt

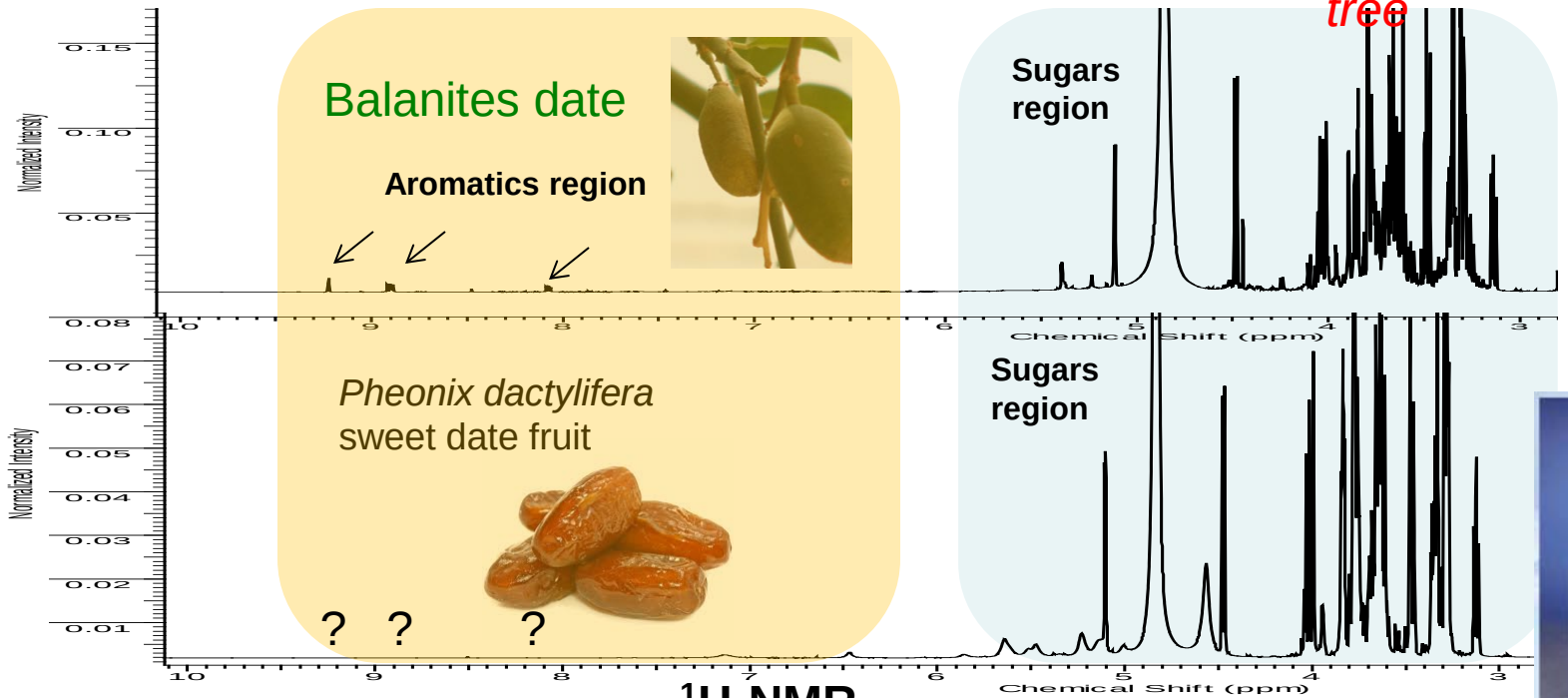


Balanitin



Balanites aegyptiaca tree

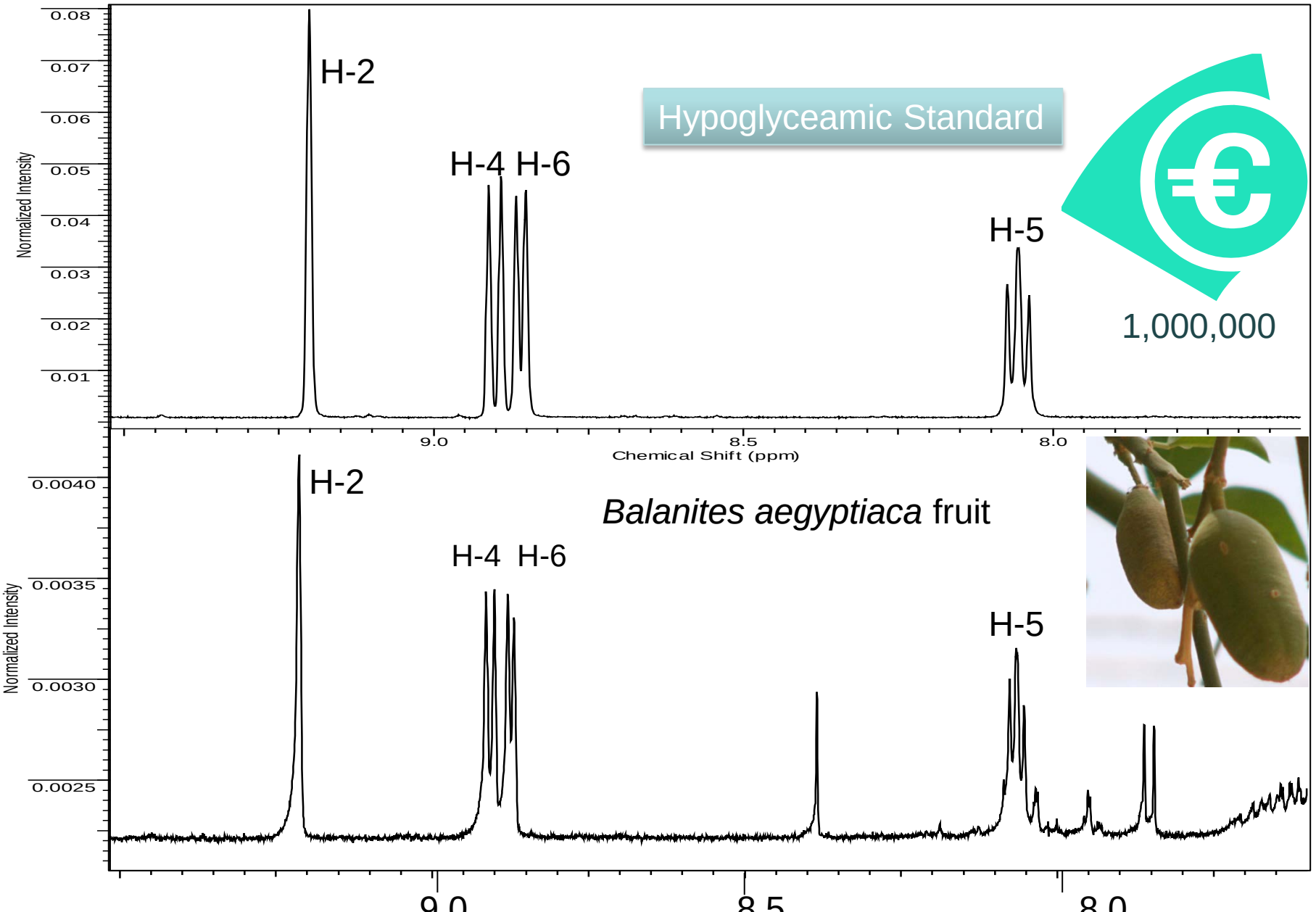
-Known as ``slave date`` due to its bitter taste, not like other date fruit.
-The fruit exhibits marked antidiabetic effect.
-Active constituents, claimed to be steroidal saponins (Balanitin)



