



Centre for Functional Genomics Applied to Agriculture and Agroenergy



1st Workshop on Multi-User-Equipment and Facilities
04/06/2014

Luiz L. Coutinho and Carlos A. Labate
llcoutinho@usp.br and calabate@usp.br

Presenter: Mônica T. Veneziano Labate



Centre for Functional Genomics Applied to Agriculture and Agroenergy



Laboratório Multiusuários
Centralizado
Genômica Funcional Aplicada
à Agropecuária e Agroenergia

Apoio: Finep, Fapesp, USP

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Laboratório Multiusuários Centralizado

Esta iniciativa é um esforço de várias instituições de ensino e pesquisa, capitaneado pela ESALQ, para construir uma Facility nas áreas de Genômica e Genômica Funcional. Esta Facility deverá atender pesquisadores de várias Instituições de ensino (USP, UNESP, Unicamp, Unifesp, UFSCAR), pesquisa (Embrapa, IAC), Empresas (Agroceres, Suzano), Incubadoras de Empresas e o Pólo Tecnológico de Piracicaba, além de outras instituições da comunidade científica do Estado de São Paulo.

O Laboratório Multiusuários já conta com recursos aprovados pela FINEP, no valor de R\$ 2.000.000,00 (Proc. Nº 35 0655/05, 41 1036/06, 0119/07, 0114/08) para as obras de construção civil do prédio que irá abrigar os equipamentos multiusuários, pesquisadores e técnicos de apoio. O Laboratório Multiusuários é um importante centro aglutinador para o desenvolvimento de pesquisas básicas e aplicadas, formação de pessoal e desenvolvimento tecnológico da agropecuária, setor florestal e agroenergia em São Paulo.

Coordenadores: Luiz Lehmann Coutinho e Carlos A. Labate

<http://genfis40.esalq.usp.br/multi/>

PESQUISA

Que tipo de Serviço
você tem interesse?

- Sequenciamento
- Genotipagem
- Transcritoma
- Proteômica
- Bioinformática

VOTAR

RESULTADOS

QUEM ESTÁ ONLINE

Nós temos 1
visitante online



Scientific Committee

Antonio Figueira - Cena/USP

Flavio Meirelles - FZEA-USP

Heloisa Araujo - UFSCAR

Junior Barrera - IME-USP

Luciana Regitano - Embrapa

Marcos Machado - IAC

Marilia Buzalaf - FCF – USP/Bauru

Ricardo Vencio - USP – FFCLRP

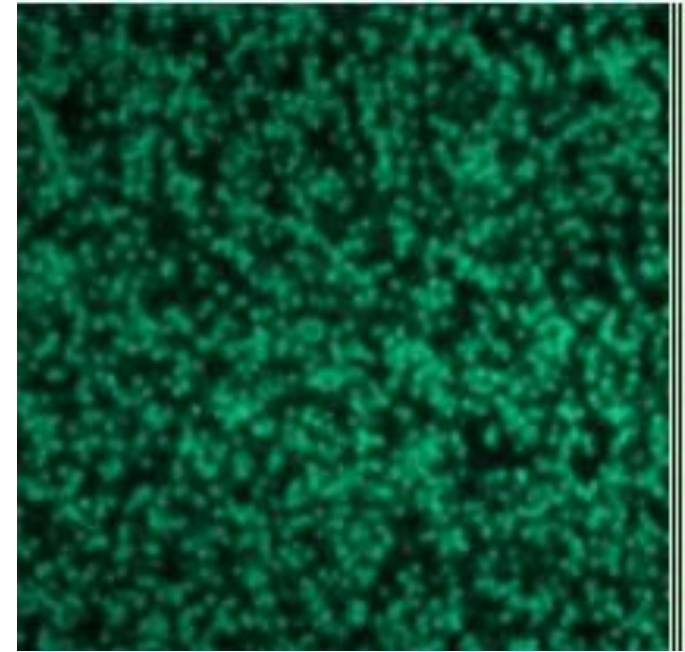
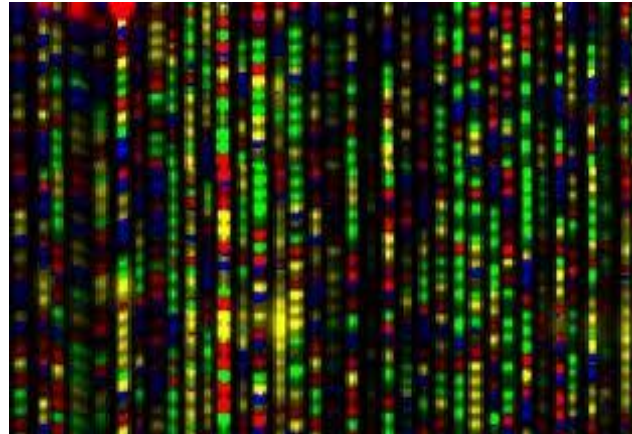
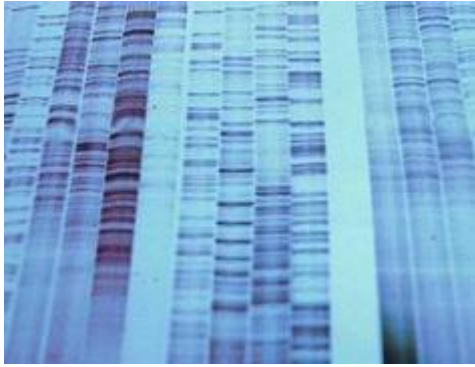


