# Una aproximación "-ómica" a la deficiencia y toxicidad de metales en plantas

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Análisis "targeted"

Metabolómica

Análisis de imagen

**Proteínas**

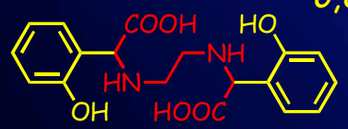
Proteómica 2-DE

Proteómica HPLC-MS

## Clorosis Férrica en el Valle del Ebro, España

EDDHA

o,oEDDHA



La deficiencia de Fe en plantas: clorosis férrica

## Efectos del Cd en la parte aérea de tomate

control | 10 µM Cd | 100 µM Cd

López-Millán et al. (2011) Env Exp Bot

## Efectos de la toxicidad de Zn en la parte aérea de remolacha

1.2 μM Zn

100 μM Zn

300 μM Zn

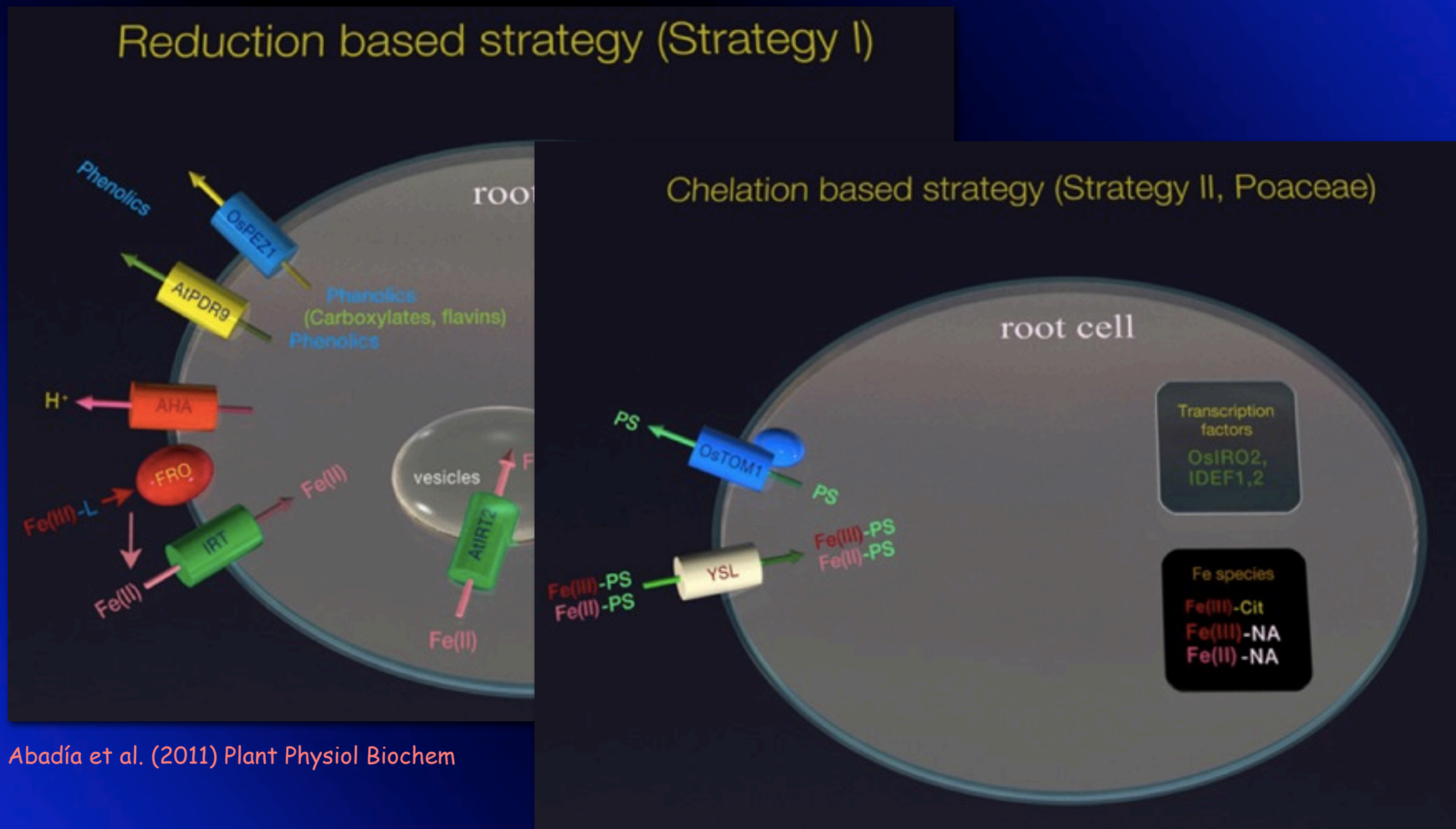
Sagardoy et al. (2009) Plant Biol

## Efectos del Cd en la raíz de tomate

control 10 μM Cd 100 μM Cd

Rodríguez-Celma et al. (2010) J Proteomics

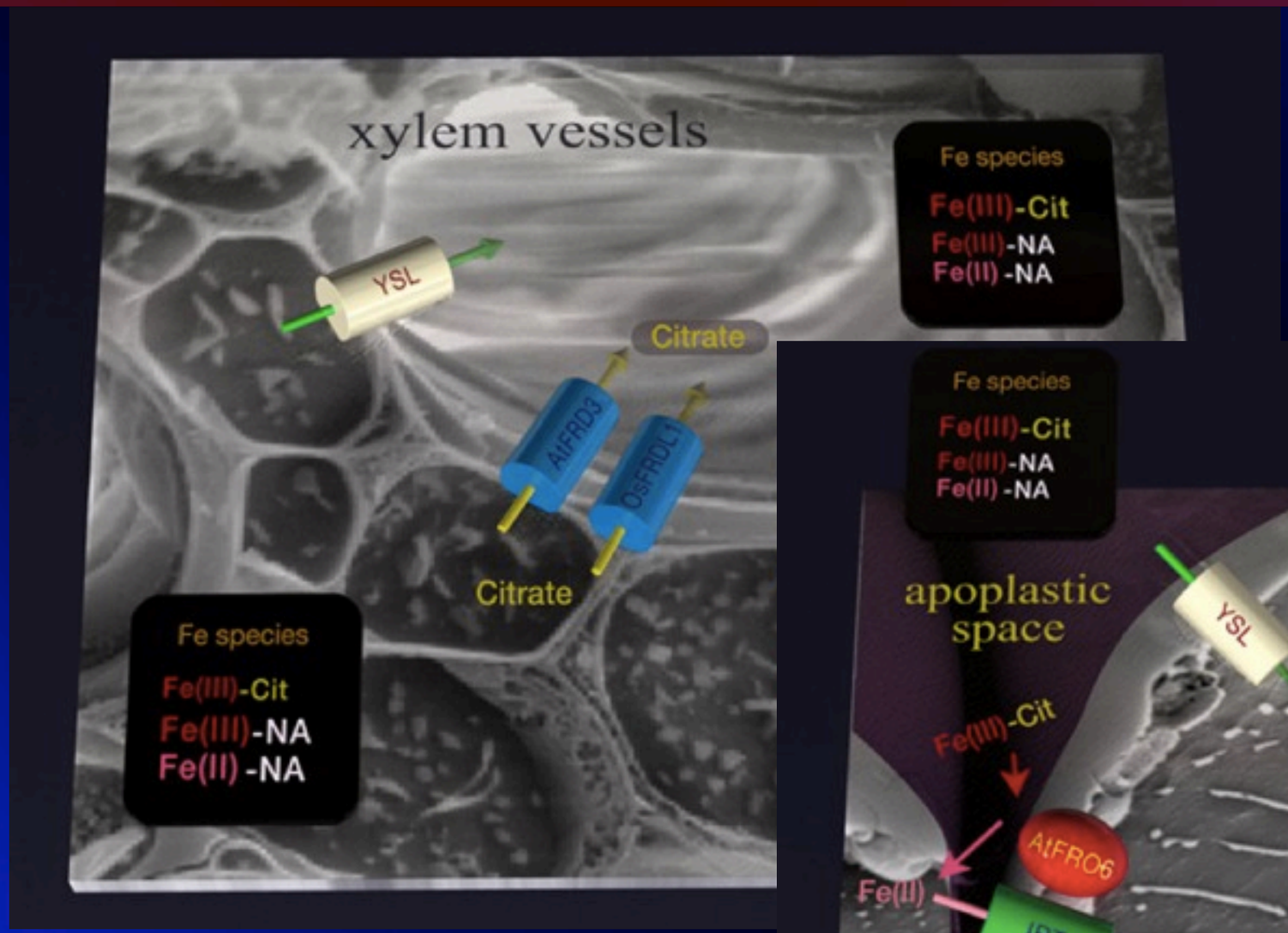
La deficiencia de Fe en plantas: clorosis férrica



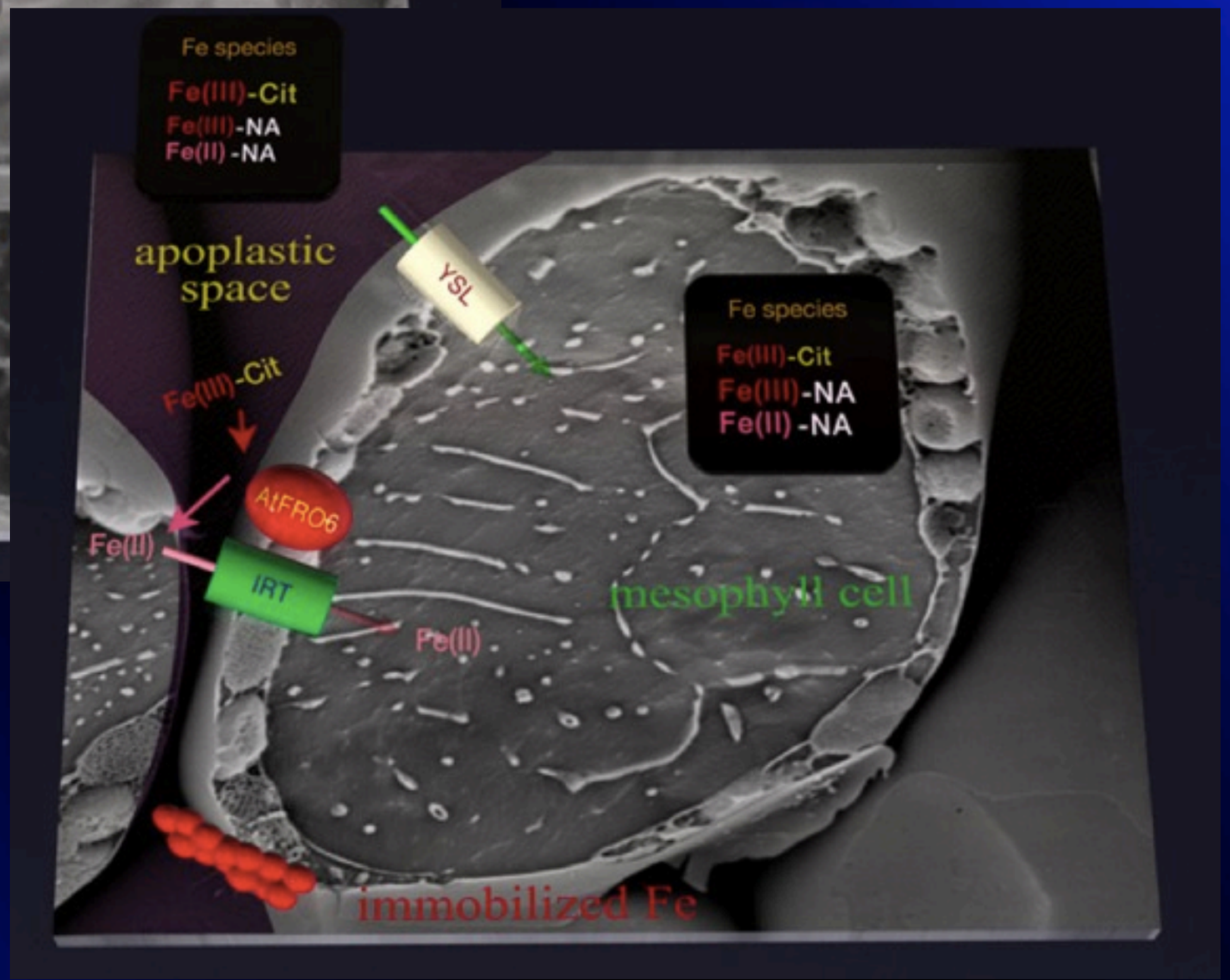

Abadía et al. (2011) Plant Physiol Biochem

# ¿Por qué estudiar los metales en plantas?

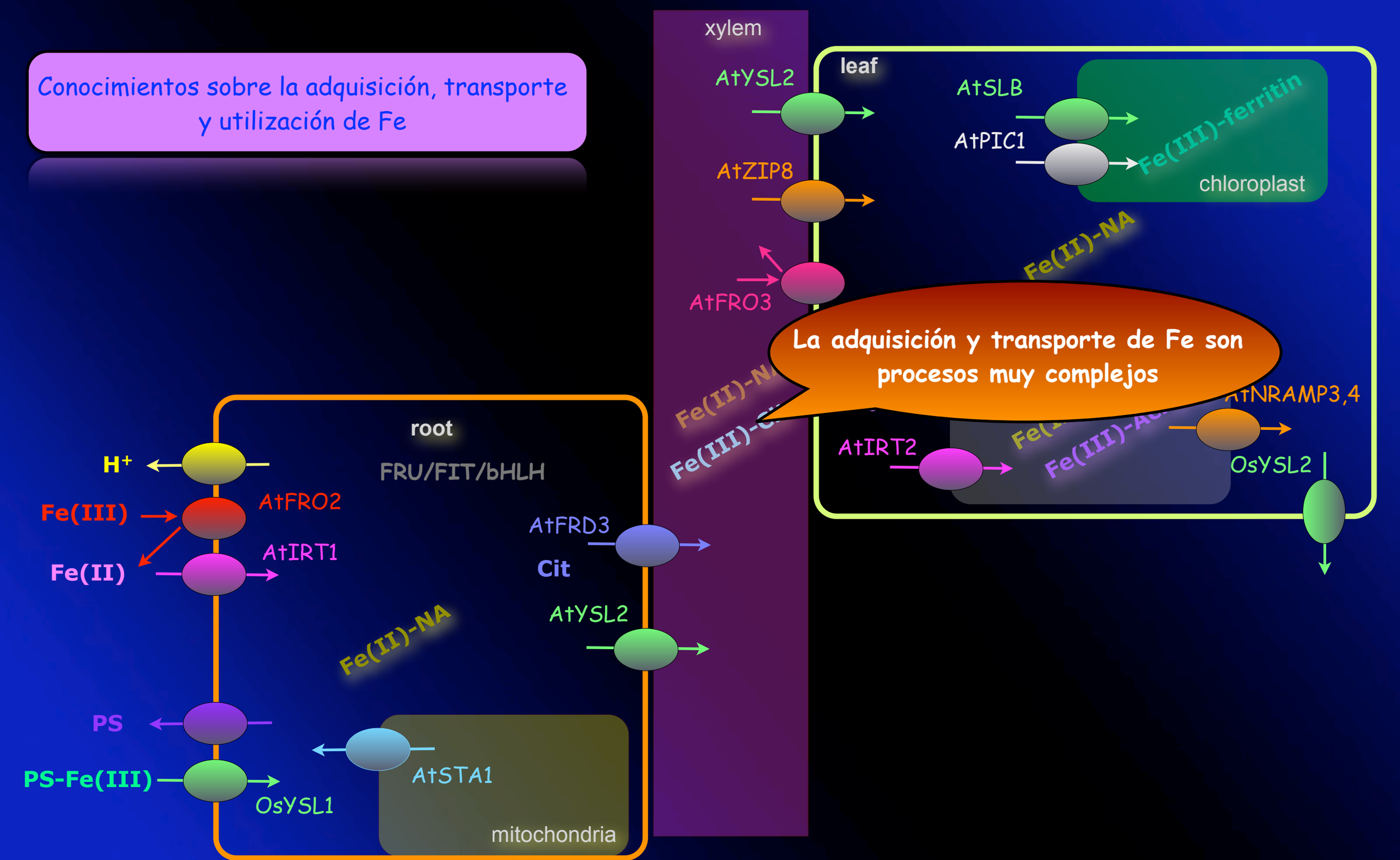
Deficiencia de Fe




Abadía et al. (2011) Plant Physiol Biochem



Conocimientos sobre la adquisición, transporte y utilización de Fe



# ¿Hasta qué punto el sistema es complejo?

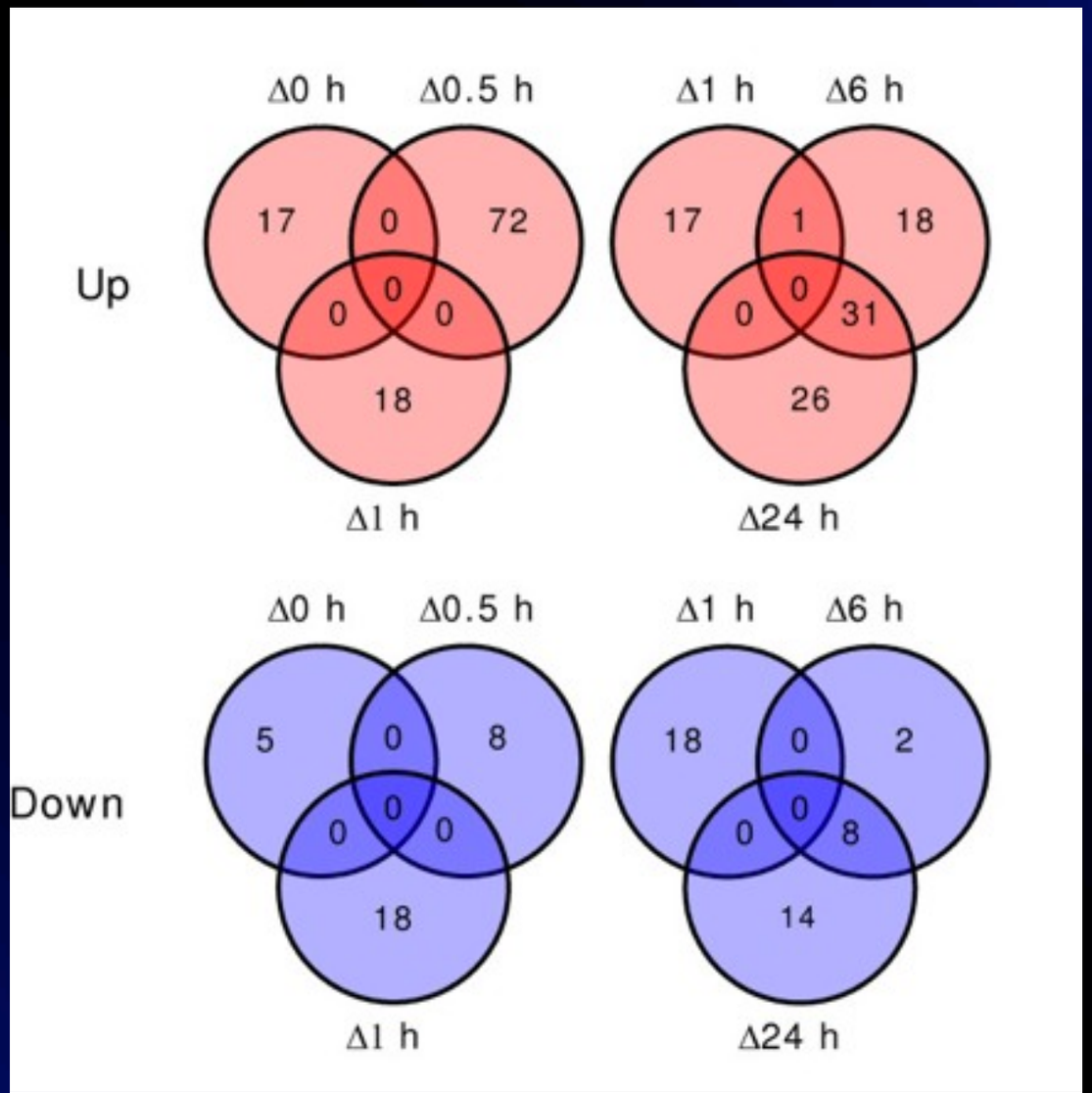
>200.000 metabolitos (10.000 primarios)