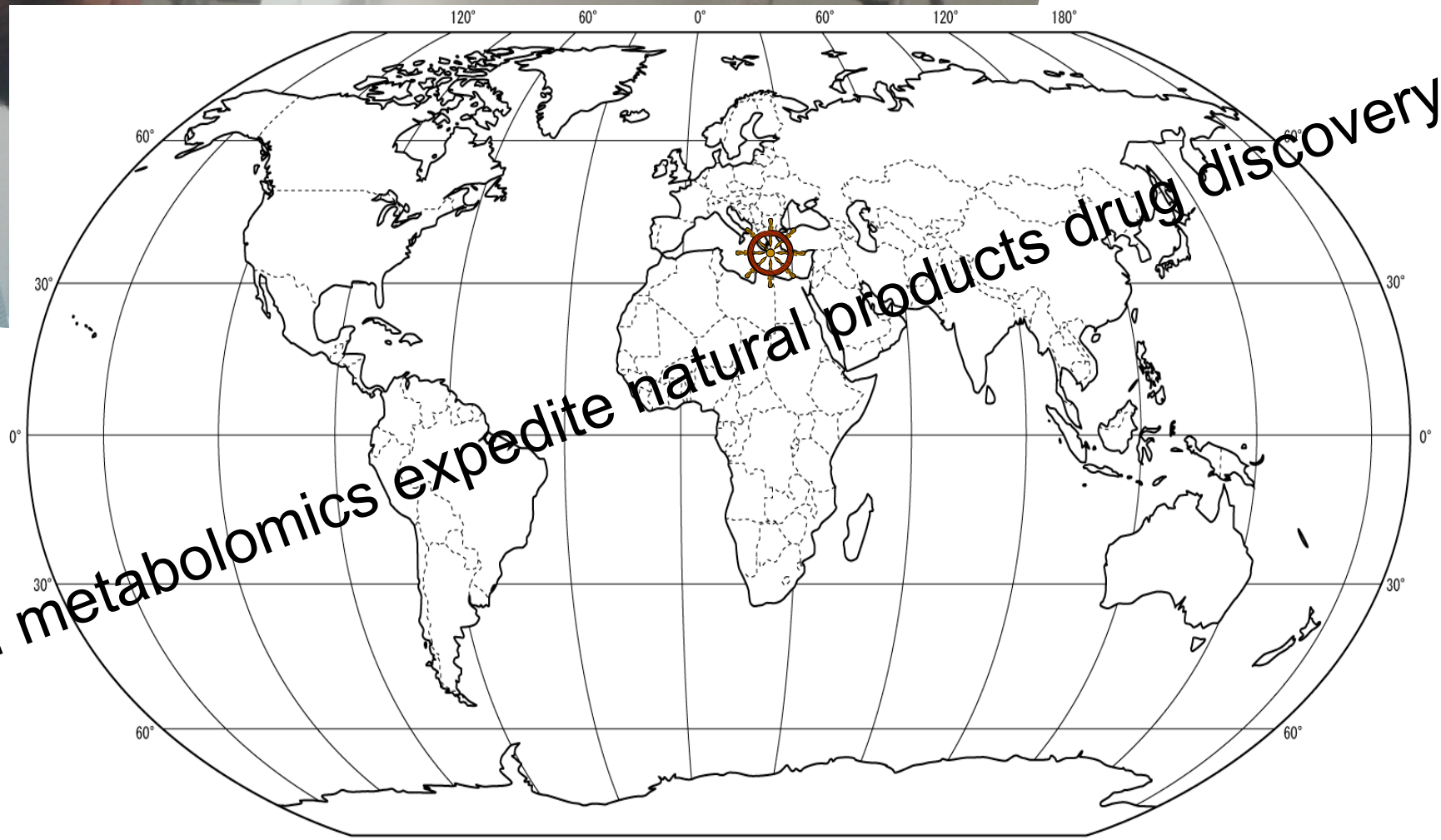
^{1}H-NMR

NMR metabolomics unravel the antidiabetic agent in balanites date fruit

^{1}H-NMR (ppm)



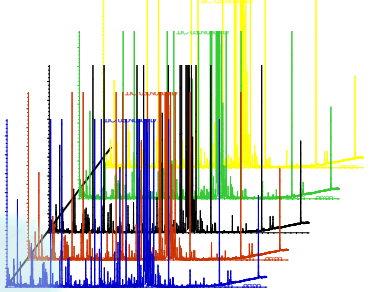

120°
60°
0°
60°
120°
180°
60°
30°
0°
30°
60°



Could metabolomics expedite natural products drug discovery

Metabolomics , a multidisciplinary science to bridge different fields

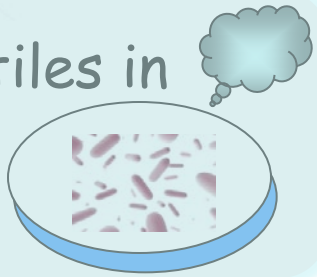
Metabolomics: New Applications, New Science



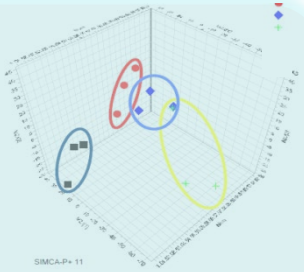
1-Functional genomics of natural products in legumes (*Medicago truncatula*)

How and Why?