1st User`s Workshop 2012





Luiz Lehmann Coutinho



Whole genome sequencing, genotyping and RNAseq



Equipments Available for DNA sequencing



Acquired by Fapesp



Donated by Affymetrix



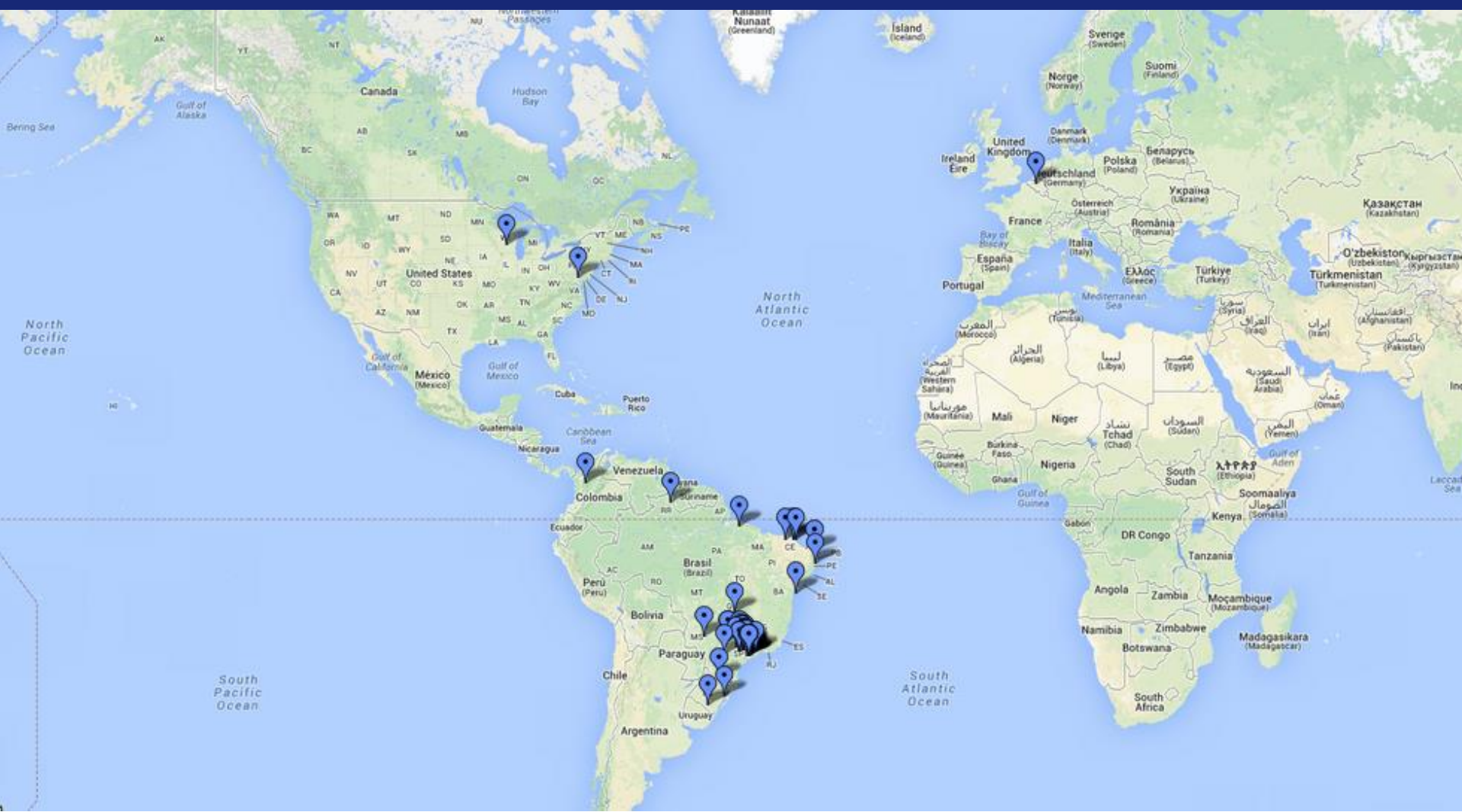
Acquired by USP and upgraded with Fapesp



Acquired with funding from Capes



Users - DNA Sequencing



Over 70 projects in the last 3 years

Most of the groups using the DNA sequencing facility received training for the preparation of the samples, interpretation of the results and bioinformatics analysis



1st RNAseq Training Course 2013





DNA Sequencing Team



- Gustavo Gasparin (Post-doc, libraries and sequencing)
- Ricardo Brassaloti (Procontes-USP technician, genotyping)
- Sonia Andrade (Post-doc, bioinformatics)
- Nirlei Silva (Technician BA - USP, sequencing and support)
- Jorge Andrade (Technician - USP, bioinformatics and technical support)
- Marcela Paduan (Technician, libraries - Fealq)



Proteomics and Metabolomics Team



- Thais Cataldi – PhD – Procontes-USP
- Livia Maria Franceschini – Bioinformatics (Uniemg)
- Mônica T. Veneziano Labate – Post-doc (Uniemg)
- Simone Gonzalez Guidette – Post-doc (Fapesp)
- Ilara G. Frasson Budzinsk – Post-doc (Fapesp)



Laboratório Multiusuários Centralizado
Genômica Funcional Aplicada à Agropecuária e Agroenergia

PROTOCOLS AVAILABLE FOR FUNCTIONAL GENOMICS

A⁺ A⁻ A⁻ pesquisar...