Pichersky and Lewinsohn, Ann Rev Plant Biol 2011

Arabidopsis Affymetrics Chips 22.500 probe sets

60 a 6 h

79 a 24 h




Buckhout et al. (2009) BMC Genomics

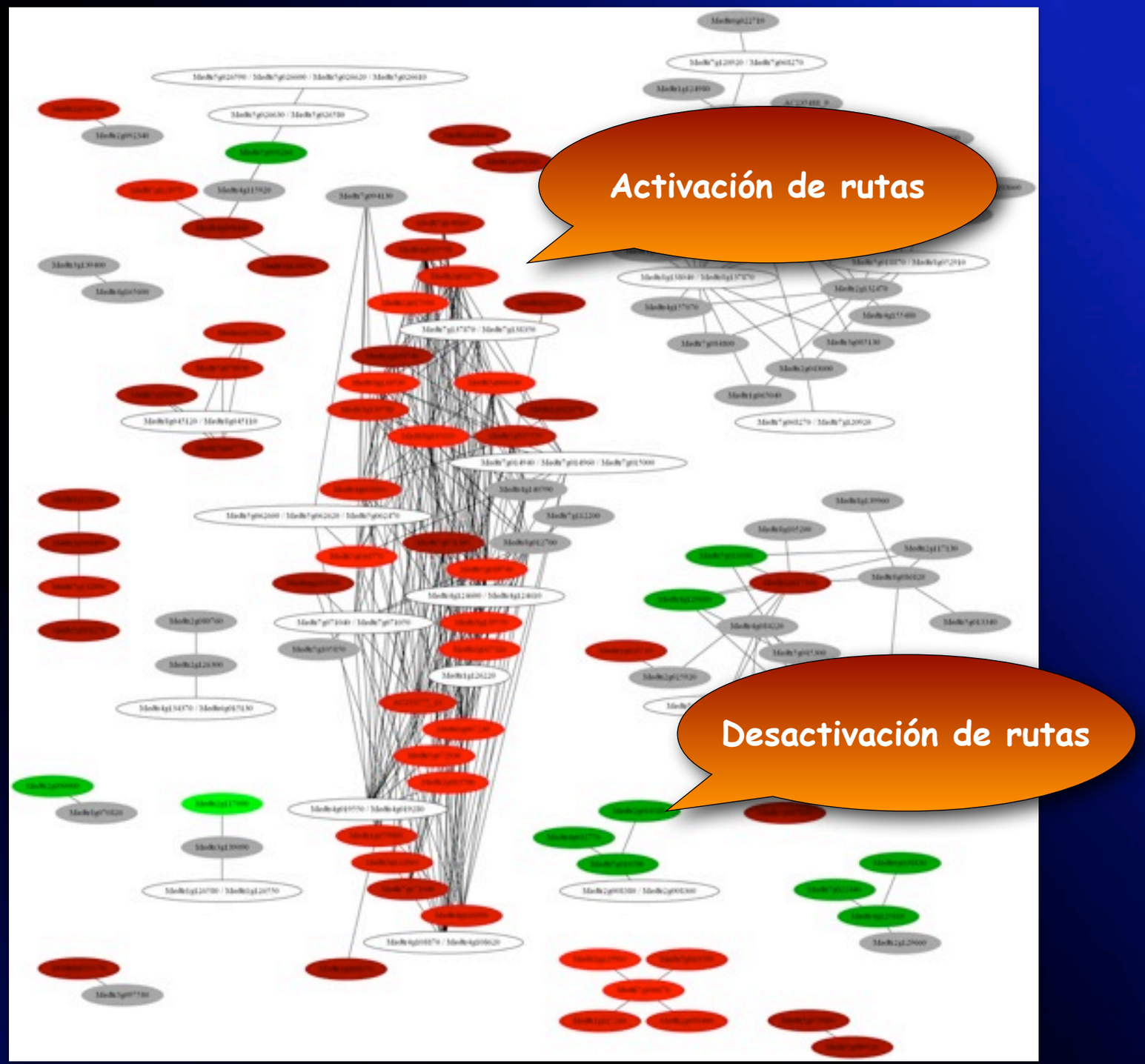
Total RNA

cDNA

secuenciación

Medicago truncatula
roots

0 µM Fe, pH 5,5
vs.
Control




Rodríguez-Celma et al., en preparación

Análisis de expresión génica

Schmidt's Lab, Academia Sinica, Taiwan

Perfiles transcriptómicos: RNAseq

Solexa II

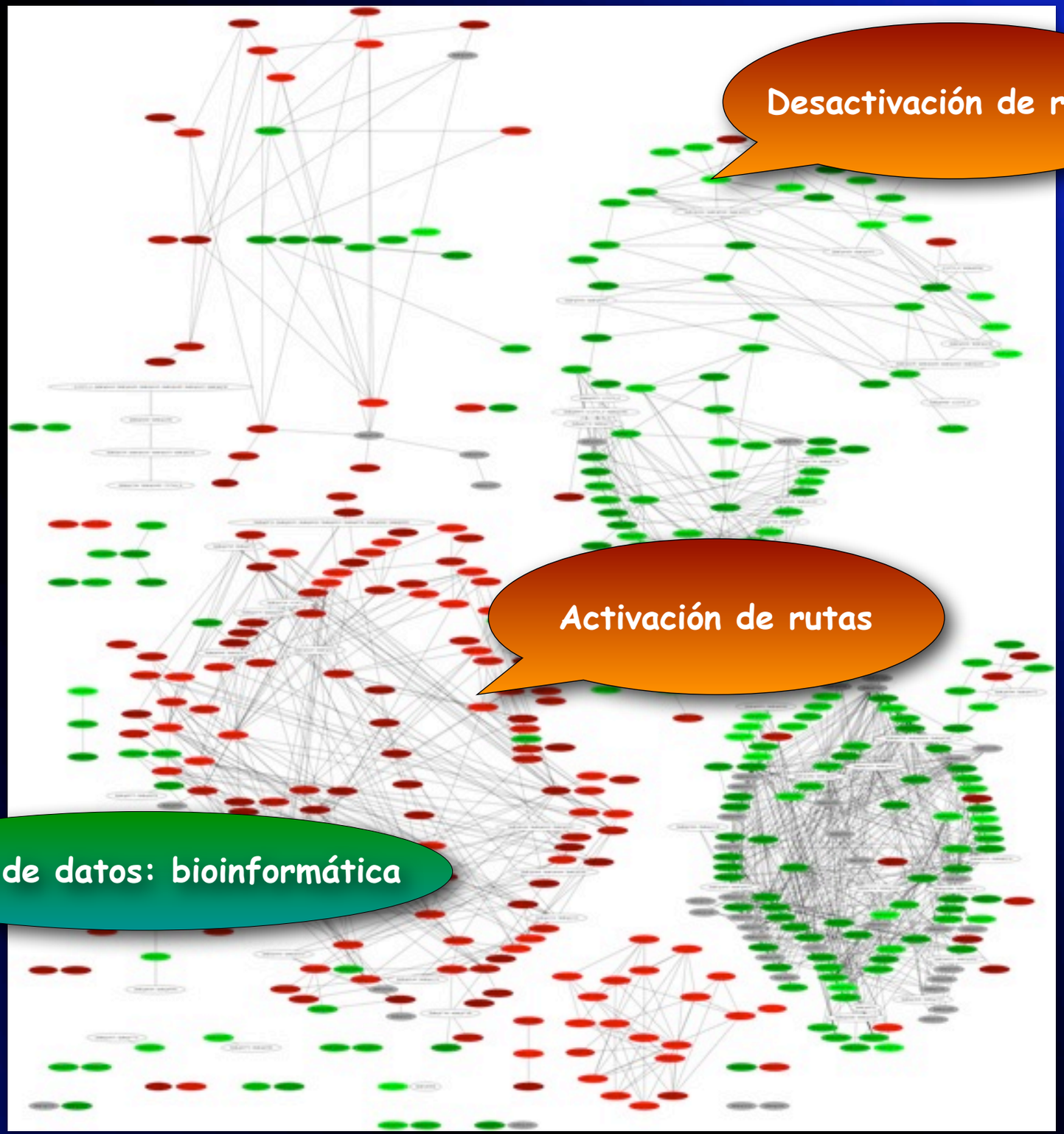
**Genes**

Medicago truncatula roots

0 µM Fe + $CaCO_3$, pH 7.7

vs.

Control




Miles de datos: bioinformática

47.800 genes en bases de datos

Rodríguez-Celma et al., en preparación

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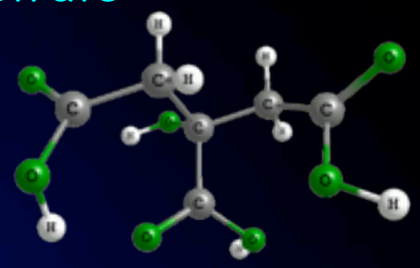
Citrate



Metabolitos

## Análisis de metabolitos

Sabemos que es importante, vamos a por ello

De uno en uno

Dentro de una clase: Análisis "targeted" (HPLC-MS)

Muchos: Análisis metabolómico "untargeted" y "high throughput" (GC-MS)

"Pescando" cambios desconocidos

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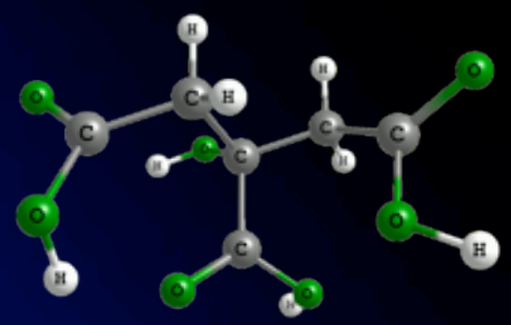
# Análisis de metabolitos (de uno en uno)

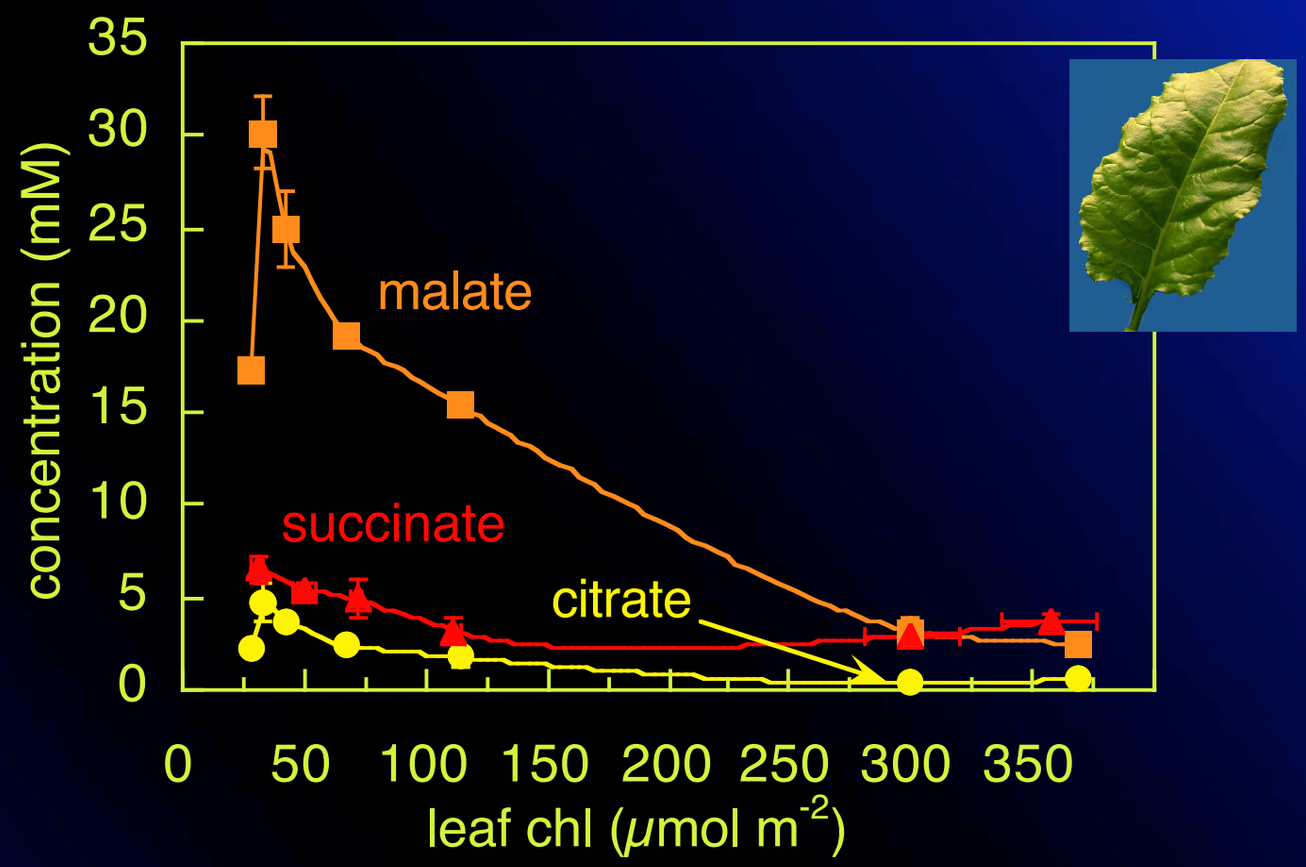
Metabolitos

Citrato



Citrate Assay Kit

Los ácidos orgánicos en xilema aumentan con la clorosis




López-Millán et al. (2000) Plant Physiol

# Dentro de una clase: Análisis "targeted" (HPLC-MS)

**Metabolitos**

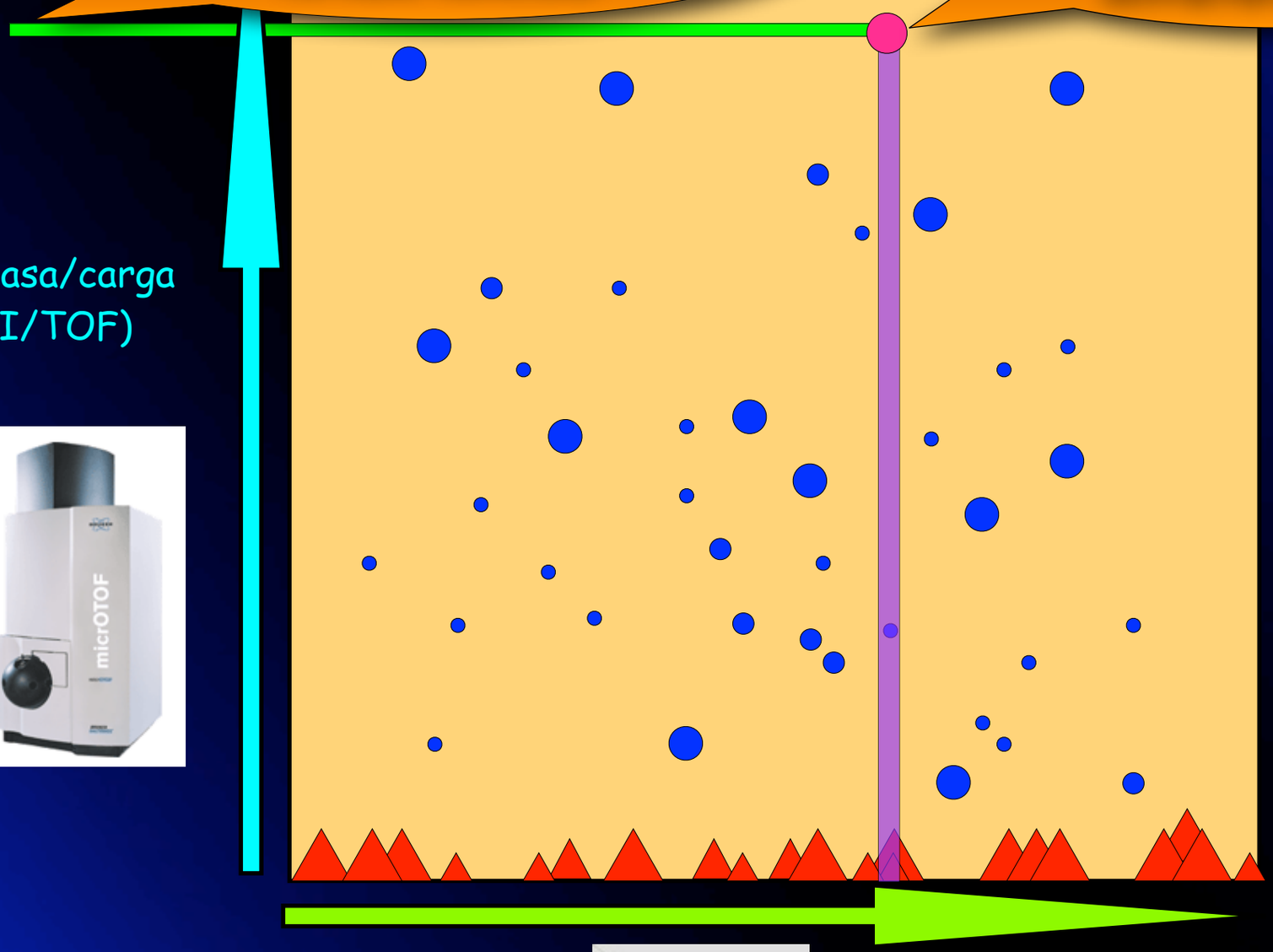
HPLC-MS

Separación por técnicas 2-D

**m/z única para cada compuesto: fórmula molecular**

**el compuesto se puede romper por MS/MS: estructura molecular**

relación masa/carga
MS (ESI/TOF)



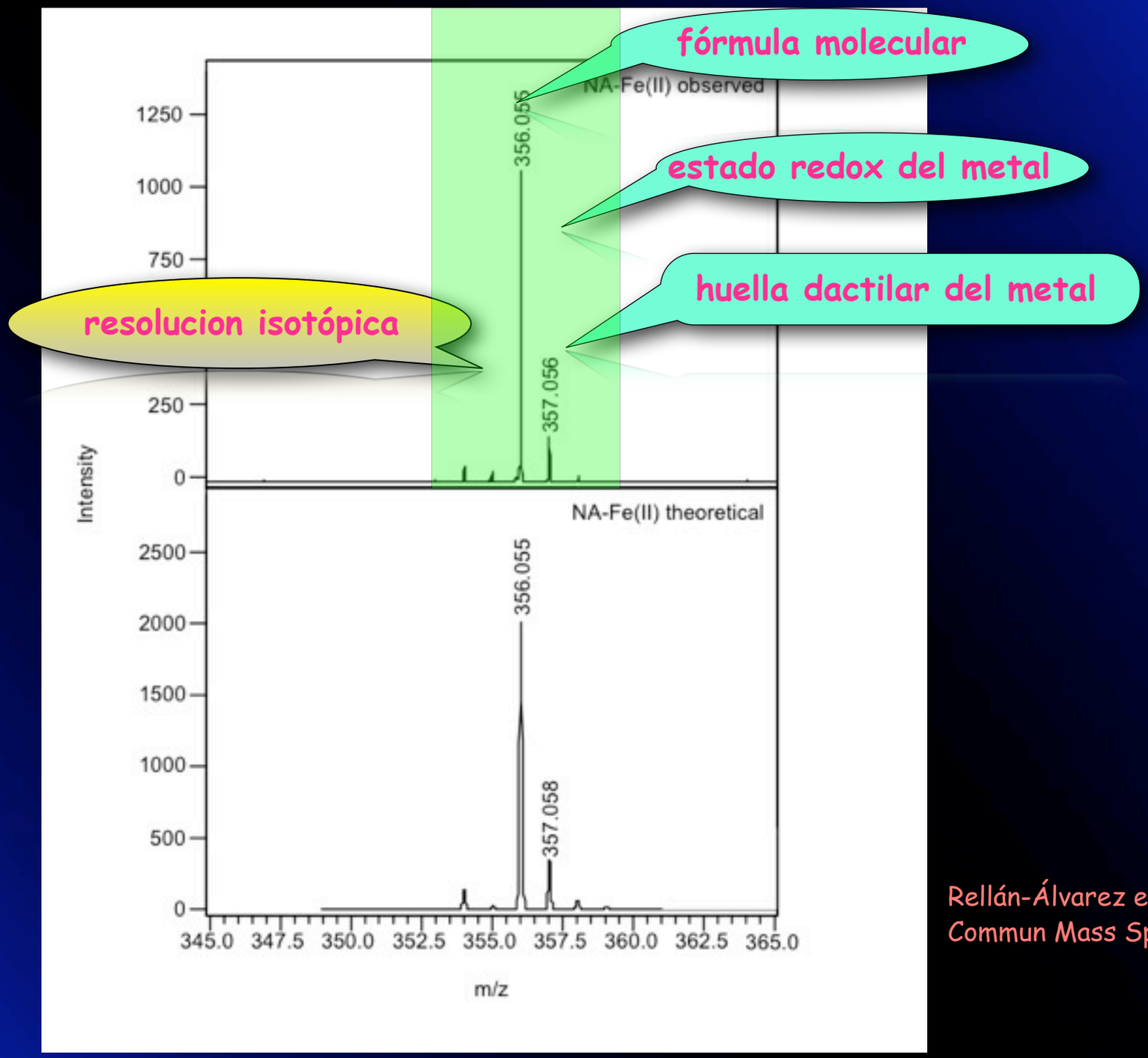
Tiempo (HPLC)

# Dentro de una clase: Análisis "targeted" (HPLC-MS)

**Metabolitos**

HPLC-MS

Separación por técnicas 2-D




Rellán-Álvarez et al. (2008) Rapid Commun Mass Spectrom

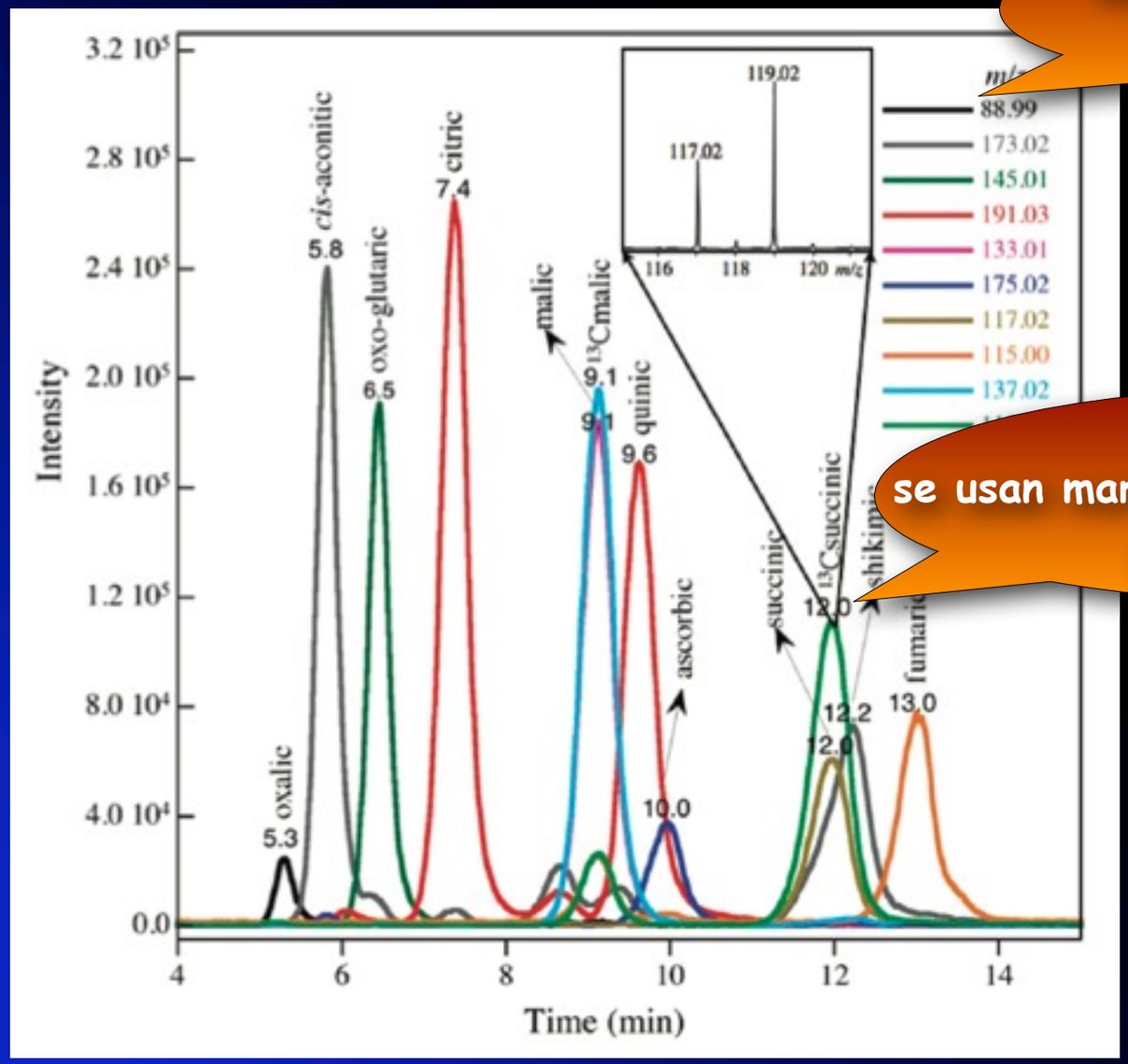
# Dentro de una clase: Análisis "targeted" (HPLC-MS)