2-Metabolic Profiling of Volatiles in Soil Bacteria PGPR



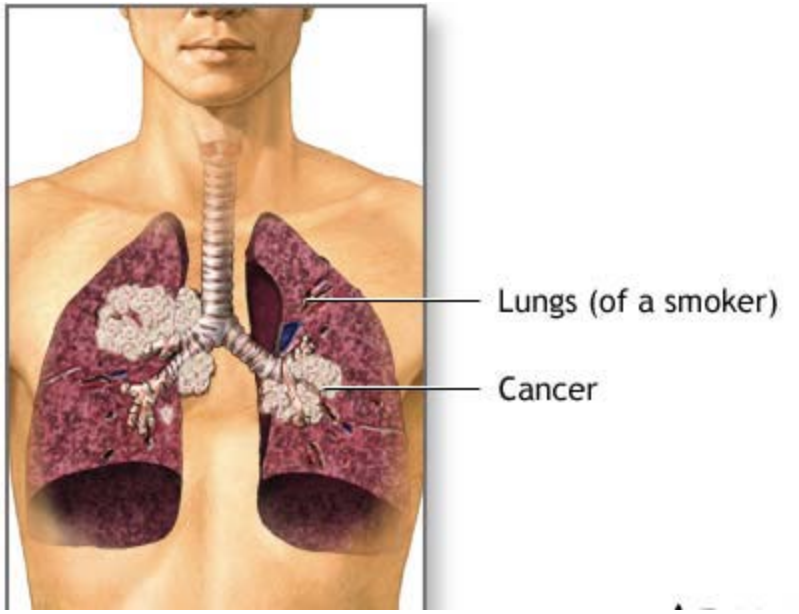
3-Metabolomics in quality control analysis of phytomedicines



4-Metabolomics in biomarker discovery and disease diagnosis

2-Isotopomer Metabolomics Approach in Lung Cancer

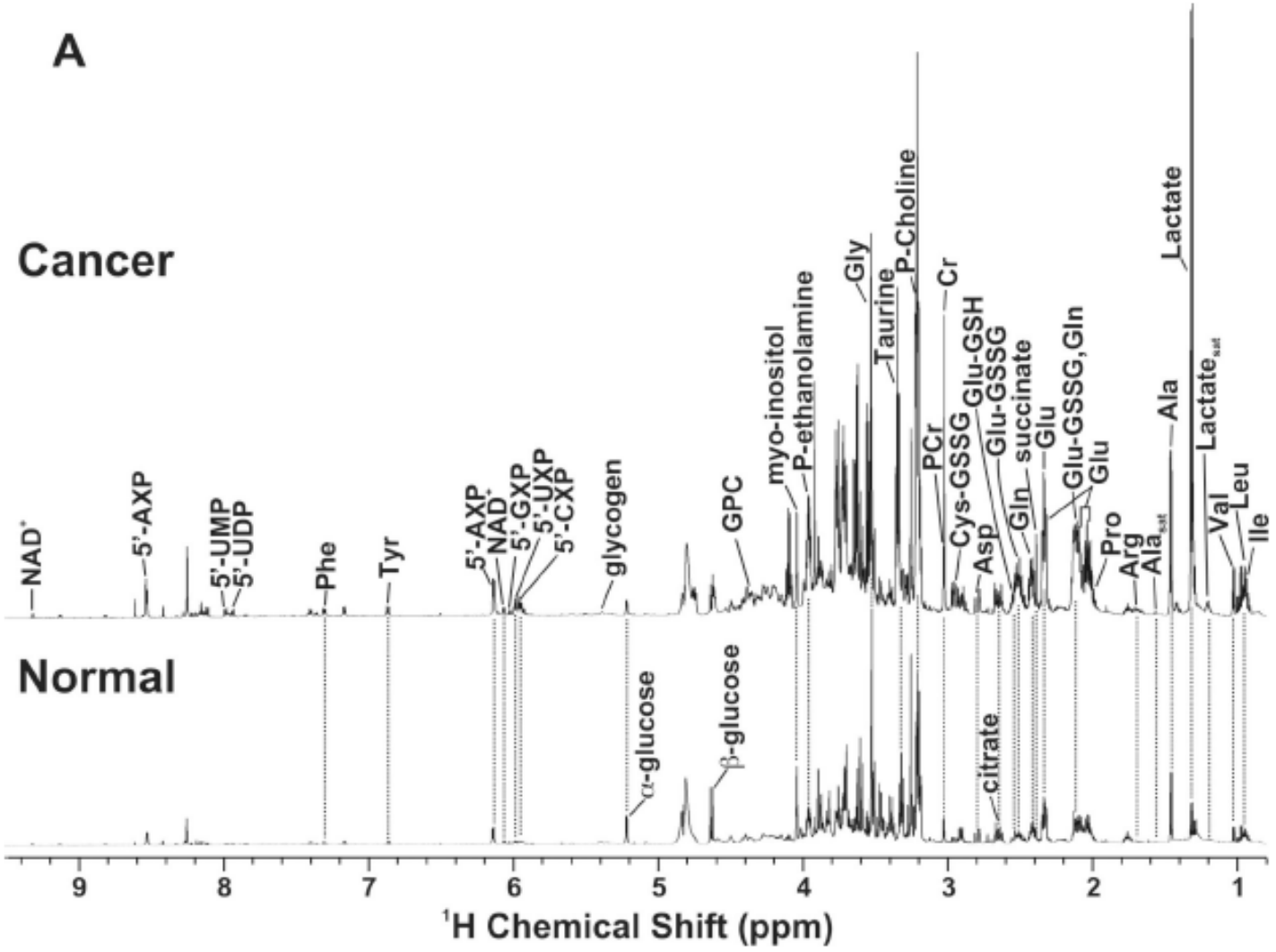
Lung cancer is currently the third most common cancer in the Western World.