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SERVIÇOS

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Downloads

- Agilent Protein Kits (5)
- Sequenciamento (1)
- Proteoma (10)
- Genotipagem (0)
- Transcritoma (0)
- Bioinformática (0)

genfis40.esalq.usp.br/multi/index.php?option=com_phocadownload&view=category&id=8&proteomail&Itemid=62

Aplicativos www.mconline.org/si... Proteomics and Signal ... Nova guia New Objective : Innov... USP Mail: Responder HMDB: MS Search

Proteoma

- Enriched Peptide Agilent Search Proteoma.pdf (13/09/10) **Download**
- Curso BRAProteoma.pdf **Download**
- Questão em Gen Proteoma.pdf (02/09/10) **Download**
- Carteira Proteoma, Dados, Elenco, Entenda, Download (01/02/10) **Download**
- QSEARCH Wordcloud (18/01/10) **Download**
- Uniprot Anotado, Compara, Base de Dados, Explicação de FFL, Download (09/01/10) **Download**

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- Projeto Executado
- Projeto em Espera

PESQUISA

Que tipo de Serviço você tem interesse?

Sequenciamento

Genotipagem

Proteoma

Transcritoma

Bioinformática

VOTAR

RESULTADOS

AGENDA

< Maio 2014 >

	27	28	29	30	1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

QUEM ESTÁ ONLINE

Não temos visitantes online



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à Agropecuária e Agroenergia

PRICES OF SERVICES FOR PROTEOMICS

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Proteômica		
MALDI-TOF/TOF		
	Universidade	Empresa
Análise por MS + Processamento dos dados (R\$/por poço da placa de MALDI)*	R\$80,00	R\$110,00
Digestão das amostras a partir dos spots do gel+análise por MS+ Processamento dos dados (R\$/por poço da placa de MALDI)	R\$150,00	R\$180,00
* Amostra já digerida seguindo os protocolos do site http://genfis40.esalq.usp.br/multi		
Synapt-G2-(LC-MSE)		
(até 5 frações com 3 replicatas técnicas cada)		
	Universidade	Empresa
Análise por MS + Processamento dos dados (R\$/por vial)*	R\$5.000,00	R\$6.500,00
Digestão das amostras complexas+análise por MS+ Processamento dos dados (R\$/por vial)	R\$6.000,00	R\$7.500,00
* Amostra já digerida seguindo os protocolos do site (http://genfis40.esalq.usp.br/multi)		



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PRICES OF SERVICES FOR METABOLOMICS

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Metabolômica e Lipidômica		
LC-MS (Q-TOF Última da Waters)		
(3 replicatas técnicas por amostra)		
	Universidade	Empresa
Análise por MS das amostras extraídas e filtradas (R\$/por vial)*	R\$140,00	R\$200,00
* Filtro: unidade filtrante descartável millex GV 0,22 um, membrana durapore PVDF-Millipore		
# As análises serão realizadas em triplicadas técnicas		



Finalized Projects – MALDI-TOF/TOF-ABSciex

Date	Research Group	Institution	Analysis	Quantity
Jan-12	Flávio Vieira Meirelles	USP	Proteomica	79
Feb-12	Luiz Filipe Protasio Pereira	UEL	Proteomica	87
Feb-12	Maysa Furlan	UNESP-Araraquara	Proteomica	50
Feb-12	Reginaldo Bruno Gonçalves	UNICAMP	Proteomica	99
Feb-12	Eny Lopes	USP	Proteomica	106
Feb-12	Marcia Braga	Instituto Biológico-SP	Proteomica	106
Feb-12	Glenda Nicioli da Silva	UNESP-Botucatu	Proteomica	233
Feb-12	Heloísa Sobreiro Selistre de Araújo	UFSCAR	Proteomica	146
Mar-12	Sônia Andrade	Butantã	Proteomica	13
May-12	João Nascimento	USP	Proteomica	14
May-12	Marcos Machado	Centro Citricultura	Proteomica	75
Jun-12	José Santista	CFNA (USP)	Proteomica	31
Jul-12	Paola Talia	Instituto de Biotecnología CICVyA, CNIA, INTA Castelar-Argentina	Proteomica	27
Sep-12	Maria José Giannini	UNESP-Araraquara	Proteomica	620
Oct-12	Henrique Ferreira	UNESP-Rio Claro	Proteomica	78
Oct-12	Marília Afonso Rabelo Buzalaf	USP	Proteomica	240
Dec-12	Grupo Boticário	-	Proteomica	16
Jan-13	Deborah Schechtman	Incor-USP	Proteomica	110
Jan-13	Flávio Vieira Meirelles	USP	Proteomica	102
Jan-13	Márcio Lambais	ESALQ	Proteomica	59
Feb-13	Ricardo Azevedo	ESALQ	Proteomica	122
Mar-13	Marília Afonso Rabelo Buzalaf	USP	Proteomica	27
Apr-13	Grupo Boticário	-	Proteomica	22
Jun-13	Ana Augusta Pagnano Derussi	Unesp_Botucatu	Proteomica	38
Sep-13	Joao Nascimento	USP	Proteomica	146
Oct-13	Luisa Lina Villa	Instituto do Câncer do Estado de São Paulo	Proteomica	20
Oct-13	Evandro Antonio de Lima	CTBE	Proteomica	8
Jan-14	Claudia Vitorello	USP	MALDI IMAGING	-
Feb-14	Marcelo Menossi	UNICAMP	Proteomica	32
Apr-14	Frederico Ozanam Papa	Unesp_Botucatu	Proteomica	50
May-14	Marcelo Menossi	UNICAMP	Proteomica	4