**Metabolitos**

HPLC-MS

## Carboxilatos

**m/z única para cada compuesto: fórmula molecular**




**se usan marcajes con isótopos estables para calibración**

**Table 1. Molecular Formula of the Organic acids Analyzed by HPLC-ESI-TOFMS and the Theoretical and Experimental Mass-to-Charge (m/z) ratios of the corresponding $[M-H]^-$ Ions**

| organic acid | molecular formula | theoretical *m/z* | experimental *m/z* | error (mDa) |
|---|---|---|---|---|
| [illegible] | [illegible] | 88.9880 | 88.9849 | 3.17 |
| [illegible] | [illegible] | [illegible].0092 | 173.0083 | 0.90 |
| [illegible] | [illegible] | 145.0142 | 145.0131 | 1.18 |
| citric | $C_6H_8O_7$ | 191.0184 | 191.0185 | 1.25 |
| malic | $C_4H_6O_5$ | 133.0142 | 133.0136 | 0.69 |
| quinic | $C_7H_{12}O_6$ | 191.0561 | 191.0559 | 0.24 |
| ascorbic | $C_6H_8O_6$ | 175.0248 | 175.0248 | 0.06 |
| shikimic | $C_7H_{10}O_5$ | 173.0455 | 173.0464 | 0.86 |
| succinic | $C_4H_6O_4$ | 117.0193 | 117.0190 | 0.33 |
| fumaric | $C_4H_4O_4$ | 115.0037 | 115.0029 | 0.75 |

Rellán-Álvarez et al. (2011) J Agric Food Chem

# Dentro de una clase: Análisis "targeted" (HPLC-MS)

**Metabolitos**

HPLC-MS

Carboxilatos

**Técnica cuantitativa**

**Calibración: límites de detección y cuantificación**

**Table 2. Calibration Data, LODs, and LOQs Obtained for the Di**

| | calibration data | | | LOD | | LOQ | |
|---|---|---|---|---|---|---|---|
| organic acid | linearity range | equation | $R^2$ | $\mu$M in solution | pmol injected | $\mu$M in solution | pmol injected |
| oxalic | 10.0–500.0 | | | 12.8 | 255 | 16.9 | 337 |
| *cis*-aconitic | 0.[illegible] | | | 0.058 | 1.16 | 0.245 | 4.91 |
| 2-oxoglutaric | 0.2–10[illegible] | | | 0.007 | 0.13 | 0.208 | 4.15 |
| citric | 0.3–500.0 | $y = 0.0093x + 0.0169$ | 0.992 | 0.063 | 1.27 | 0.140 | 2.80 |
| malic | 0.1–1000.0 | $y = 0.0085x + 0.0416$ | 0.996 | 0.003 | 0.05 | 0.162 | 3.24 |
| quinic | 0.2–70.0 | $y = 0.0115x + 0.0269$ | 0.991 | 0.043 | 0.85 | 0.184 | 3.68 |
| ascorbic | 1.0–500.0 | $y = 0.0035x - 0.0236$ | 0.995 | 1.25 | 24.9 | 2.417 | 48.3 |
| shikimic | 0.1–100.0 | $y = 0.0085x + 0.0213$ | 0.994 | 0.050 | 1.00 | 0.367 | 7.35 |
| succinic | 0.4–1000.0 | $y = 0.0088x + 0.0652$ | 0.998 | 0.021 | 0.42 | 0.158 | 3.15 |
| fumaric | 1.0–1000.0 | $y = 0.0069x + 0.0567$ | 0.997 | 0.540 | 10.8 | 1.87 | 37.5 |

[a] All data were obtained by analyzing standard mixtures prepared in 2% metaphosphoric acid.

Rellán-Álvarez et al. (2011) J Agric Food Chem

# Dentro de una clase: Análisis "targeted" (HPLC-MS)

**Metabolitos**

HPLC-MS

**Repetibilidad: Intra-día, inter-día**

Table 3. Intraday ($n$ = 10) and Interday ($n$ = 5) Re
ities of the HPLC−ESI-TOFMS Method[a]

| organic acid | | intraday mean | intraday RSD (%) | interday mean | interday RSD (%) |
|---|---|---|---|---|---|
| oxalic | RT | 5.36 | 0.15 | 5.35 | 0.22 |
| | $A_S/A_{IS}$ | 0.06 | 1.56 | 0.05 | 3.70 |
| *cis*-aconitic | RT | 5.87 | 0.08 | 5.87 | 0.06 |
| | $A_S/A_{IS}$ | 1.15 | 1.47 | 1.04 | 3.19 |
| 2-oxoglutaric | RT | 6.48 | 0.04 | 6.47 | 0.07 |
| | $A_S/A_{IS}$ | 0.61 | 2.28 | 0.66 | 2.27 |
| citric | RT | 7.42 | 0.04 | 7.41 | 0.13 |
| | $A_S/A_{IS}$ | 1.74 | 1.62 | 1.73 | 0.99 |
| malic | RT | 9.18 | 0.04 | 9.17 | 0.11 |
| | $A_S/A_{IS}$ | 0.82 | 2.32 | 0.85 | 2.91 |
| quinic | RT | 9.64 | 0.05 | 9.64 | 0.08 |
| | $A_S/A_{IS}$ | 1.06 | 0.84 | 0.88 | 4.14 |
| ascorbic | RT | 9.99 | 0.06 | 9.97 | 0.35 |
| | $A_S/A_{IS}$ | 0.22 | 2.74 | 0.25 | 19.9 |
| shikimic | RT | 12.2 | 0.03 | 12.2 | 0.10 |
| | $A_S/A_{IS}$ | 1.08 | 1.92 | 1.08 | 4.41 |
| succinic | RT | 12.1 | 0.05 | 12.0 | 0.10 |
| | $A_S/A_{IS}$ | 0.53 | 0.52 | 0.51 | 1.36 |
| fumaric | RT | 13.2 | 0.06 | 13.2 | 0.16 |
| | $A_S/A_{IS}$ | 0.730 | 1.11 | 0.72 | 1.52 |

[a] A 100 $\mu$M standard solution containing all ten organic acids and the two internal standards was used. RT = retention time, $A_S$ = area of the standard peak, and $A_{IS}$ = area of the internal standard peak.

**Recuperaciones cercanas al 100%**

Table 4. Recoveries (%) Obtained for the Different Organic Acids in Sugar Beet Leaf Extracts, Tomato Xylem Sap, and Commercial Orange Juice[a]

| organic acid | sugar beet leaves | tomato xylem sap | |
|---|---|---|---|
| oxalic | 107.8 ± 3.6 | 46.5 ± 1.0 | |
| *cis*-aconitic | 99.2 ± 1.7 | 102.8 ± 2.3 | 95.8 ± 2.0 |
| 2-oxoglutaric | 66.2 ± 0.9 | 68.8 ± 1.8 | 43.9 ± 0.7 |
| citric | 100.0 ± 1.8 | 109.6 ± 2.2 | 96.7 ± 1.5 |
| malic | 104.0 ± 1.4 | 100.6 ± 1.0 | 101.0 ± 0.5 |
| quinic | 98.0 ± 0.4 | 96.7 ± 2.4 | 98.2 ± 1.4 |
| ascorbic | 21.5 ± 0.4 | 85.5 ± 1.9 | 76.4 ± 0.5 |
| shikimic | 103.2 ± 0.8 | 97.4 ± 2.5 | 91.9 ± 1.9 |
| succinic | 100.4 ± 0.6 | 96.8 ± 2.5 | 96.4 ± 2.5 |
| fumaric | 93.2 ± 0.5 | 94.8 ± 3.1 | 93.5 ± 2.7 |

[a] Results are means ± SE ($n$ = 5).

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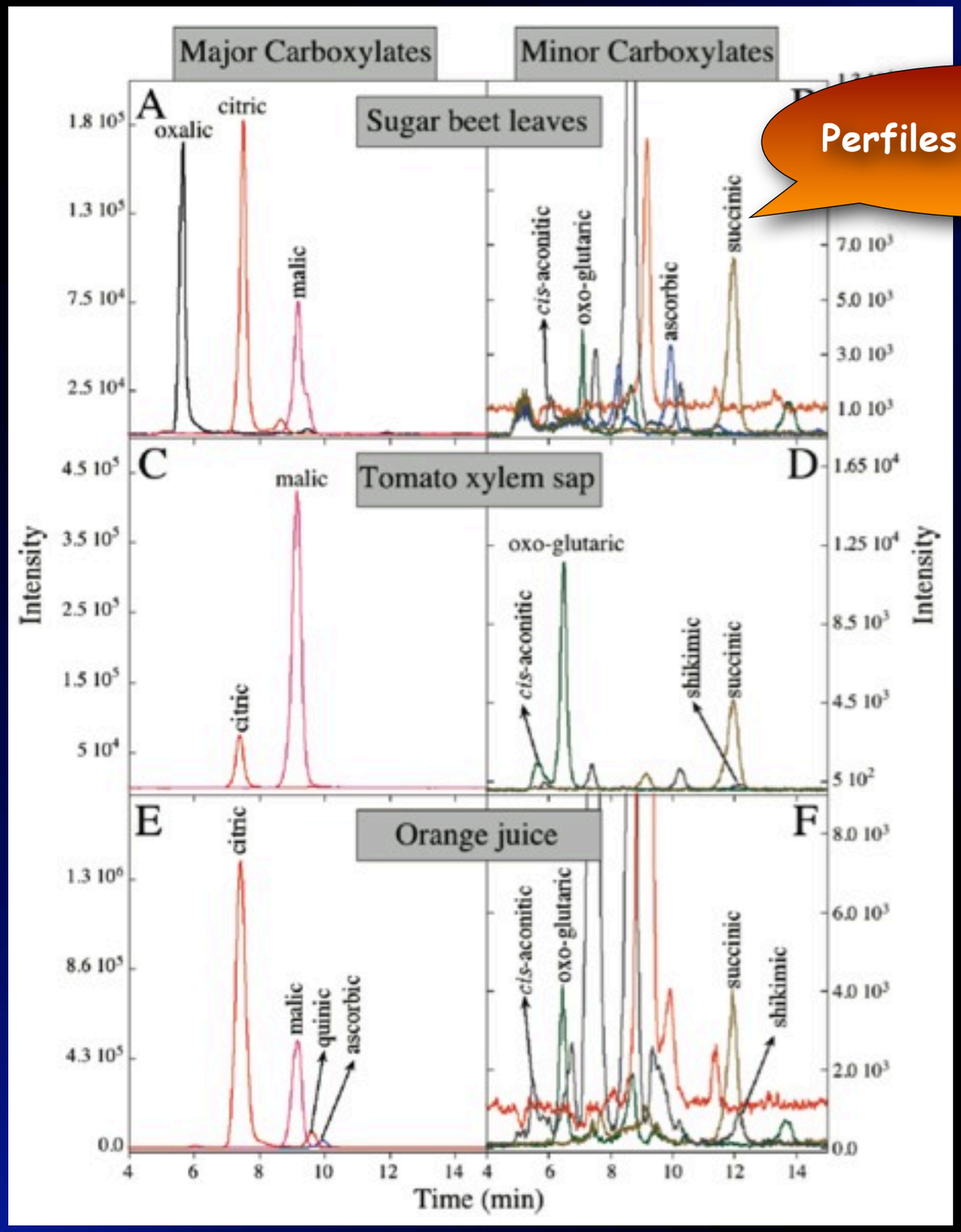
# Dentro de una clase: Análisis "targeted" (HPLC-MS)

**Metabolitos**

HPLC-MS

Carboxilatos




**Perfiles metabólicos**

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J Agric Food Chem

# Análisis metabolómico "untargeted" y"high throughput" (GC-MS)

Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**

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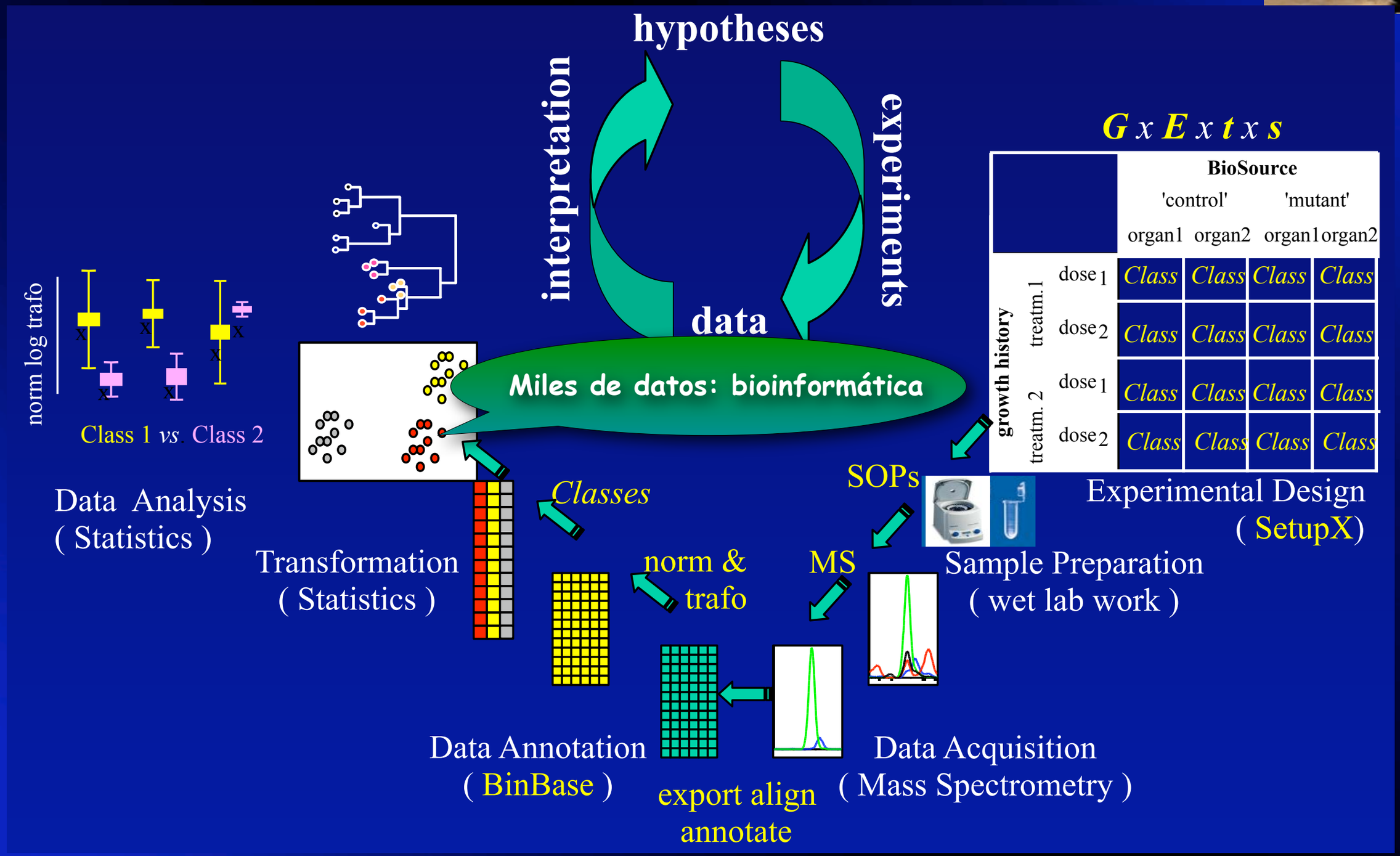
# Análisis metabolómico "untargeted" y"high throughput" (GC-MS)

Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**



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**Metabolitos**

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Plant Stress Physiology

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# Análisis metabolómico "untargeted" y"high throughput" (GC-MS)

Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**

**En busca de cambios consistentes**

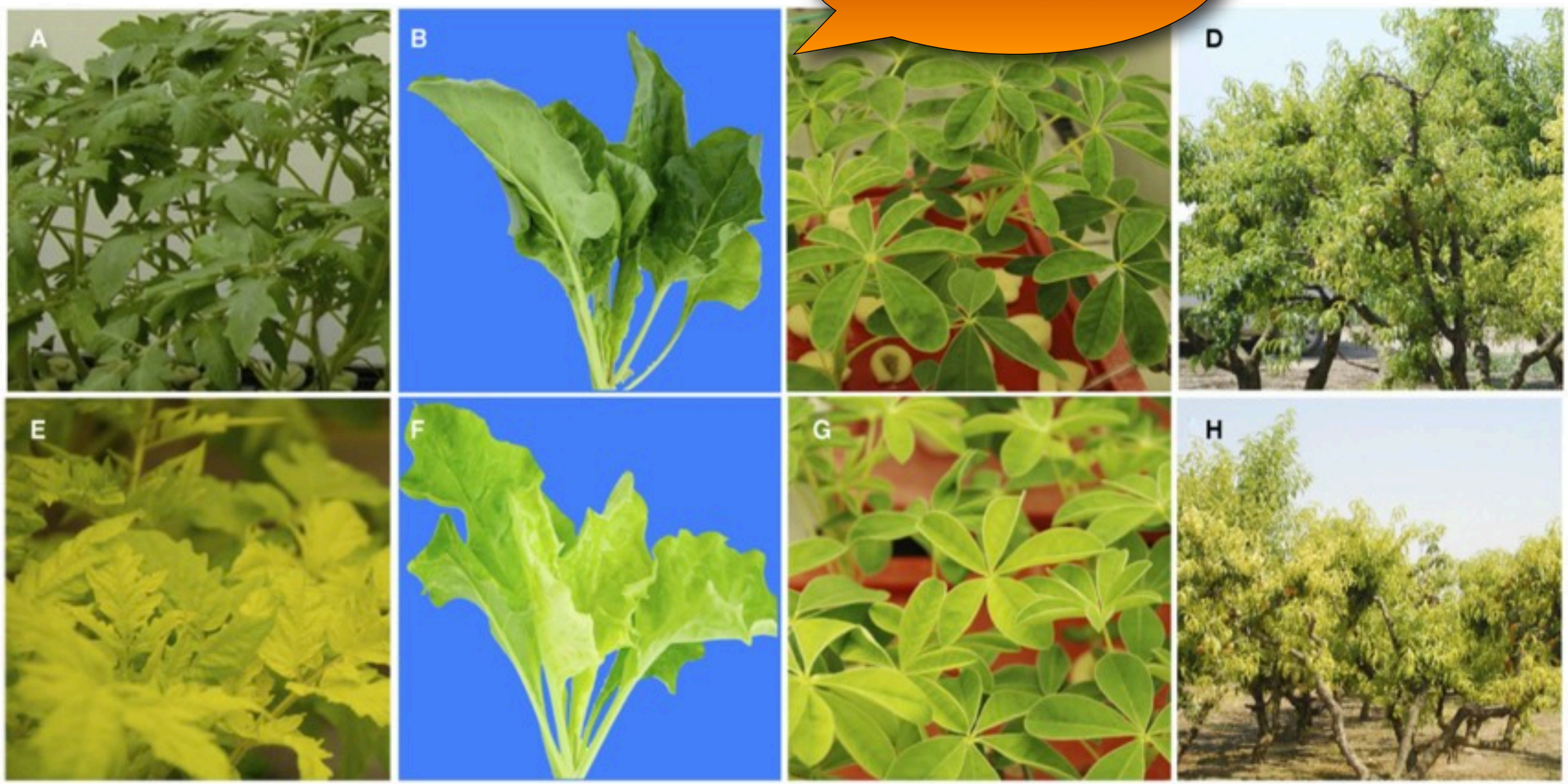


**FIGURE 1 | Plant appearance at sampling time**. Plants grown in Fe-sufficient conditions: **(A)** tomato, **(B)** sugar beet, **(C)** lupine, and **(D)** peach tree. Plants grown in Fe-deficient conditions: **(E)** tomato, **(F)** sugar beet, **(G)** lupine, and **(H)** peach trees.