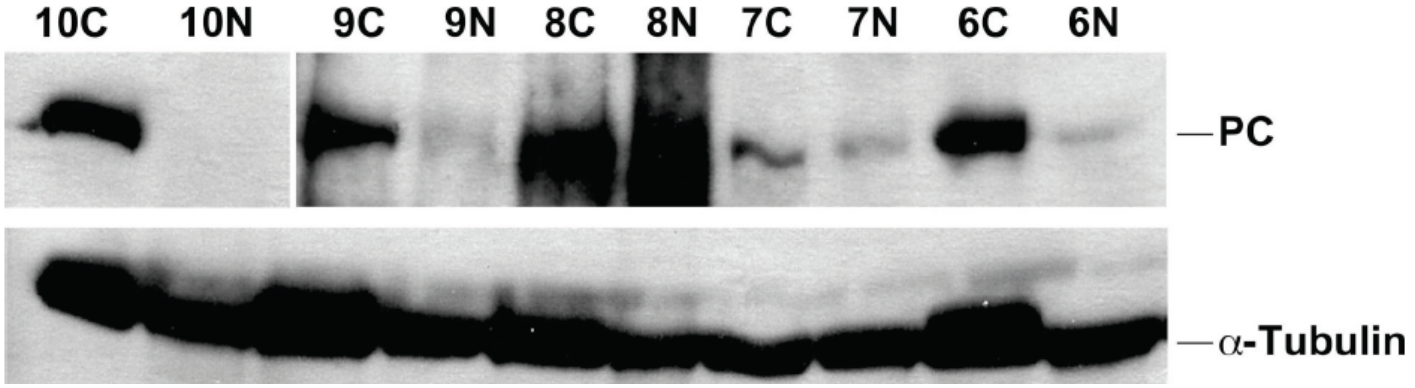
It is one of the most preventable kinds of cancer, as at least four out of five cases are associated with cigarette smoking.