Finalized Projects- **Synapt G2 Waters**

Date	Research Group	Institution	Analysis	Quantity
Dec-11	Carlos Alberto Labate	ESALQ	Proteoma nuclear	19
Dec-11	Sônia Andrade	Butantã	Proteômica Total	4
Jan-12	Carlos Alberto Labate	ESALQ	Proteômica Total	6
Jul-12	Carlos Alberto Labate	ESALQ	Proteômica Total	2
Jul-12	Luiz Lehmann Coutinho	ESALQ	Proteômica Total	36
Feb-13	Carlos Alberto Labate	ESALQ	Proteômica Total	24
Feb-13	Mônica Veneziano Labate	ESALQ	Proteômica Total	16
Apr-13	Simone Guidetti	ESALQ	Proteômica Total	20
Apr-13	Leonardo César Ferreira	ESALQ	Proteômica Total	10
Aug-13	Regina Helena Pires	UNESP	Proteômica Total	4
Sep-13	Ligia Macedo	UNICAMP	Proteômica Total	6
Sep-13	Joao Nascimento	USP	Proteômica Total	24
Oct-13	Carlos Alberto Labate	ESALQ	Proteômica Total	36
Nov-13	Carlos Alberto Labate	ESALQ	Proteômica da parede celular	6
Nov-13	Carlos Alberto Labate	ESALQ	Proteômica da parede celular	12
Dec-13	Deborah Schechtman	Incor-USP	Proteômica Total	22
Jan-14	Julio Balieiro	USP	Proteômica Total	32



Finalized projects - Metabolomics/Lipidomics Q-TOF Ultima Waters

Date	Research Groups	Institution	Analysis	Quantity
Apr-12	Edson Guimarães Lo Turco	UNIFESP	lipidômica	11
Oct-12	Edson Guimarães Lo Turco	UNIFESP	Metabolomica/lipidômica	24
Oct-12	Alberto Cavalheiro	UNESP	Metabolomica	55
Nov-12	Carlos Alberto Labate	ESALQ	Metabolomica	10
Dec-12	Carlos Alberto Labate	ESALQ	Metabolomica	72
Dec-12	André Ricardo Alcarde	ESALQ	Metabolomica	15
Jan-13	Flávio Vieira Meirelles	USP	lipidômica	62
Feb-13	Silvia Daher	UNIFESP	lipidômica	90
Feb-13	Simone Guidetti	ESALQ	Metabolomica	60
Feb-13	Leonardo César Ferreira	ESALQ	Metabolomica	12
May-13	Benedicte Favreau	Cirad-Montpellier-France	Metabolomica	48
Jul-13	Carlos Alberto Labate	ESALQ	Metabolomica	72
Jul-13	Empresa Delta	-	Metabolomica	2
Sep-13	Fernando Cotinguiba da Silva	ESALQ	Metabolomica	100
Sep-13	Mônica Labate	ESALQ	Metabolomica	10
Oct-13	Edson Guimarães Lo Turco	UNIFESP	lipidômica/metabolomica	110
Nov-13	Mariana Silva Souza	ESALQ	lipidomica	90
Nov-13	Jocelem Salgado	ESALQ	Metabolomica	12
Feb-14	Fabiano Pinheiro	Hospital das Clínicas USP	lipidômica	90
Apr-14	Ilara Budzinski	ESALQ	Metabolomica	324



Equipments available for Proteomics and Metabolomics



Synapt G2 HDMS – *Label Free Proteomics*



Acquired by Fapesp

Q-TOF Ultima API



Maldi TOF/TOF 5800



Acquired by Fapesp



GC/GC-TOF-MS Pegasus 4D Leco – Acquired by USP



Synapt G2 HDMS– *Label free quantitative proteomics* - Phosphoproteomics, Glycoproteomics