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# Análisis metabolómico "untargeted" y"high throughput" (GC-MS)

Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**

**Table 1 | Main effects of iron deficiency on xylem sap metabolite levels.**

| Tomato | | Lupine | | | |
|---|---|---|---|---|---|
| **AMINOACID AND NITROGEN METABOLISM** | | | | | |
| Suberyl glycine (57) | 7.1 | Maleic acid (38) | | | |
| Tryptophan (68) | 6.4 | Shikimic acid (60) | | | |
| Hydroxyglutaric acid (31) | 4.6 | Spermidine (42) | | | |
| Maleic acid (38) | 4.0 | Tyrosine (70) | | | |
| Asparagine (6) | 2.5 | Glycine (7) | | | |
| Serine (59) | −2.0 | Tryptophan (68) | | | |
| Alanine (3) | −2.3 | Alanine (3) | | | |
| Phenylalanine (50) | −2.8 | Oxoproline (48) | | | |
| Urea (71) | −2.9 | Beta alanine (9) | | | |
| | 3.0 | Putrescine (52) | | | |
| | 3.1 | | | | |
| | 3.1 | | | | |
| | 3.2 | | | | |
| | 3.6 | | | | |
| | 3.8 | | | | |
| | 3.9 | | | | |
| | 4.2 | | | | |
| | 4.3 | | | | |
| | 5.1 | | | | |
| | 6.7 | | | | |
| | 11.8 | | | | |
| | | | | | |
| | 91 | Aconitic acid (2) | | | |
| | 6.2 | Succinic acid (62) | | | |
| | 5.7 | | | | |
| Succinic acid (62) | 4.7 | | | | |
| Aconitic acid (2) | 2.8 | | | | |
| **CARBOHYDRATE METABOLISM** | | | | | |
| *S*-glycine (62) | 7.1 | Trehalose (67) | −2.3 | Gluconic acid (22) | −2.1 |
| *N*-ac-d-mannosamine (16) | −2.6 | | | | |
| Sucrose (63) | −2.7 | | | | |
| Xylose (73) | −3.8 | | | | |
| Galacturonic acid (21) | −4.1 | | | | |
| Glucuronic acid (24) | −4.2 | | | | |
| Galactinol (19) | −4.7 | | | | |
| Fructose (14) | −5.2 | | | | |
| Inulobiose (33) | −5.2 | | | | |
| Saccharic acid (58) | −7.1 | | | | |
| Arabinose (5) | −7.5 | | | | |
| **GLYCOLYSIS AND PENTOSE PHOSPHATE METABOLISM** | | | | | |
| Glucose-6-P (23) | −5.2 | PGA (49) | −2.5 | PGA (49) | −3.9 |

**Xilema**
tomate/lupino/melocotonero
253/233/251 resueltos
77/83/77 identificados
11/3/1 aumentan
32/16/5 descienden

**Hoja**
tomate/remolacha/melocotonero
254/113/239 resueltos
65/82/92 identificados
12/29/4 aumentan
8/4/3 descienden

Rellán-Álvarez et al. (2011)
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# Análisis metabolómico "untargeted" y"high throughput" (GC-MS)

Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**

**Componentes principales: se diferencian bien control y deficiente en Fe**

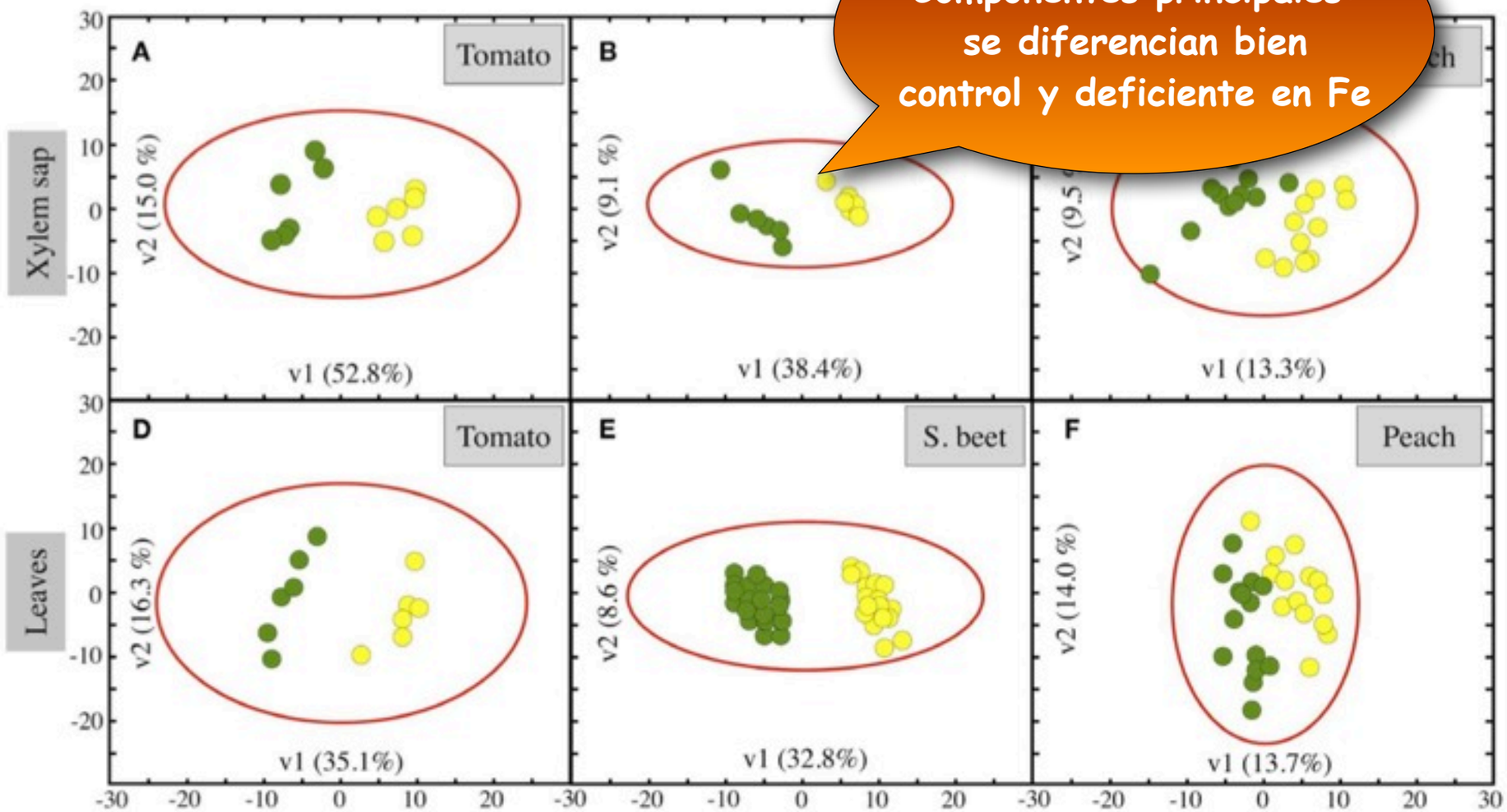


**FIGURE 2 | Partial least square (PLS) analysis of xylem sap and leaf extract metabolites as affected by Fe deficiency**. Score scatter plot of PLS vector 1 (v1) vs. PLS vector 2 (v2) of all detected metabolites (identified ones plus unknowns) in control (green circles) and Fe-deficient (yellow circles) samples. Xylem sap of **(A)** tomato, **(B)** lupine, and **(C)** peach tree. Leaf extracts of **(D)** tomato, **(E)** sugar beet, and **(F)** peach tree. The percentage of variability explained by each vector is indicated in parenthesis in the corresponding axes.

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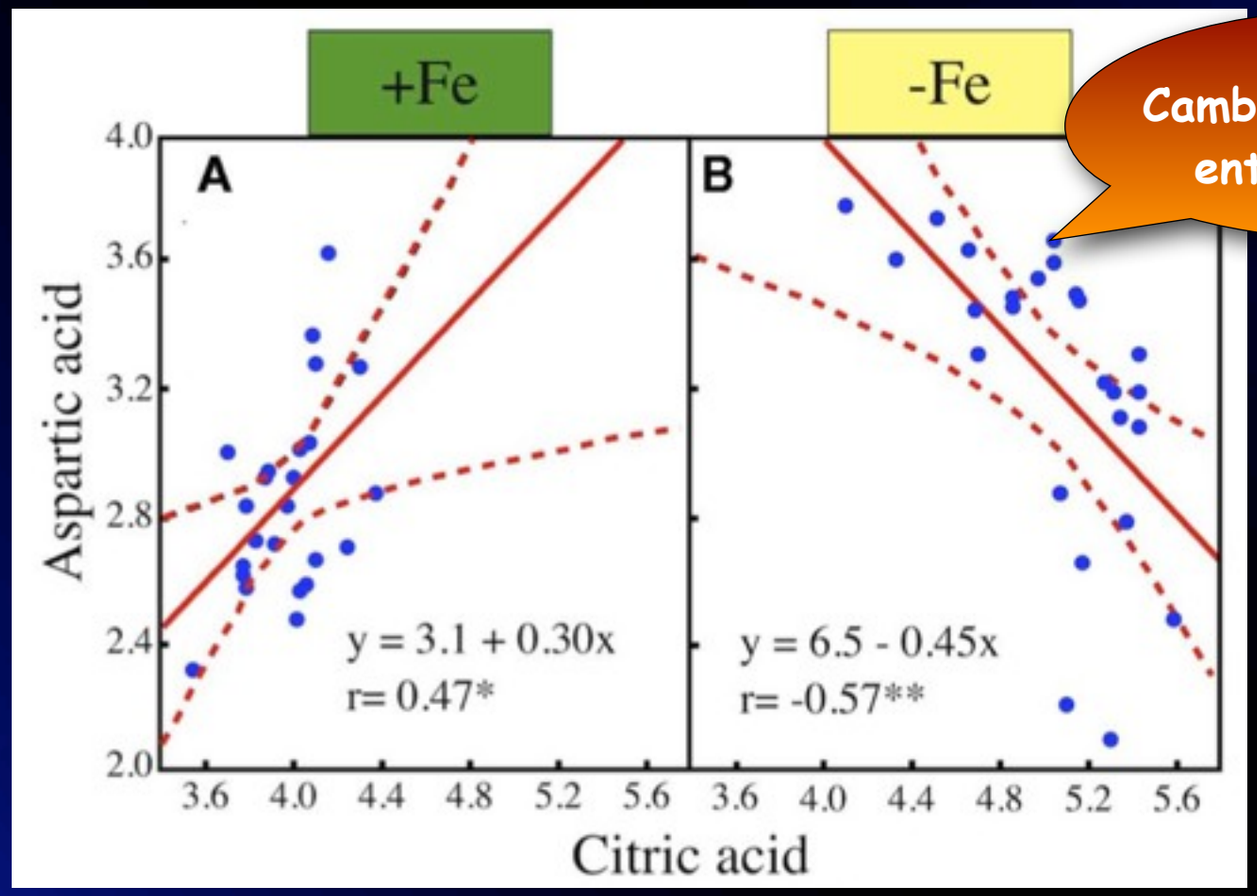
Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**




**Cambian las relaciones entre metabolitos**

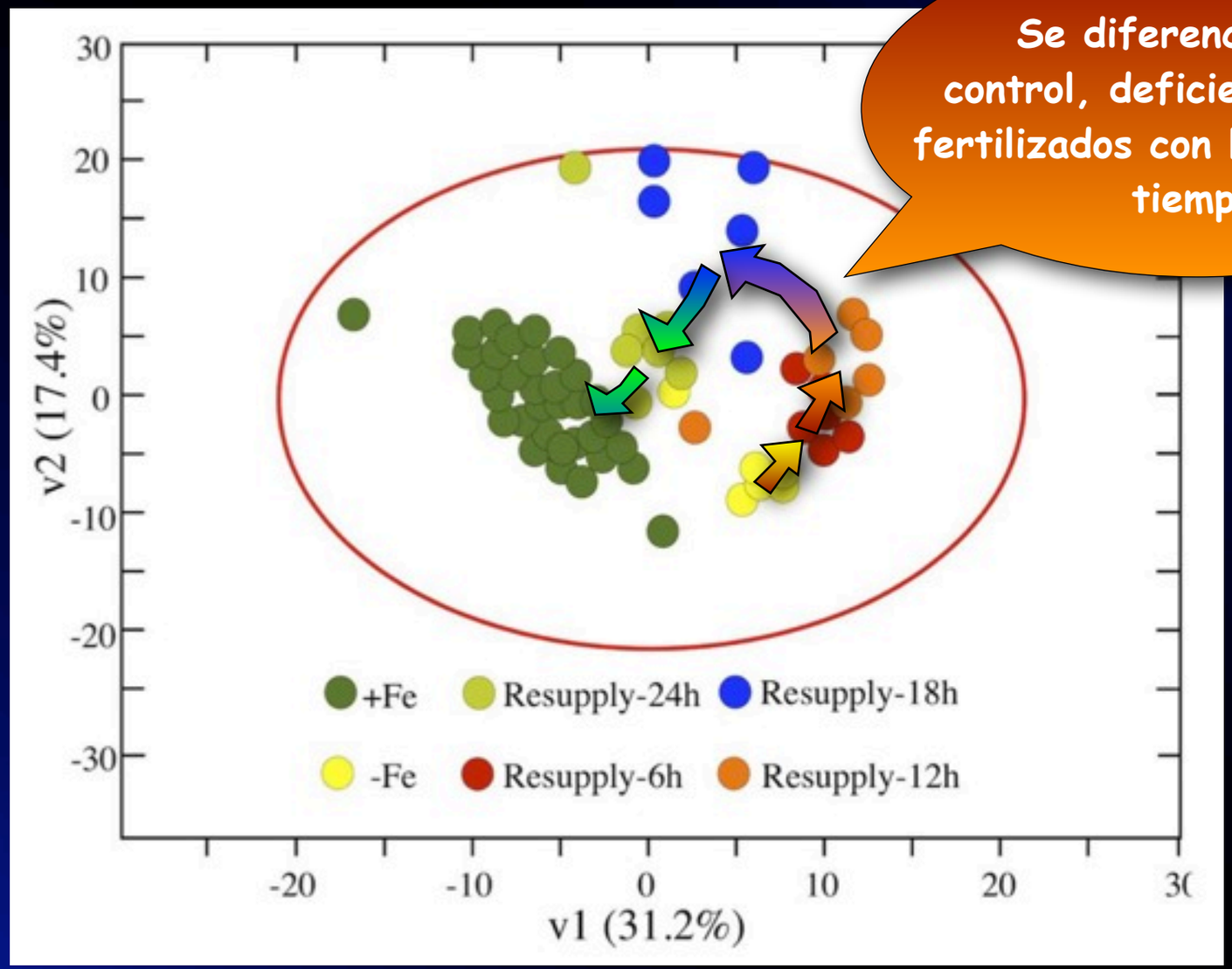
Rellán-Álvarez et al. (2011) Frontiers Plant Sci

# Análisis metabolómico "untargeted" y"high throughput" (GC-MS)

Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**

**Se diferencian bien control, deficiente en Fe y fertilizados con Fe a distintos tiempos**




Rellán-Álvarez et al. (2011) Frontiers Plant Sci

# Análisis metabolómico "untargeted" y"high throughput" (GC-MS)

Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**

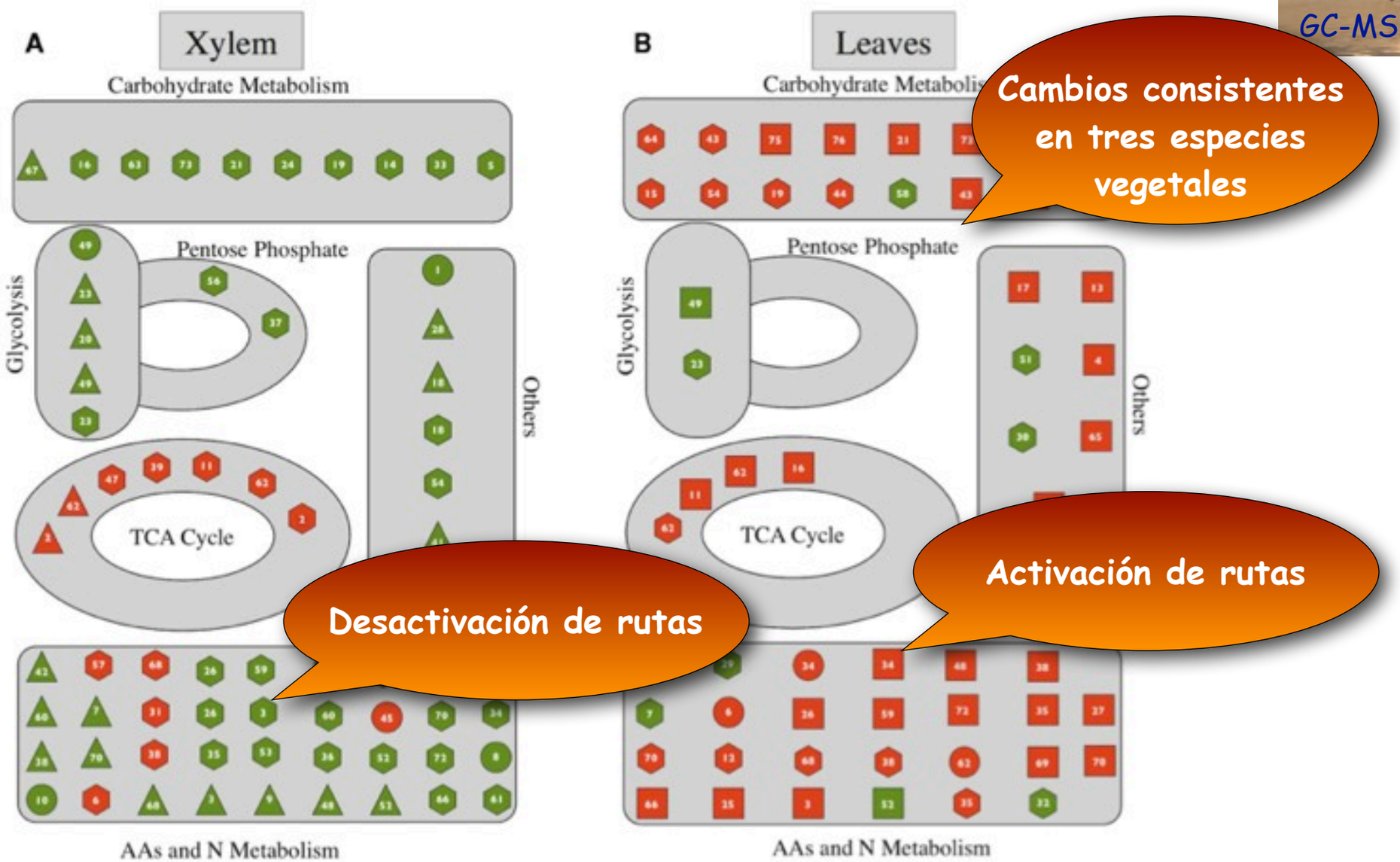



**FIGURE 7 | Changes in metabolic pathways as affected by Fe deficiency.** **(A)** Xylem sap. **(B)** Leaf extracts. Red and green symbols indicate significant ($p \leq 0.01$) increases and decreases, respectively. Octagon, circles, triangles, and squares are for tomato, sugar beet, lupine, and peach, respectively. The numbers inside each symbol correspond to metabolites shown in **Tables 1** and **2**.

Rellán-Álvarez et al. (2011) Frontiers Plant Sci

biotecnología master

Plant Stress Physiology

CSIC

# Análisis metabolómico "untargeted" y"high throughput" (GC-MS)

Metabolomics, Fiehn's lab, UC Davis

**Metabolitos**

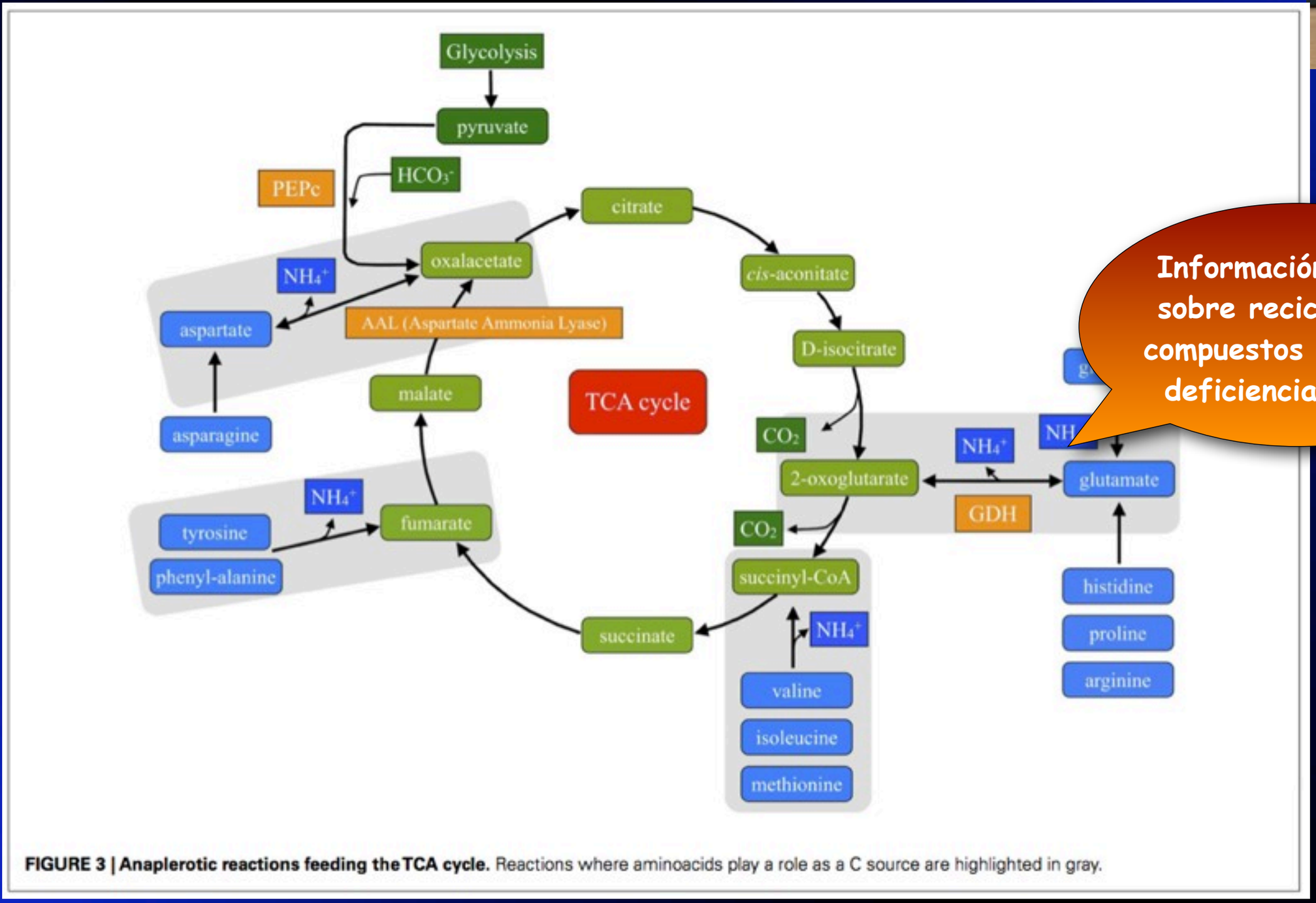



**FIGURE 3 | Anaplerotic reactions feeding the TCA cycle.** Reactions where aminoacids play a role as a C source are highlighted in gray.