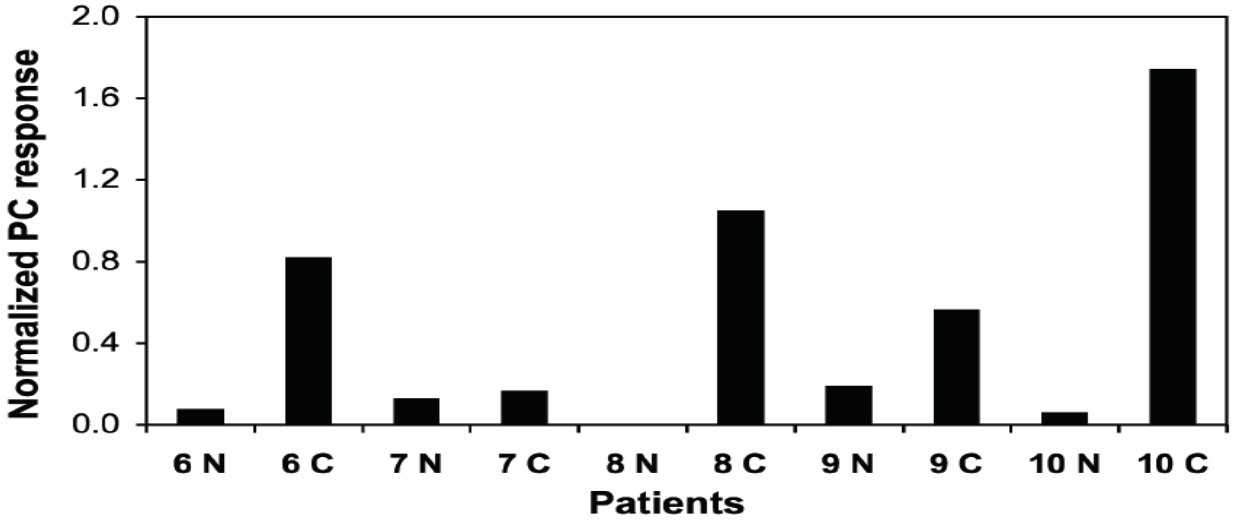
How does it start and what happens at the metabolite level??

Comparison of metabolite profiles in TCA extracts of paired normal and cancerous lung tissues using 1-D 1H NMR



Pyruvate carboxylase PC, key gene in lung cancer development



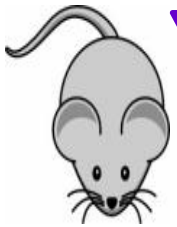


Altered Regulation of Metabolic Pathways in Human Lung Cancer Discerned by 13C Stable Isotope-Resolved Metabolomics (SIRM). *Molecular Cancer*, 2009, 8, article 41, 1-15.

Metabolomics Projects

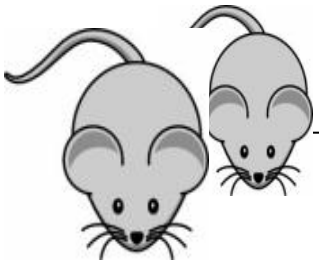
Mechanistic Evidence of Anti-Obesity Herbal Drugs, a Metabolomic Approach