SUCROSE ACCUMULATION

Sugarcane Stalks

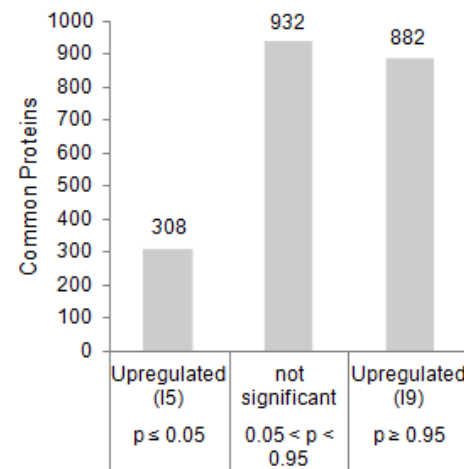
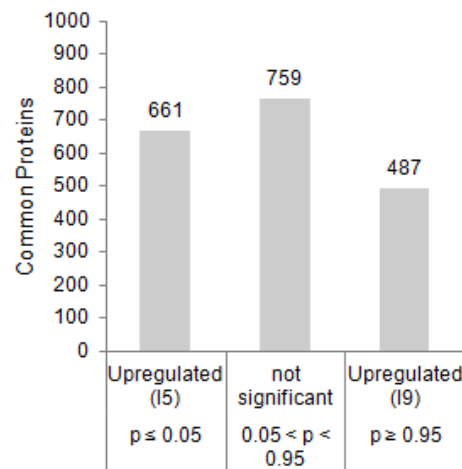
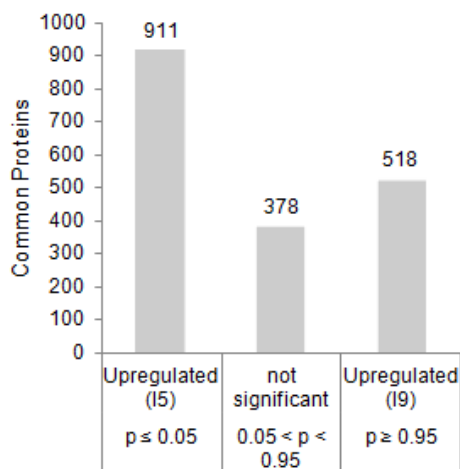
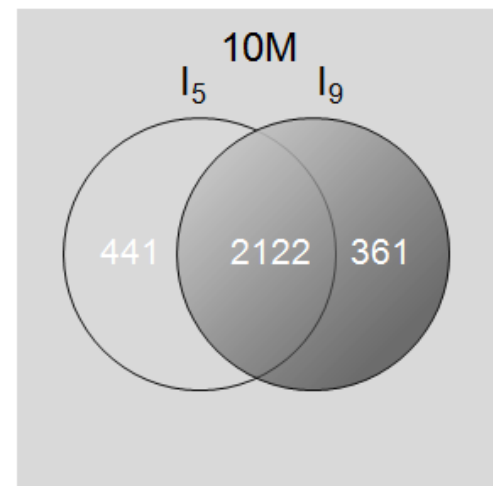
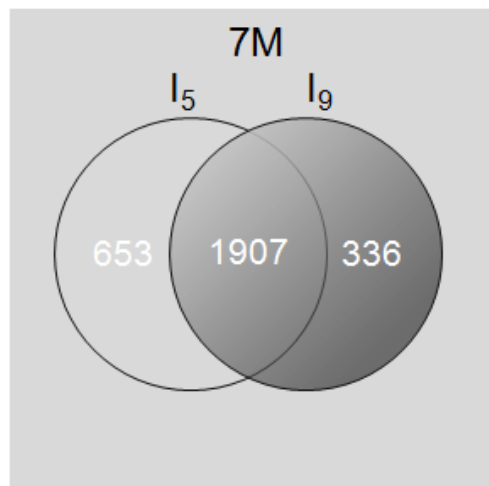
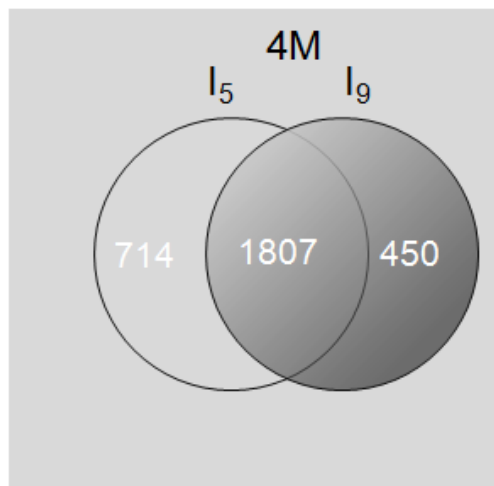


4 Months

7 Months

10 Months





Total number of expressed proteins identified by the PGLS Software, for the internodes 5 (I_5) and 9 (I_9) of **sugarcane**, analysed at 4, 7 months and after the drought stress at 10 months. (<http://sucest-fun.org/>).