**Información nueva sobre reciclaje de compuestos de N en deficiencia de Fe**

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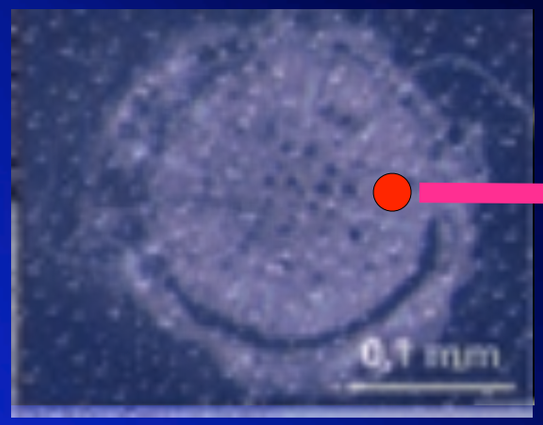
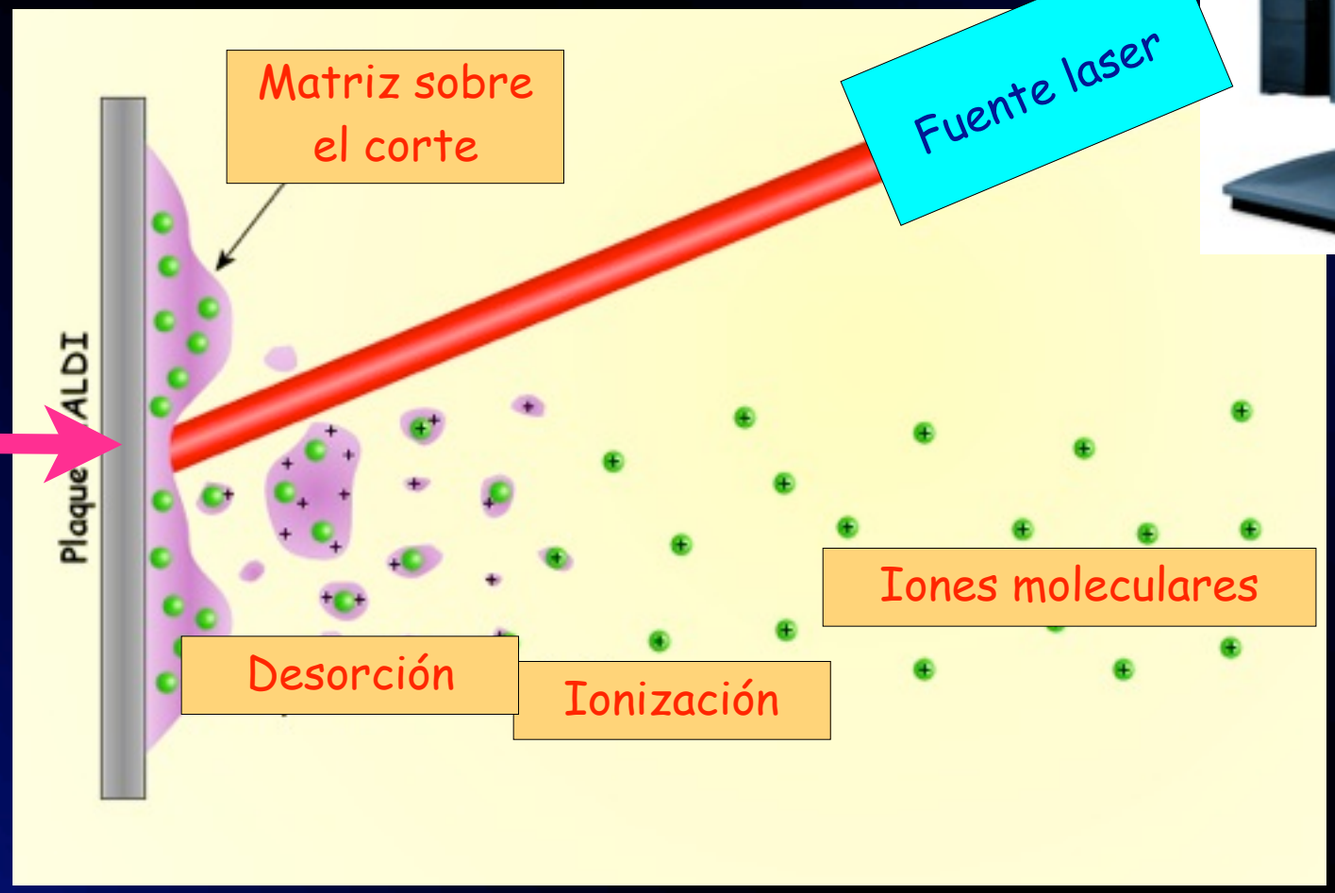
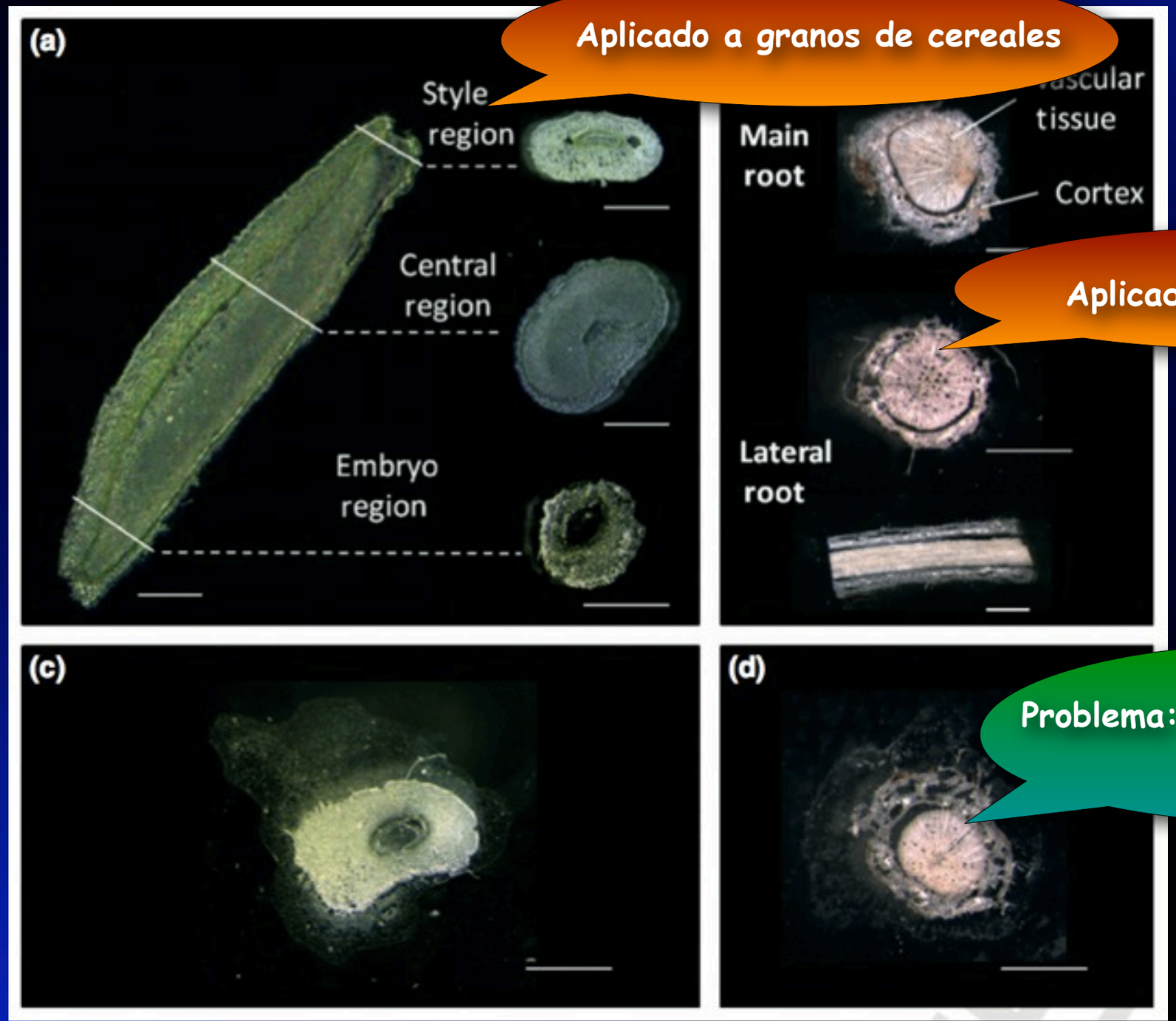
MS

**Limitación:**
**resolución de unas 20 µm**



MS




Aplicado a granos de cereales

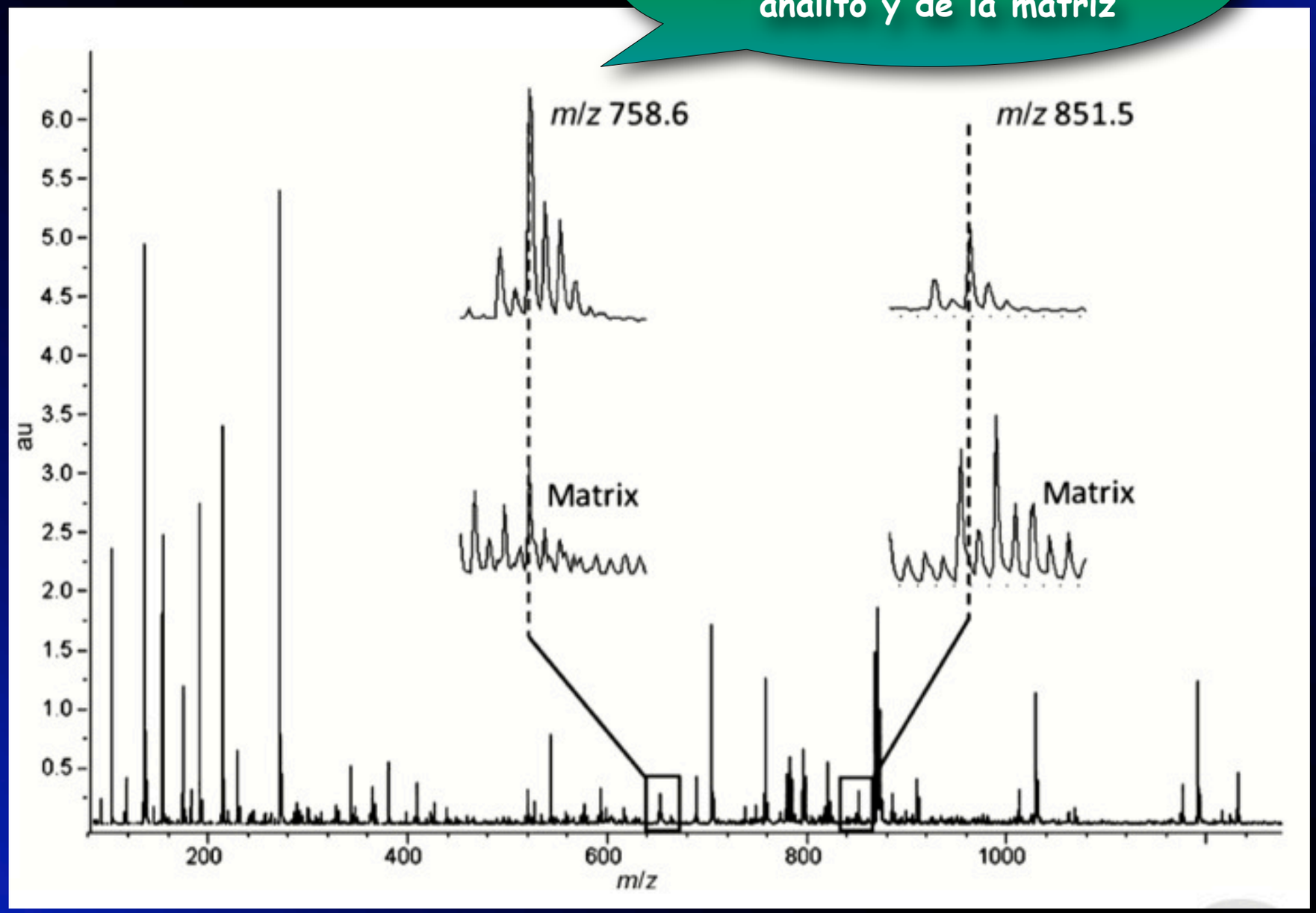
Aplicado a cortes de raíz

Peukert et al.

Problema: cómo obtener secciones de raíz

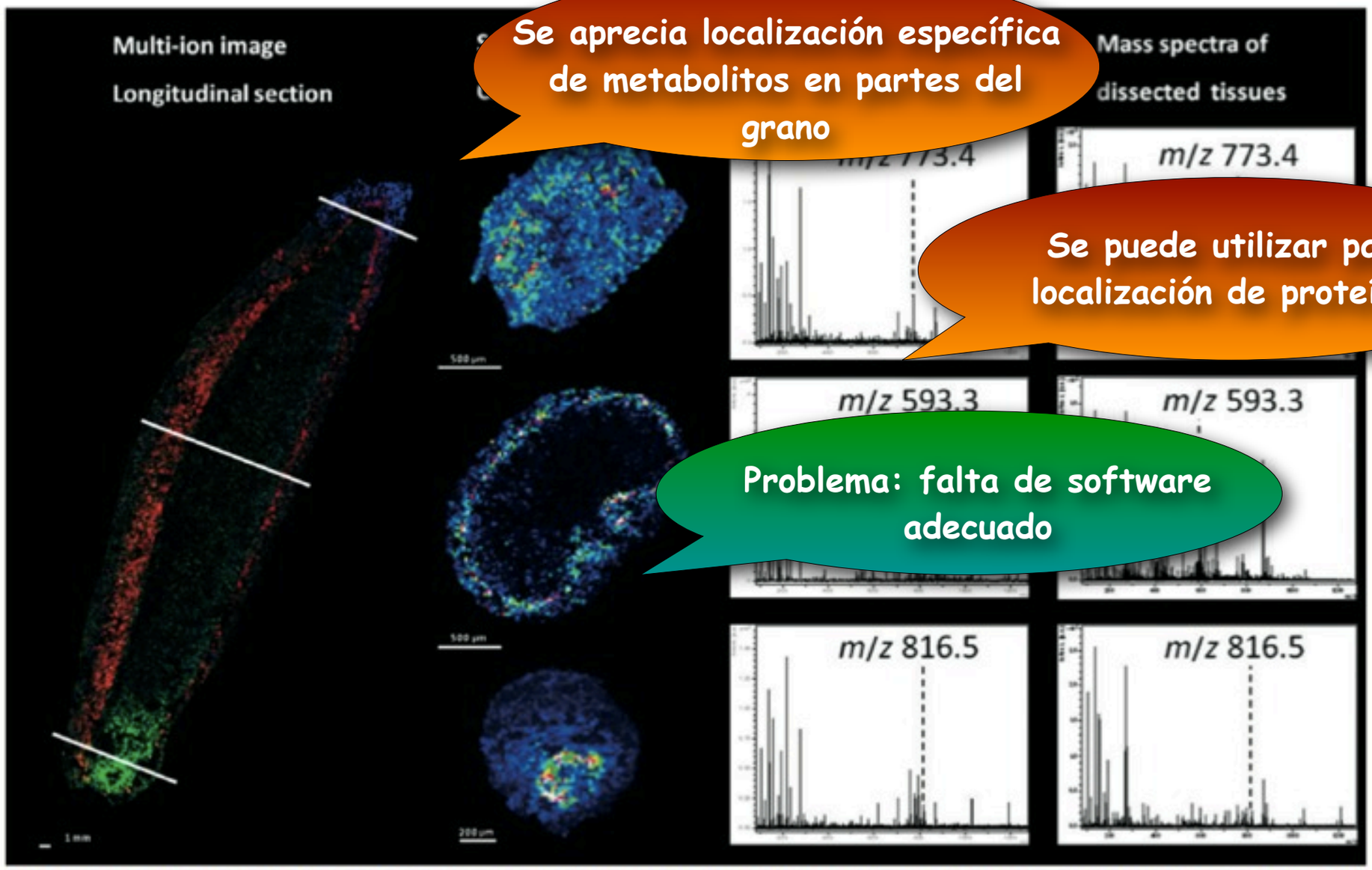
MS

Hay que diferenciar espectro de analito y de la matriz




Peukert et al. (2011) New Phytol

MS




Se aprecia localización específica de metabolitos en partes del grano

Se puede utilizar para localización de proteínas

Problema: falta de software adecuado

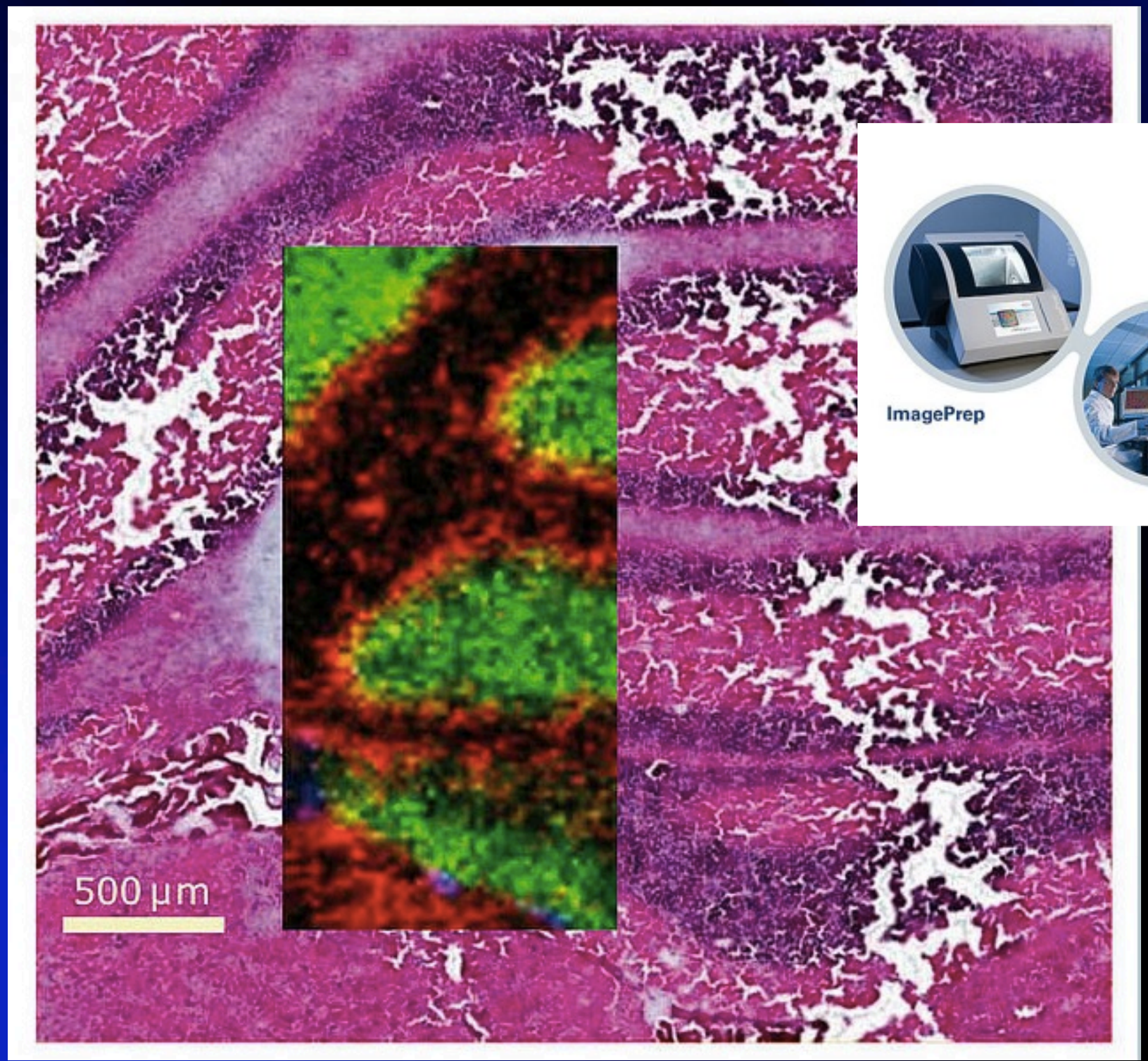
**Fig. 4** Reproducibility of matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) across tissues, showing the spectra obtained from 30-µm sections of a 7-d after pollination (DAP) developing barley (*Hordeum vulgare*) grain. The multi-ion image shows molecular masses *m/z* 773.4 (blue), *m/z* 593.3 (red) and *m/z* 816.5 (green) from a longitudinal section (left), the corresponding selected-ion images (centre) and the respective mass spectra with molecular masses indicated by a dotted line (right). The resolution was 25 µm for the longitudinal section, the cross-section of the scar and the central region, and 20 µm for the embryo cross-section.

Peukert et al, New Phytol 2011

# Laser imaging



MS

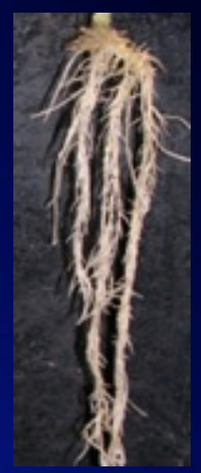


# Análisis de proteínas: proteómica

| | |
|---|---|
| Técnicas 2-DE | IEF-SDS/PAGE |
| | Blue native (PAGE-SDS/PAGE) |
| | DIGE |
| Técnicas HPLC-MS | iTraq |
| | ICAT |
| | Label free shotgun proteomics |
| Otras técnicas | Image analysis |

"Pescando" cambios desconocidos

## Extracción de proteínas



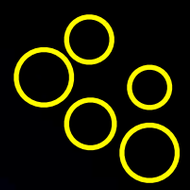
Proteínas

¿Se extractan todas?

¿Se solubilizan todas?

¿No se modifican?