Herbal drugs

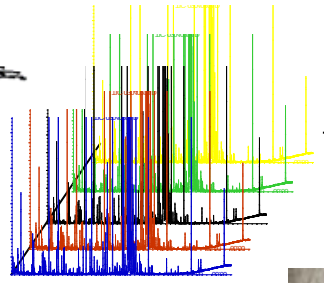


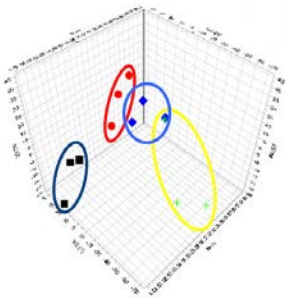
Normal

Obese

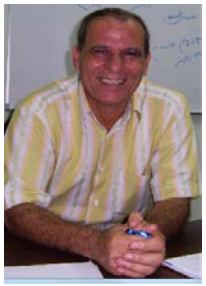


Biological fluids
i.e. serum & urine





Multivariate data analysis



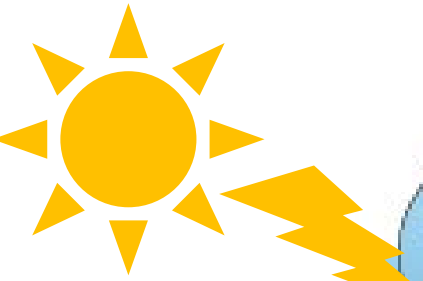
Prof. Abd el Hameed

Cairo University
Faculty of Pharmacy

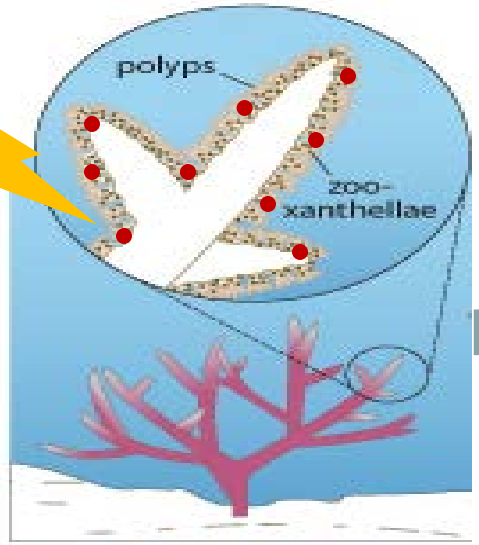
Dr. Mohamed Farag

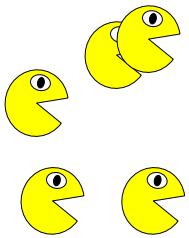
Metabolomics to Understand Coral Bleaching in Red Sea???

CORAL SECURITY

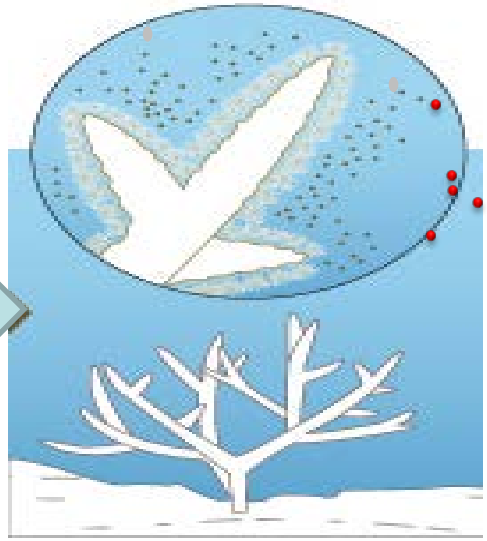


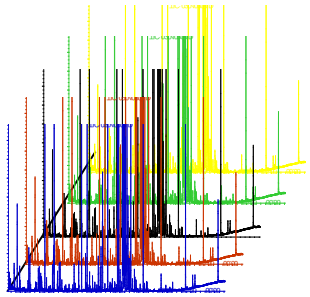
Healthy Coral



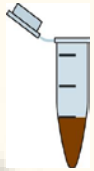


Bleached Coral

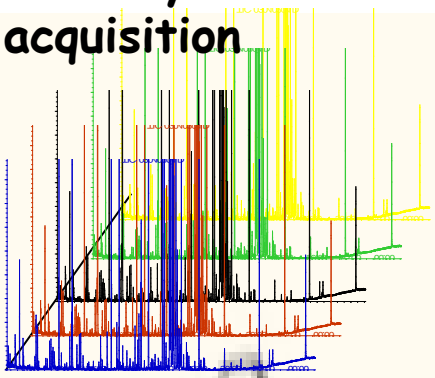


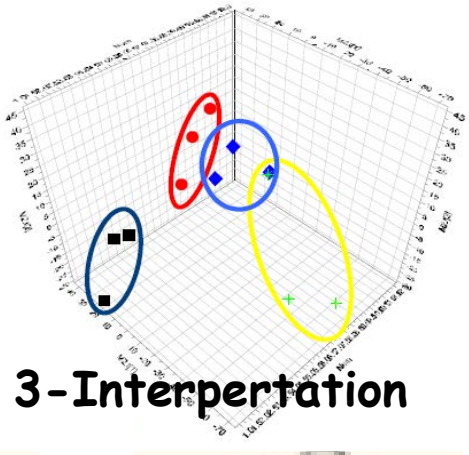


1-Sample Preparation



2-Analysis & data acquisition





3-Interpertation

Bottle necks in Metabolomics

Do not turn you back from ``Omics``

With advanced techniques and data analysis , metabolomics can INDEED open the gate to many big discoveries