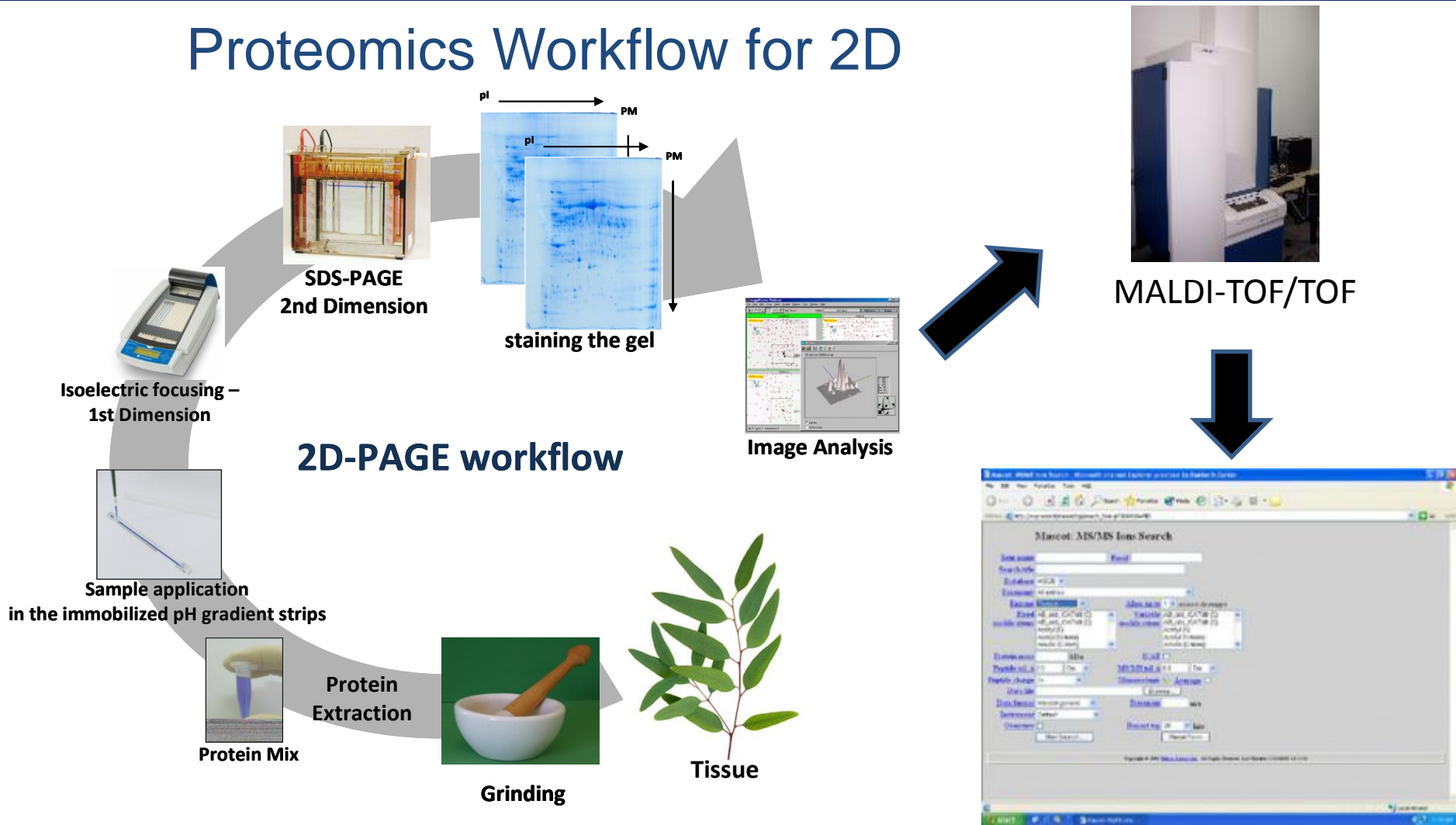


MALDI-TOF/TOF Absciex 5800 and nano LC 2D for Proteomics, Lipidomics, Phosphoproteomics, Glycoproteomics and Carbohydrates





Proteomics Workflow for 2D



We provide technical support for users at all stages of sample preparation and bioinformatics analyses

MASCOT-Matrix Science



CRYOSTAT LEICA FOR MALDI – Imaging



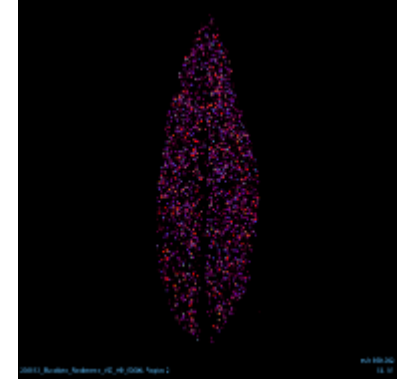
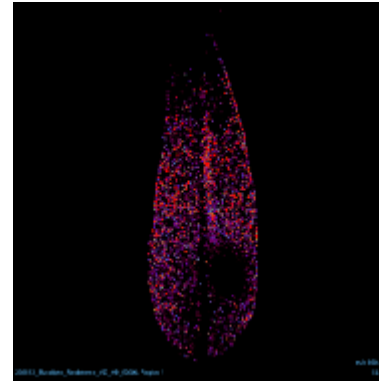
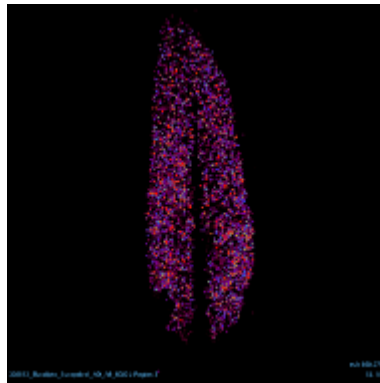
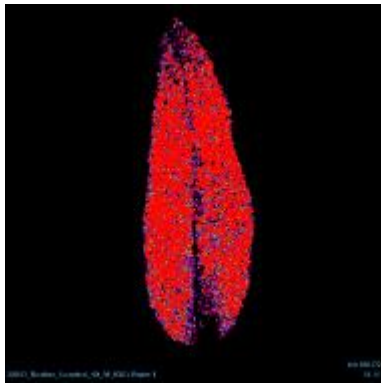
Acquired by Fapesp

MALDI Image Lipid distribution

Eucalyptus grandis leaves, Resistant and
Susceptible to *Puccinia psidii*

Susceptible

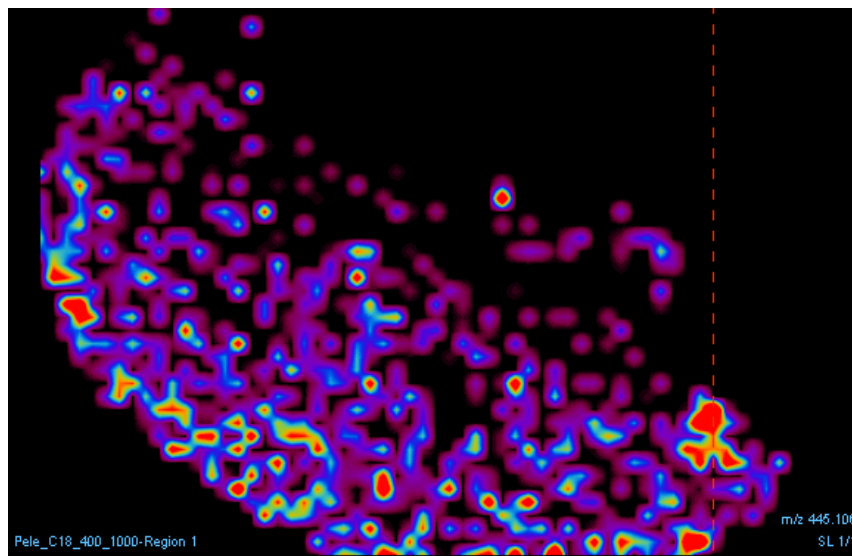
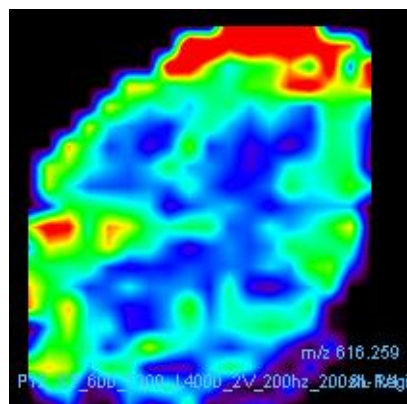
Resistant