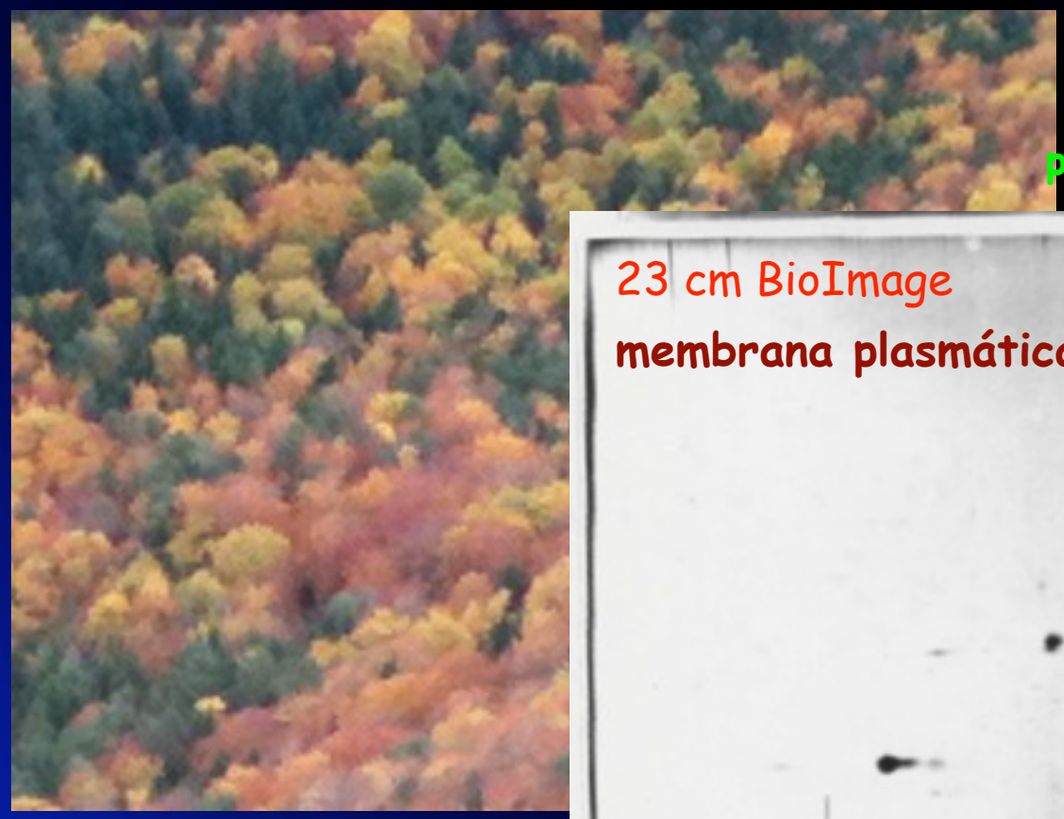
Dominios dentro de las fracciones

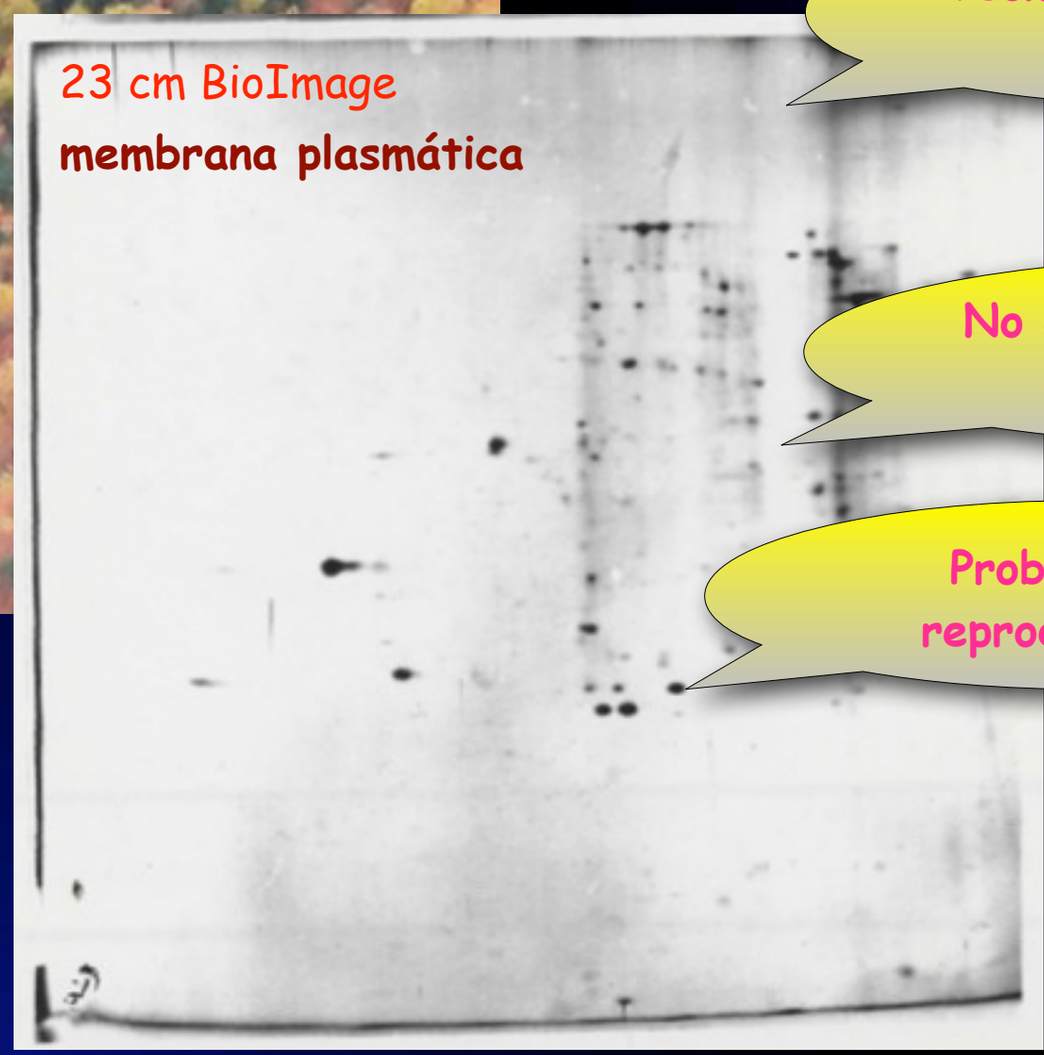


Fracciones subcelulares

# IEF-SDS/PAGE

## Historia en nuestro labo






Técnica muy laboriosa: IEF en tubo

No existía la posiblidad de identificar "spots"

Problemas de reproducibilidad

Santos Susín, 1994, Tesis Doctoral UZ

# IEF-SDS/PAGE

## Historia en nuestro labo

23 cm BioImage

¿Se pierden en la separación?

Técnica muy laboriosa: IEF en tubo

Problemas de reproducibilidad

¿Cuánta proteína hay que cargar?

Alguna identificación por anticuerpos, amino terminal bloqueado

¿Cuál es la mejor tinción?

¿Qué diseño estadístico hay que utilizar para el experimento y el análisis?

kDa

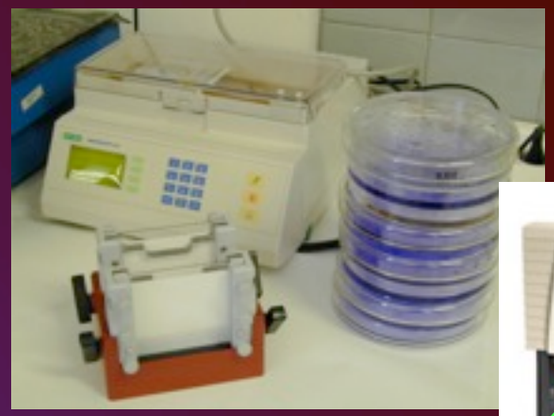
Elena González-Vallejo, 1999, Tesis Doctoral UZ

Técnicas 2-DE
EEAD-CSIC, Zaragoza

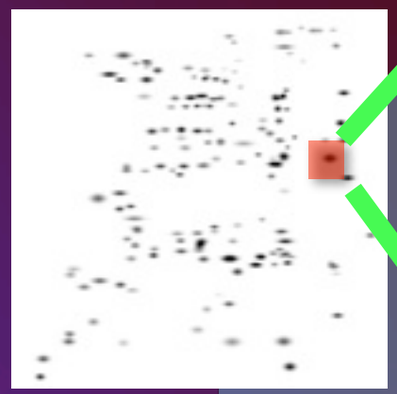
# IEF-SDS/PAGE

**Proteínas**

## 2-DE: strips IEF y geles 7 cm, Coomasie coloidal

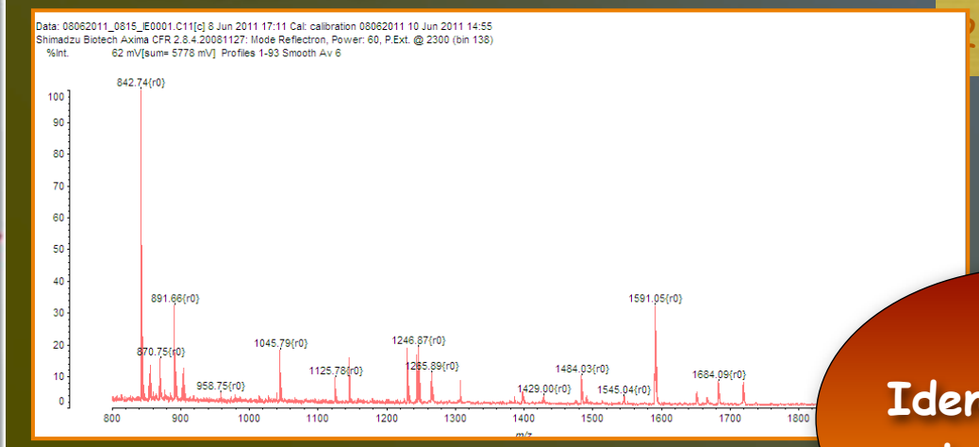


Digestión con tripsina



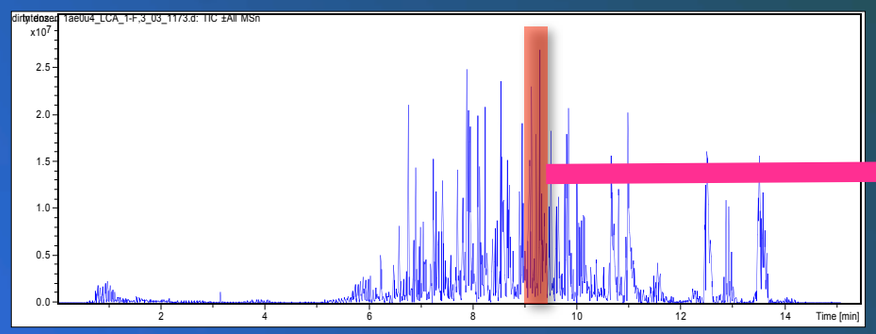
Cortador de spots

## MALDI-TOF UPM/INIA J. Kehr

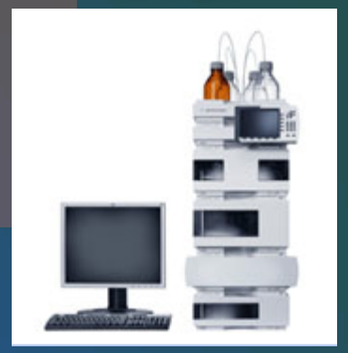



**Identificación de "spots"**

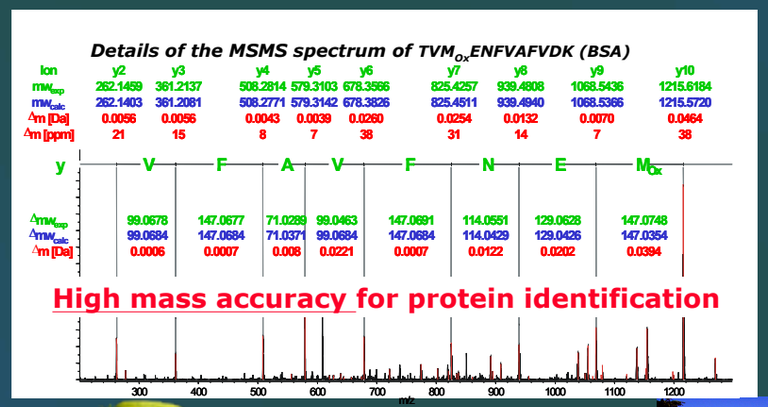
Digestión con tripsina




nHPLC



## $MS^n$ (trampa iónica)

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# IEF-SDS/PAGE

Proteínas

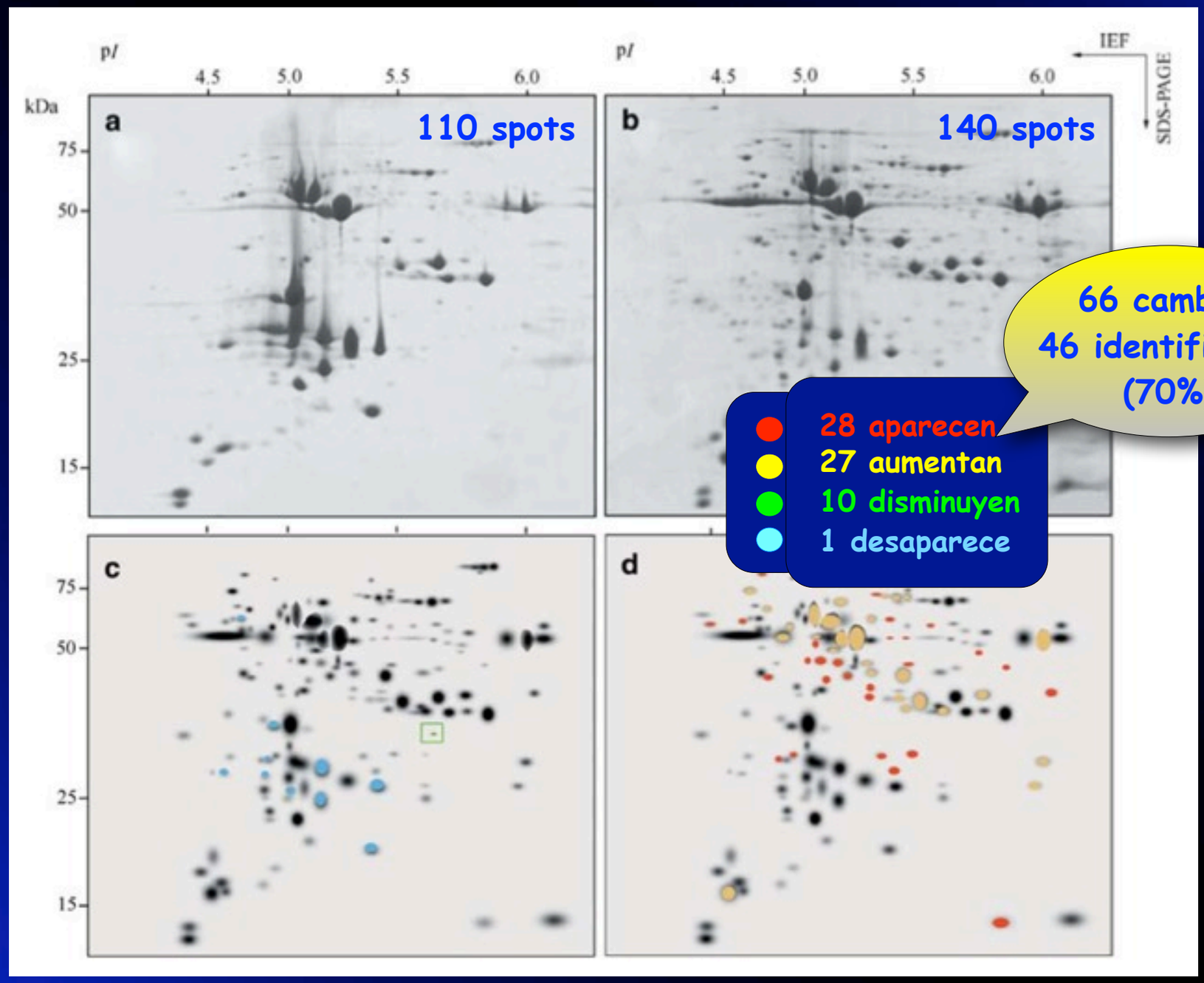
Beta vulgaris tilacoides

+ Fe

- Fe

IEF

SDS-PAGE




66 cambian
46 identificados
(70%)

- 28 aparecen
- 27 aumentan
- 10 disminuyen
- 1 desaparece

Andaluz et al. (2006)
Photosynth Res

# IEF-SDS/PAGE

Proteínas

Toxicidad de metales

2-DE

Solanum lycopersicum
Hidropónico

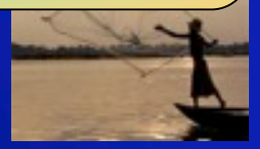
control | 10 µM Cd | 100 µM Cd

López-Millán et al. (2009) Environ Exp Bot

# IEF-SDS/PAGE

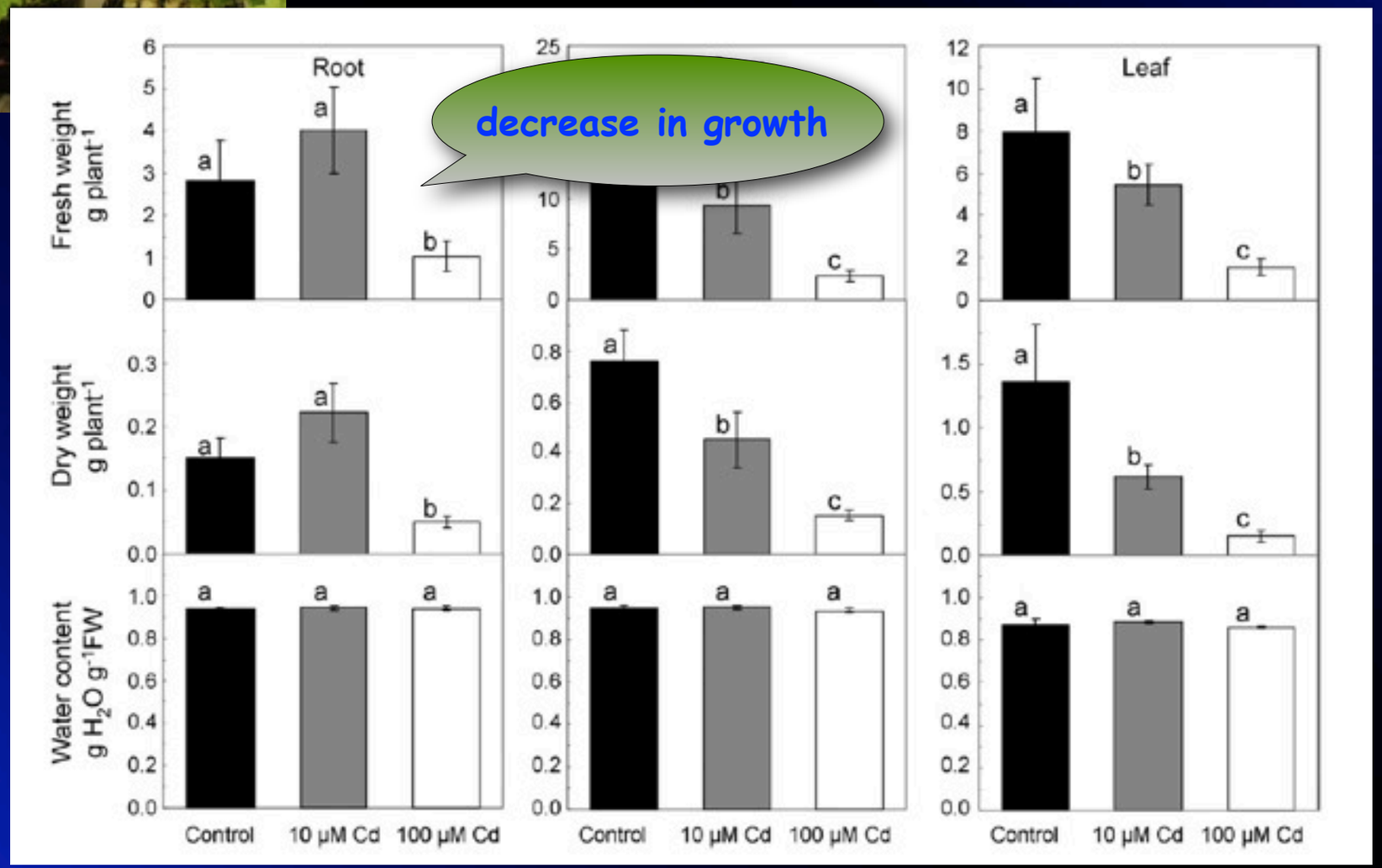
Proteínas

Toxicidad de metales



Solanum lycopersicum
Hidropónico

**Muy importante: conocer el material**




López-Millán et al. (2009) Environ Exp Bot

# IEF-SDS/PAGE

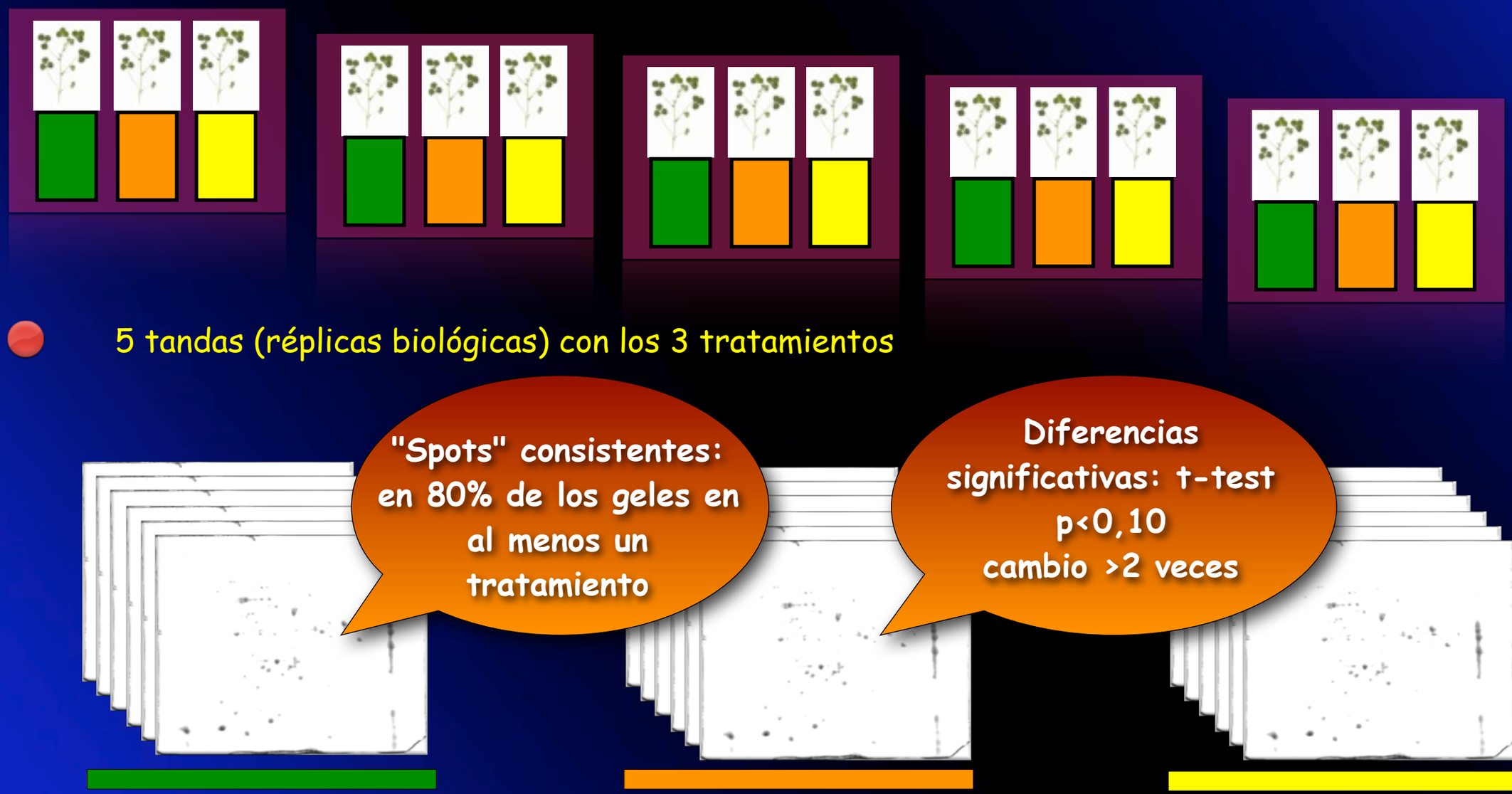


Solanum lycopersicum
Hidropónico

control 10 µM Cd 100 µM Cd

Rodríguez-Celma et al. (2010) J Proteomics

# Diseño experimental para proteómica diferencial 2-DE IEF-SDS/PAGE




- 5 tandas (réplicas biológicas) con los 3 tratamientos
- 1 gel (7 cm BioRad) por réplica biológica (5 reps. biol. x 3 tratamientos, 15 geles en total)