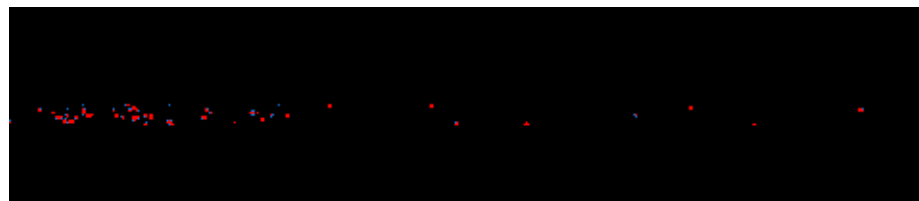
m/z 659



O Boticário



Skin



Hair



Q-TOF ULTIMA API with UPLC Acquity

Metabolomics

Secondary metabolites: phenolic compounds, terpenes

Lipidomics





PEGASUS 4D – GC-GC-TOF/MS

Metabolomics

Primary metabolites: amino acids, organic acids, sugars, sterols



Derivatization:

- N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA)
- Methylhydroxylamine Hydrochloride (MeOX) in pyridine

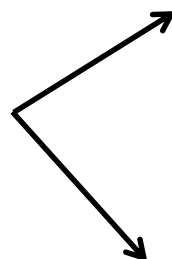
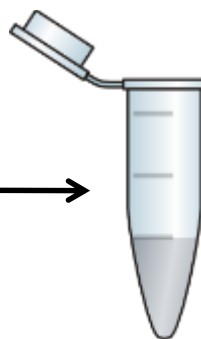


Extraction Methods

Metabolomics



Ball Mill



6% (v/v) Chloroform
2% (v/v) Methanol
Water Solution

Gullberg et al., Analytical Biochemistry, 2004

99,875% (v/v) Methanol
0,125% (v/v) Formic Acid

De Vos et al., Nature Protocols, 2007

* Sample Preparation → Quality of Analysis

→ Polar metabolites

→ Lipophilic metabolites

METABOLOMIC ANALYSIS

MarkerLynx Software

MarkerLynx XS

File Name Sample Group

1	09_212_Felp_IG1	IG1
2	09_212_Felp_RGT	RGT
3	09_212_Felp_RNT	RNT
4	09_212_Felp_SG1	SG1
5	09_212_Felp_RGT	RGT
7	09_212_Felp_RNT	RNT
8	09_212_Felp_RNT	RNT
9	09_212_Felp_SG1	SG1
10	09_212_Felp_RGT	RGT
11	09_212_Felp_RNT	RNT
12	09_212_Felp_RNT	RNT
13	09_212_Felp_SG1	SG1
14	09_212_Felp_IG1	IG1
15	09_212_Felp_RNT	RNT
16	09_212_Felp_SG1	SG1

Navigation icons: Edit Method, Process Samples, View Results, Database Manager

MarkerLynx XS Method Editor - Data Path: Data\16.ms

Method	Property	Value
Method	Function	1
Method	Analysis Type	Peak Detection
Method	Min. Retention Time	1.00
Method	Max. Retention Time	100.00
Method	Scan Range	100.00
Method	Integration	100.00
Method	% of Window (FID)	0.75
Method	Use Absolute Intensity?	<input checked="" type="checkbox"/> No
Method	Apply Peak Ratio Parameter	<input checked="" type="checkbox"/> Yes
Method	Ratio Window (TS - Height (mV))	5.00
Method	Ratio Peak Normalization	5.00
Method	Apply Normalization	<input checked="" type="checkbox"/> Yes
Method	Calculate Statistics	<input checked="" type="checkbox"/> Yes
Method	Use Peak Intensity within Window?	<input checked="" type="checkbox"/> Yes
Method	Match Name	0.75
Method	Retention Time Window	0.75
Method	Mass Number Level	0.00
Method	Match Name	<input checked="" type="checkbox"/> Yes

Create Dataset

Tracking Options

- Repeat Samples
- Project Data
- Create Tables
- Calculate Statistics
- Save Data

Project: <New Project> [New]

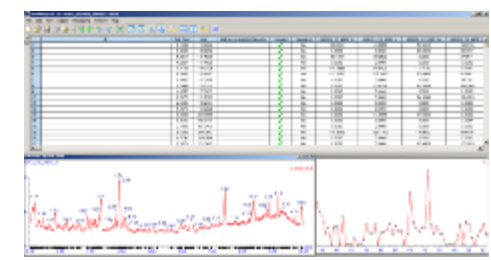
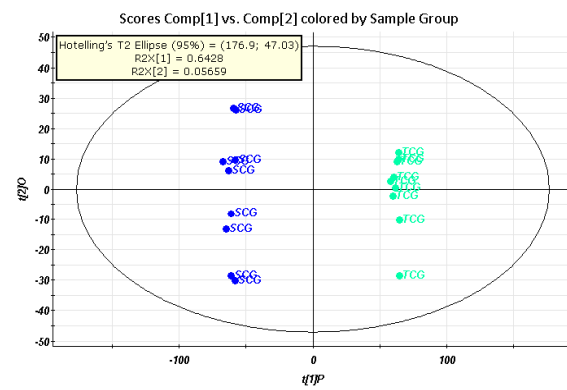
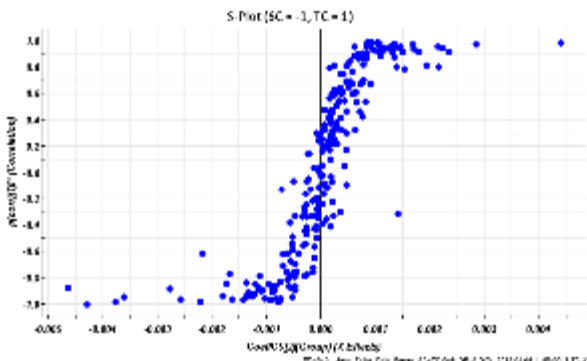
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Sample: 144

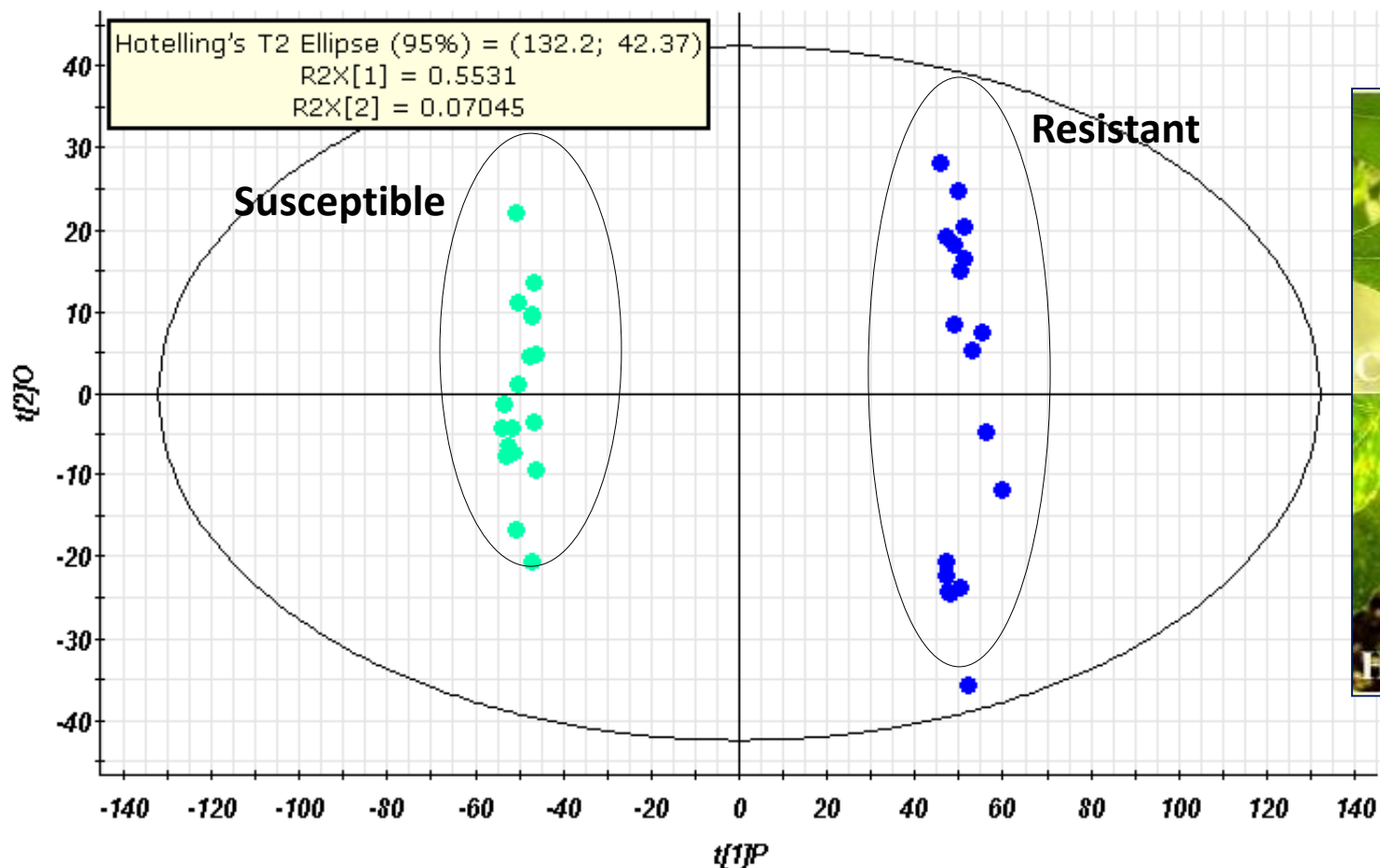
Method: <New Method> [New]

Method: <New Method> [New]

OK Cancel



Comparative metabolomic analysis of *Eucalyptus grandis* leaves from susceptible and resistant genotypes to *Puccinia psiidi*





PROBLEMS AND SUGGESTIONS TO IMPROVE THE FACILITIES

- ✓ For DNA sequencing the users have no problems in paying for services. However, for proteomics and metabolomics analyses users tend to ask for collaborations. In many cases they have not requested funding to pay for analysis.
- ✓ We need funding to maintain the equipments and consumables, such as Liquid N₂, special gases. Until last year USP was providing such funds, but with the financial problems that it is facing today, we don't have enough support.
- ✓ The costs of running the facilities are high and the prices charged are not enough. We need to have support from Fapesp or Universities in order to maintain good standards.



- ✓ We also need technicians to give support to users. Most of our technicians are payed by our own projects and are Post-docs that provide support to users. It is necessary to have a continuous support from Fapesp, provinding technical scholarships (TT).
- ✓ If the Facilities could have a Fapesp Project and users (with Fapesp`s projects) could transfer the costs of services, directly to our account. This would avoid having to pay 20-25% costs of overhead to foundations.