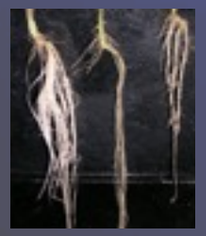
# IEF-SDS/PAGE

Proteínas

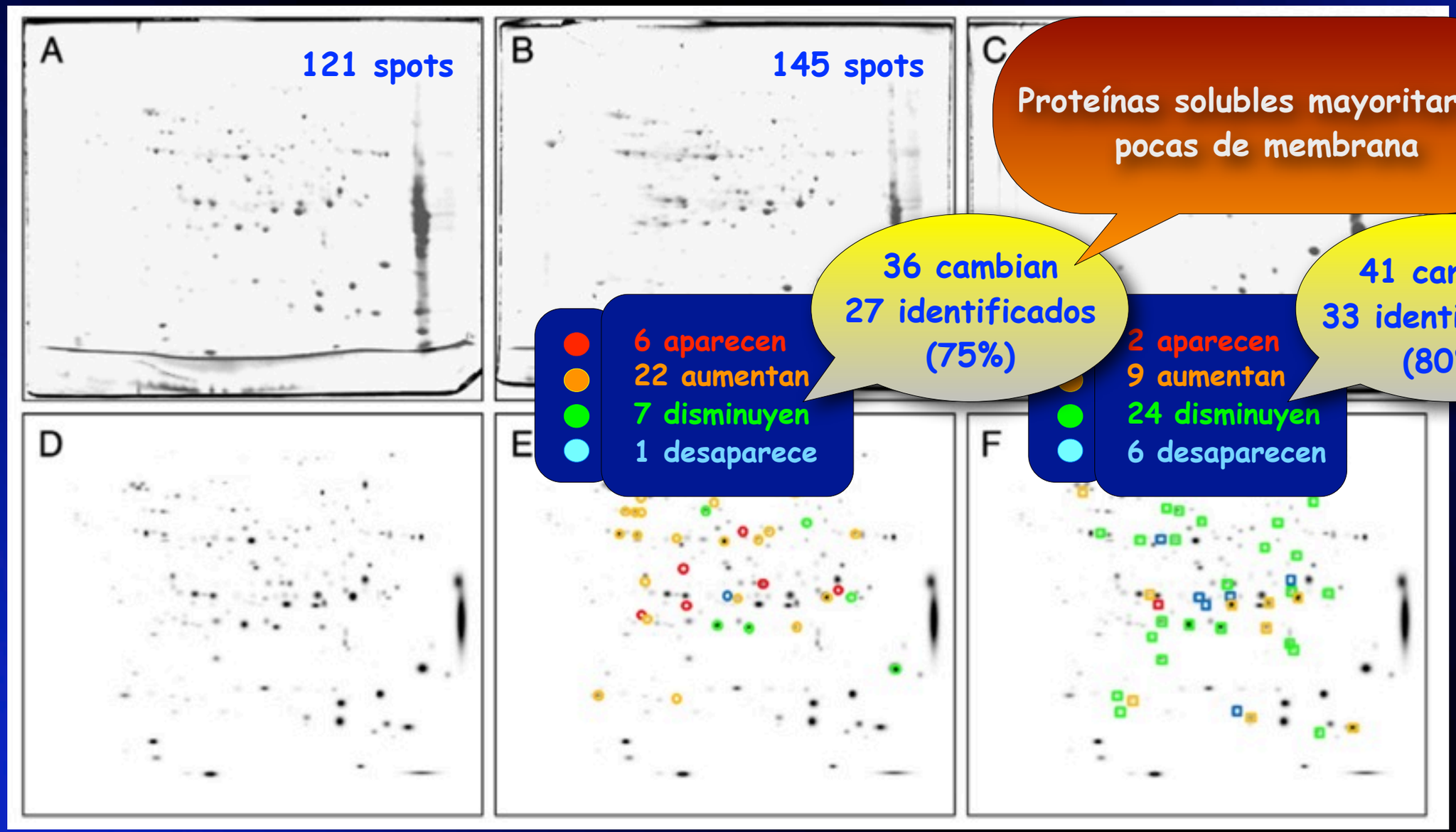
Toxicidad de metales

Solanum lycopersicum

raíces



Control | 10 µM Cd | 100 µM Cd




Rodríguez-Celma et al. (2010) J Proteomics

# IEF-SDS/PAGE

Proteínas

Toxicidad de metales

Solanum lycopersicum

raíces

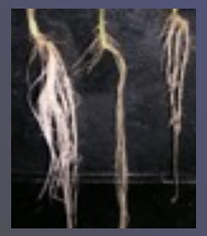


**Table 1 – Proteins identified in 2-DE IEF–SDS PAGE gels. MS[1] Protein score is –10*Log (P), where P is the probability that the observed match is a random event. Protein scores >76 were considered significant ($p<0.05$). – indicates no changes in relative abundance.**

| No. | Teor. MW/pI | Exp. MW/pI | vs. 10 Cd/ vs. 100 Cd | Cov. | Score/pep/ion | ID | Homology | Species | Matched pathway in KEGG |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 62/5.2 | 67/5.8 | +3.0/– | 44 | 240/20/3 | gi\|300265 | HSP68 heat stress DNAK homolog | *Solanum perivarum* | Chaperones and folding catalysts; pores ion channels; MAPK signaling pathway |
| 2 | 38/5.3 | 43/5.9 | +5.6/– | 31 | 139/11/2 | gi\|3219772 | Actin-51 | *Solanum lycopersicum* | Cytoskeleton proteins |
| 3 | 27/5.6 | 28/6.1 | +2.2/– | 75 | 328/15/3 | gi\|77999303 | Proteasome-like protein alpha subunit | *Solanum tuberosum* | Peptidases; proteasome |
| 4 | 42/5.8 | 40/6.6 | +4.5/– | 56 | 459/23/4 | gi\|48478827 | UDP-glucose:protein transglucosylase-like protein Sl-UPTG1 | *Lycopersicon esculentum* | |
| 5 | 48/5.7 | 50/6.4 | +3.0/– | 63 | 890/22/5 | gi\|119354 | Enolase 2-phosphoglycerate-dehydratase | *Solanum lycopersicum* | Glycolysis/gluconeogenesis |
| 6 | 61/7.9 | 52/6.8 | +2.5/– | 41 | 276/21/3 | gi\|27463709 | Neutral leucine aminopeptidase preprotein | *Solanum lycopersicum* | Peptidases |
| 7 | 65/5.8 | 70/7.0 | +2.4/– | 17 | 83/7/2 | gi\|28973653 | Putative TPR-repeat protein | *Arabidopsis thaliana* | Chaperones and folding catalysts |
| 8 | 38/6.6 | 35/7.0 | +2.7/+28.9 | 56 | 481/19/4 | gi\|461978 | Glucan endo 1–3 beta glucosidase A | *Solanum lycopersicum* | Starch and sucrose metabolism |
| 9 | 98/5.8 | 96/7.0 | +3.0/– | 46 | 535/32/4 | gi\|30407706 | Aconitase | *Solanum pennellii* | Citrate cycle (TCA cycle); reductive carboxylate cycle (CO2 fixation); glyoxylate and dicarboxylate metabolism |
| 10 | 70/5.9 | 64/6.4 | +5.0/– | 31 | 210/16/2 | gi\|15240075 | SDH1-1 | *Arabidopsis thaliana* | Citrate cycle (TCA cycle); oxidative phosphorylation |
| 11 | 63/5.7 | 59/5.8 | +11.6/– | 37 | 332/18/5 | gi\|1762130 | Chaperonin 60 beta subunit | *Solanum tuberosum* | Chaperones and folding catalysts |
| 12 | 48/5.7 | 51/6.1 | +4.5/– | 64 | 735/24/5 | gi\|119354 | Enolase 2-phosphoglycerate-dehydratase | *Solanum lycopersicum* | Glycolysis/gluconeogenesis |

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Rodríguez-Celma et al. (2010) J Proteomics

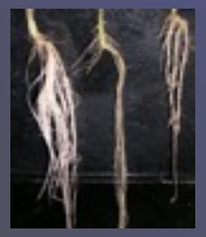
# IEF-SDS/PAGE

Proteínas

Toxicidad de metales

Solanum lycopersicum

raíces



- aparecen
- aumentan
- disminuyen
- desaparecen

10 µM Cd

100 µM Cd

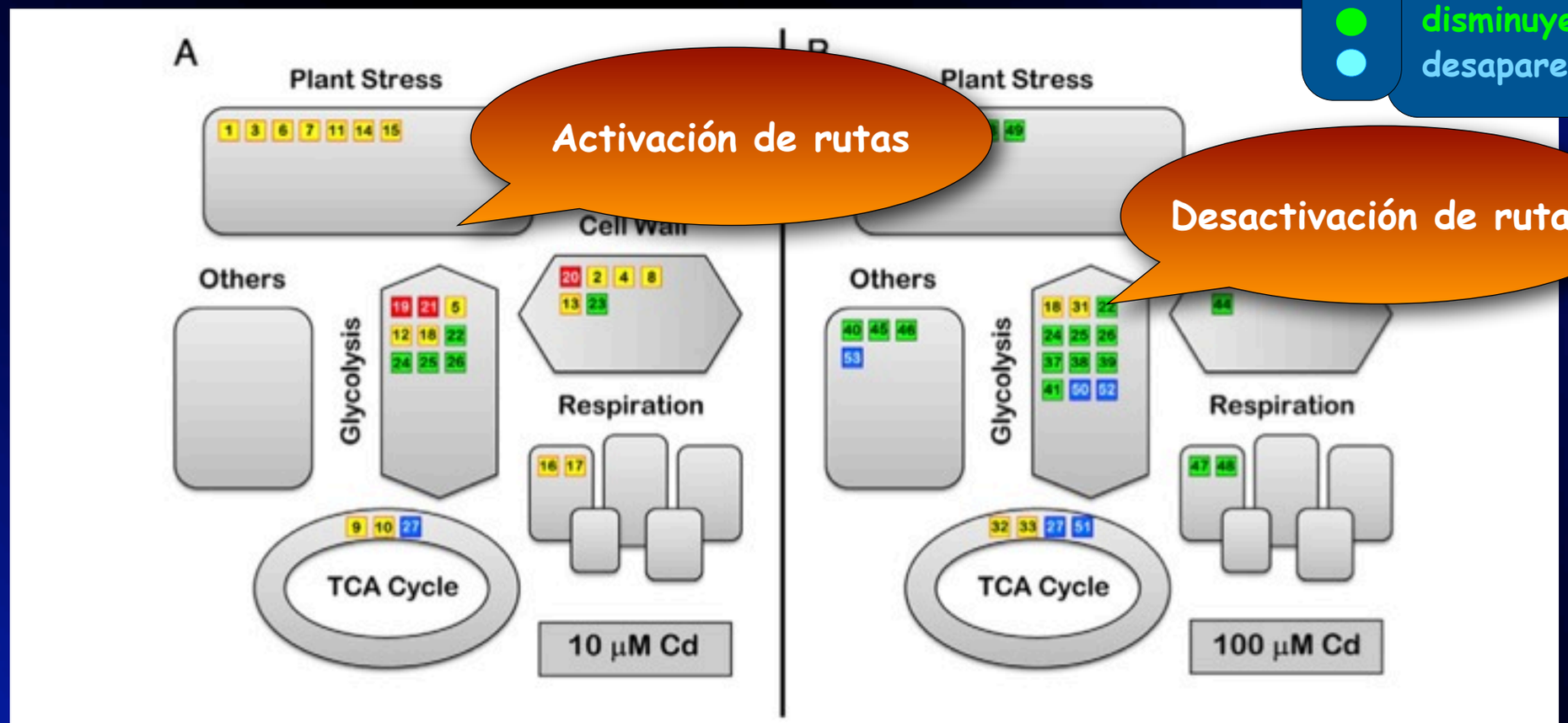



**Fig. 4 – Changes in metabolic pathways as affected by Cd. Panels A and B are for 10 and 100 µM Cd treated plants, respectively. Pathways related to the identified proteins were integrated according to the KEGG database. A statistical Student t-test was performed to show relevant changes between samples. Red symbols mean newly detected proteins in Cd treated roots and yellow symbols proteins showing increases in intensity compared to control (using a 2-fold threshold change). The same threshold (decreases larger than 50%) was selected for proteins showing decreases in intensity (green symbols). Blue symbols indicate proteins not detected in Cd treated roots. Numbers correspond to those in Table 1.**

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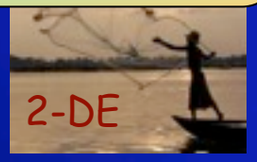
# IEF-SDS/PAGE

Proteínas

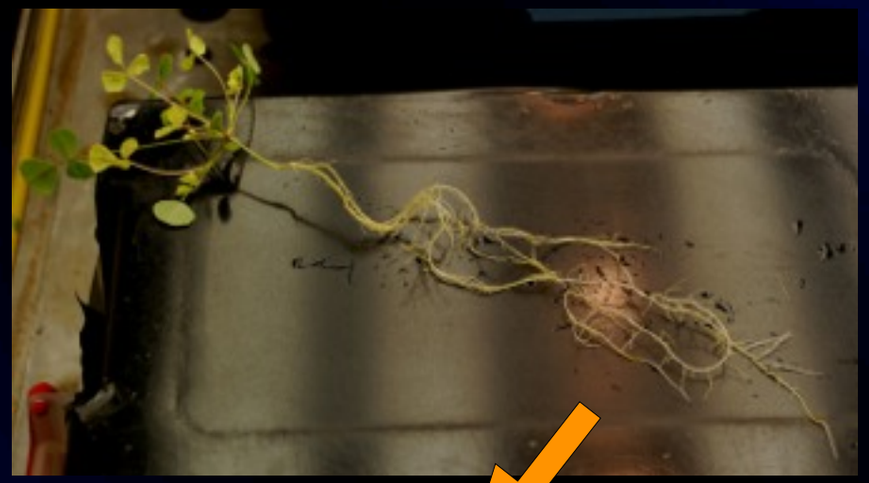
Deficiencia de Fe




Medicago truncatula
Hidropónico

Control

0 µM Fe, pH 5,5

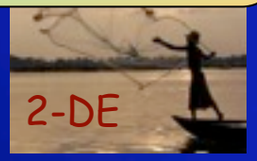


0 µM Fe + $CaCO_3$, pH 7,7

# IEF-SDS/PAGE

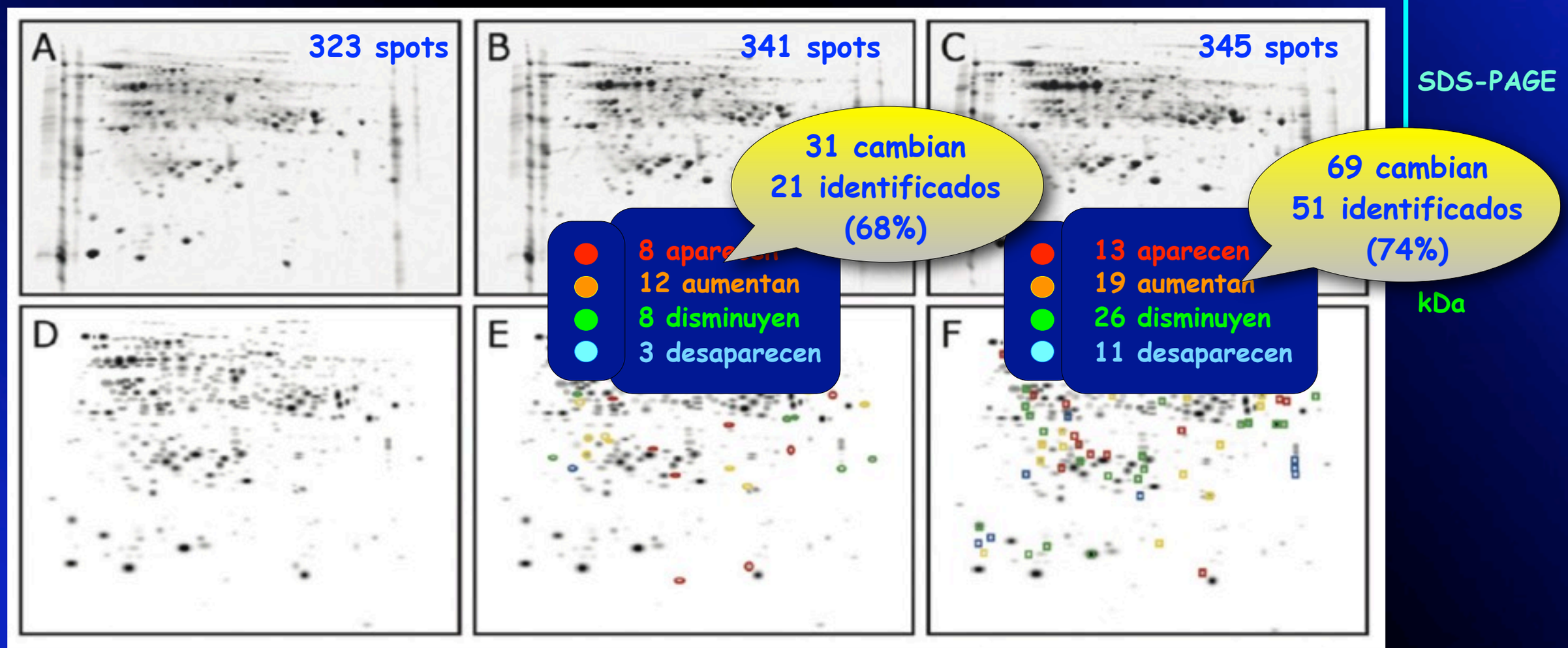
Proteínas

Deficiencia de Fe




Medicago truncatula
raíces

Control | 0 µM Fe, pH 5,5 | 0 µM Fe + $CaCO_3$, pH 7.7

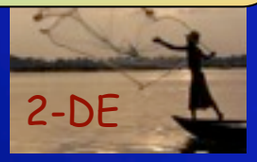



Rodríguez-Celma et al. (2011) J Proteome Res

# IEF-SDS/PAGE

Proteínas

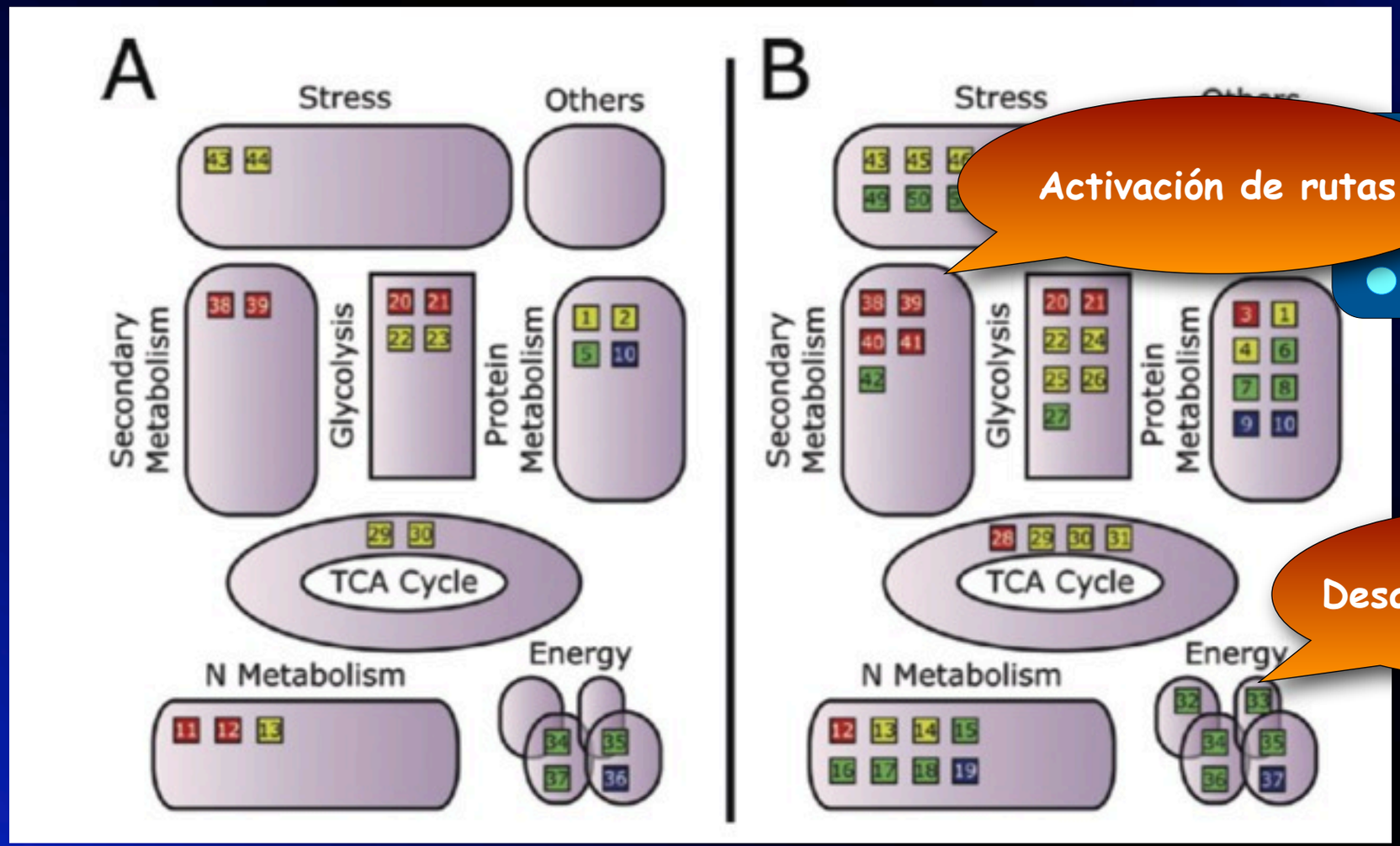
Deficiencia de Fe




Medicago truncatula
raíces

0 µM Fe, pH 5,5

0 µM Fe + $CaCO_3$, pH 7,7




Activación de rutas

Desactivación de rutas

aparecen
aumentan
disminuyen
desaparecen

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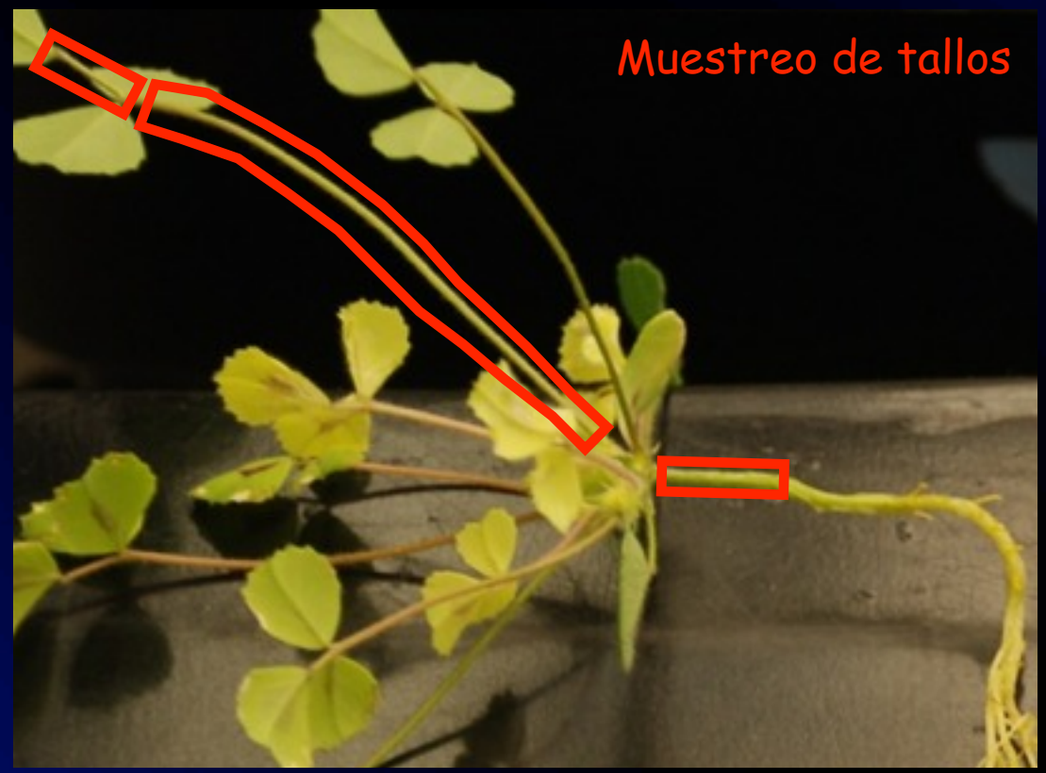
# IEF-SDS/PAGE



Medicago truncatula
Hidropónico



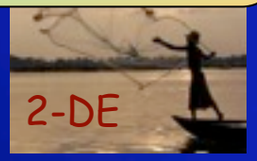

Rodríguez-Celma et al., en preparación

Técnicas 2-DE
EEAD-CSIC, Zaragoza

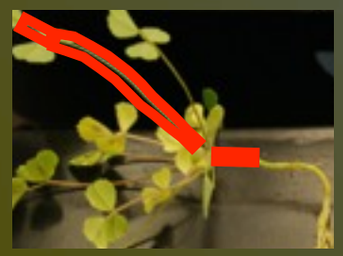
IEF-SDS/PAGE

Proteínas

Deficiencia de Fe




Medicago truncatula
tallos



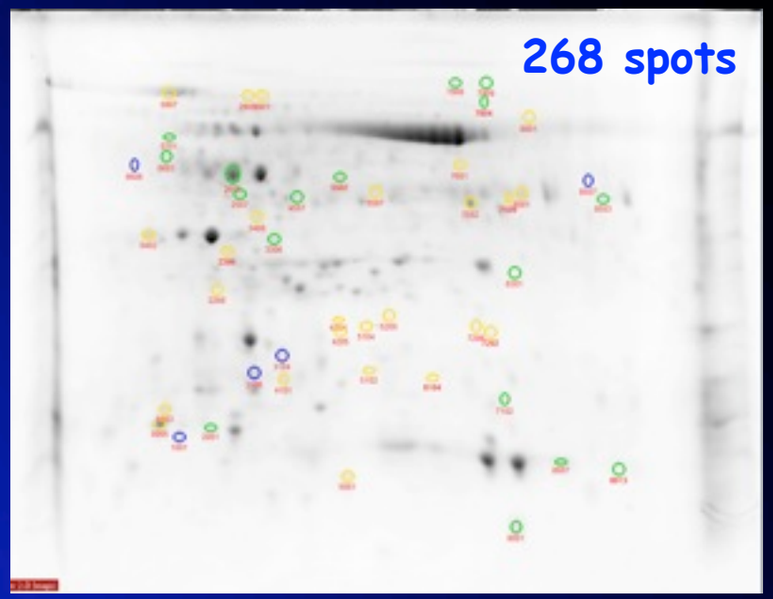
Control | 0 μM Fe, pH 5,5 | 0 μM Fe + $CaCO_3$, pH 7.7

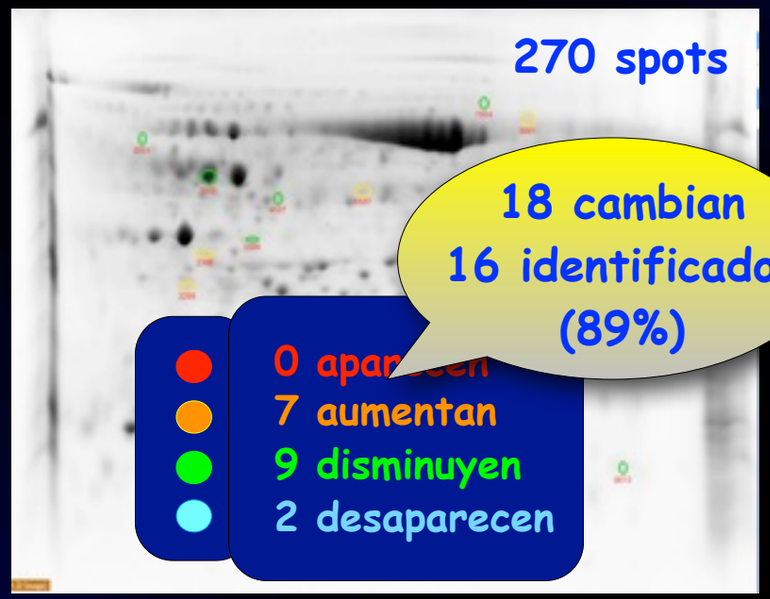
IEF

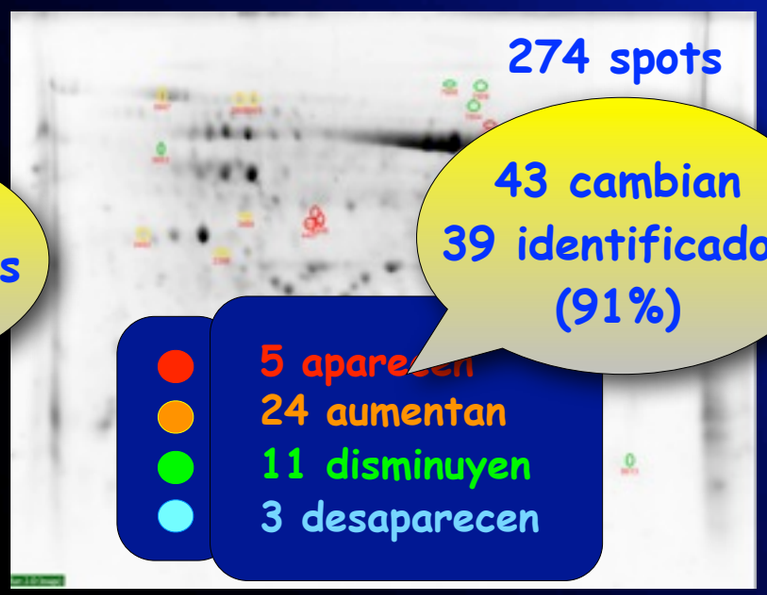
pI

SDS-PAGE

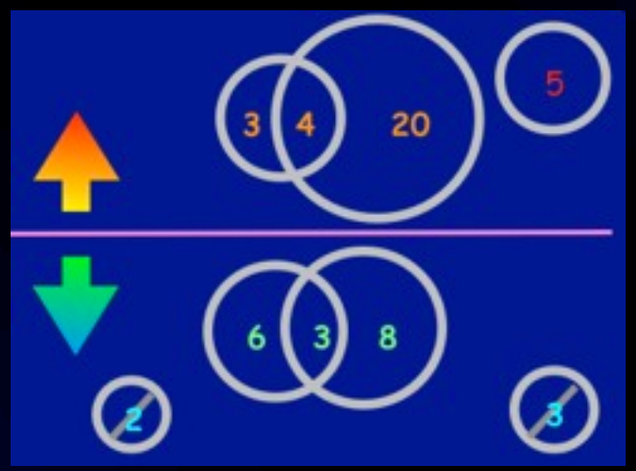
kDa




Rodríguez-Celma et al., en preparación




biotecnología master

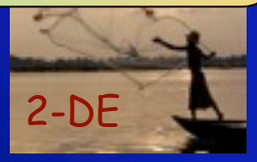
Plant Stress Physiology

CSIC

# IEF-SDS/PAGE

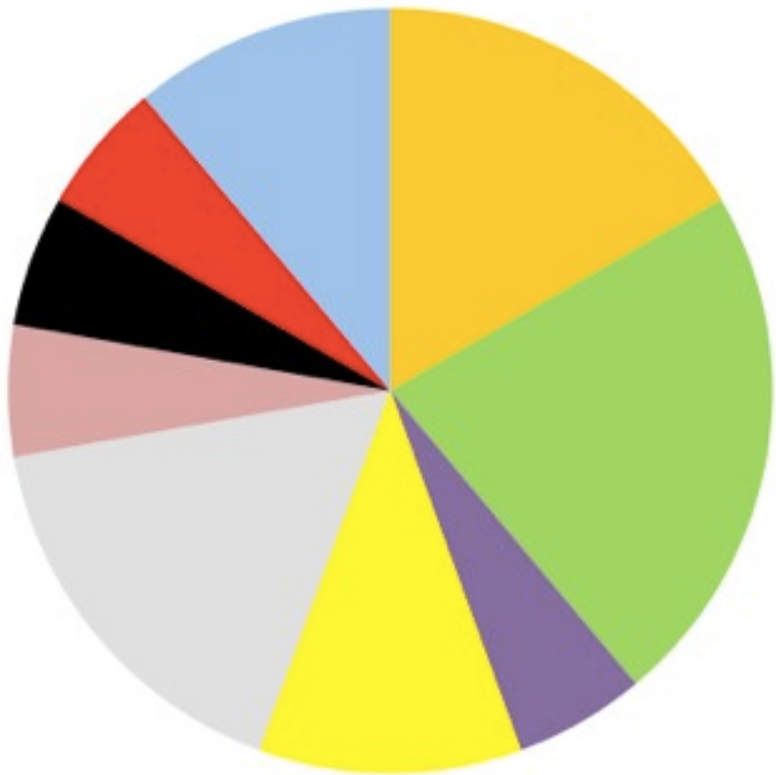
Proteínas

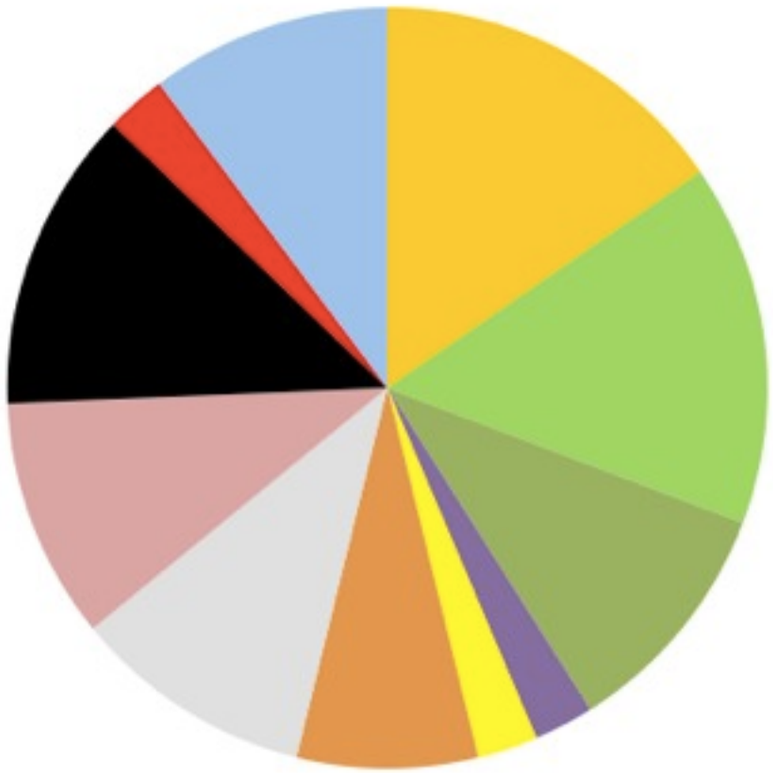
Deficiencia de Fe



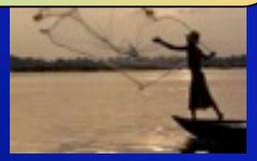

Medicago truncatula
tallos

| | 0 µM Fe, pH 5.5 | 0 µM Fe + $CaCO_3$, pH 7.7 |
|---|---|---|
| Protein metabolism | 3 | 6 |
| Carbohydrate metabolism | 4 | 6 |
| Cell wall | 0 | 4 |
| Secondary metabolism | 1 | 1 |
| Transport | 2 | 1 |
| Metal homeostasis | 0 | 3 |
| Stress | 3 | 4 |
| Photosynthesis | 1 | 4 |
| RNAmetabolism | 1 | 5 |





Rodríguez-Celma et al., en preparación



## Número de "spots" por gel

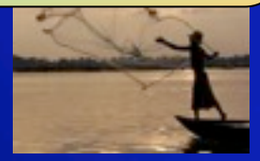
| | "spots" | "spots" con cambios significativos |
|---|---|---|
| Geles 7 cm | 200-350 | 18-69 |
| Geles 20 cm | 1000-1300 | bastante similar |

¿Cuál

# IEF-SDS/PAGE

Proteínas

Deficiencia de Fe



- Número de "spots" En un sistema dado

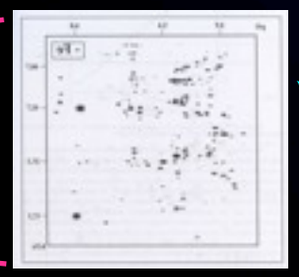
300 "spots"

3-10 pI

kDa
10-100

300 "spots"

5-6 pI

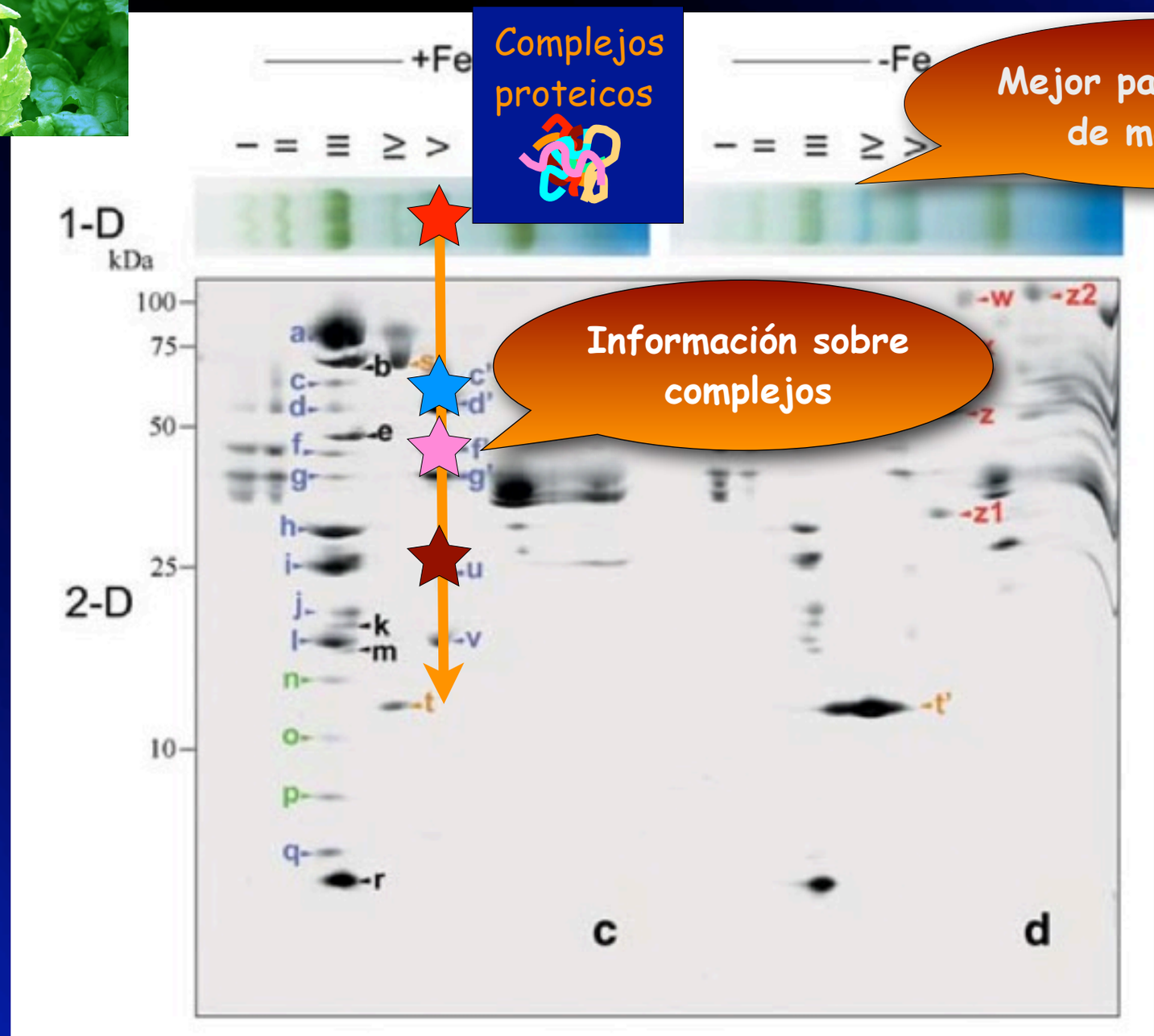


kDa
20-30

# Blue Native 2-DE

Beta vulgaris tilacoides

Blue native → Blue native →




Complejos proteicos

Mejor para proteínas de membrana

Información sobre complejos

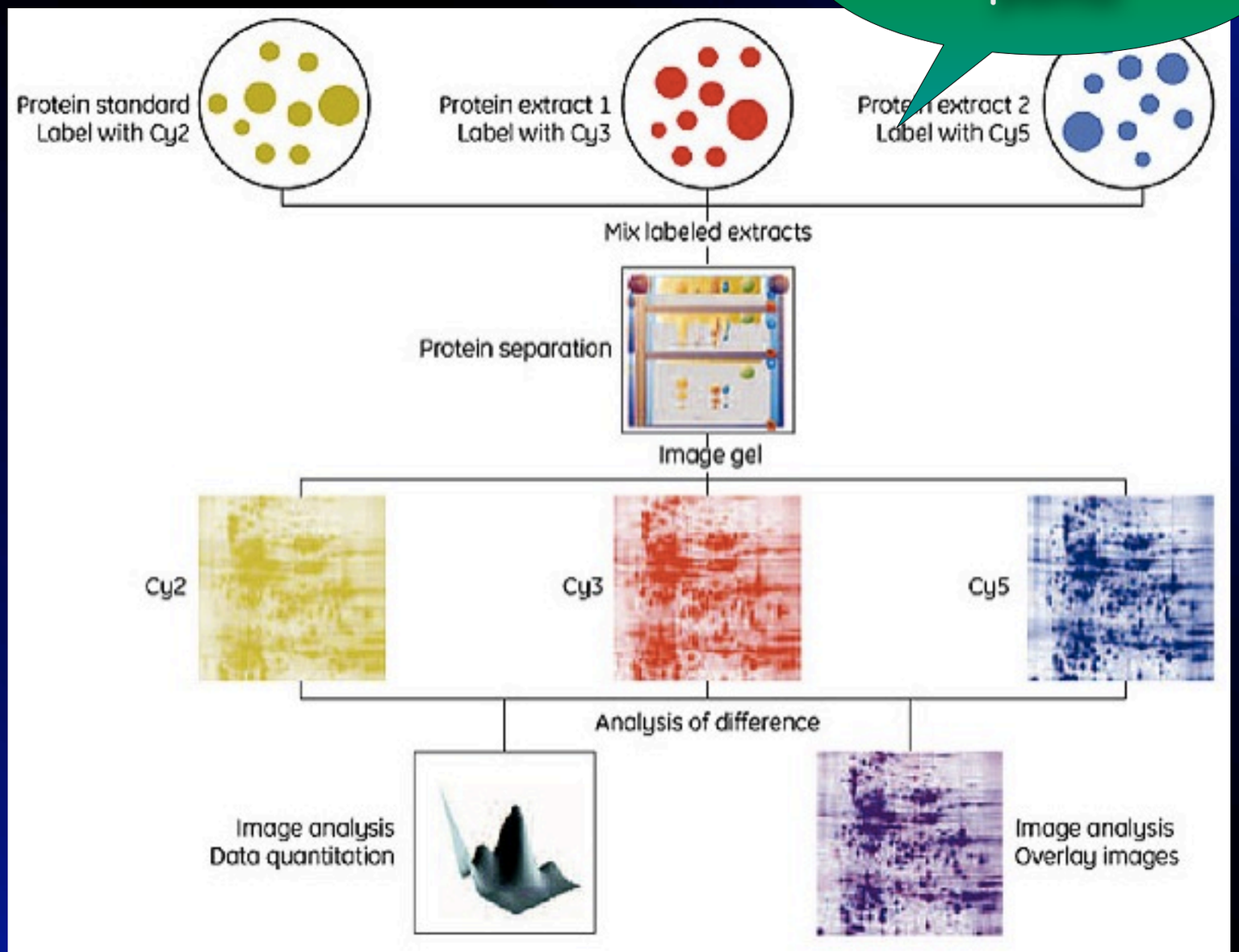
SDS-PAGE

kDa

Andaluz et al. (2006)
Photosynth Res

# DIGE

No muy optimizado para plantas

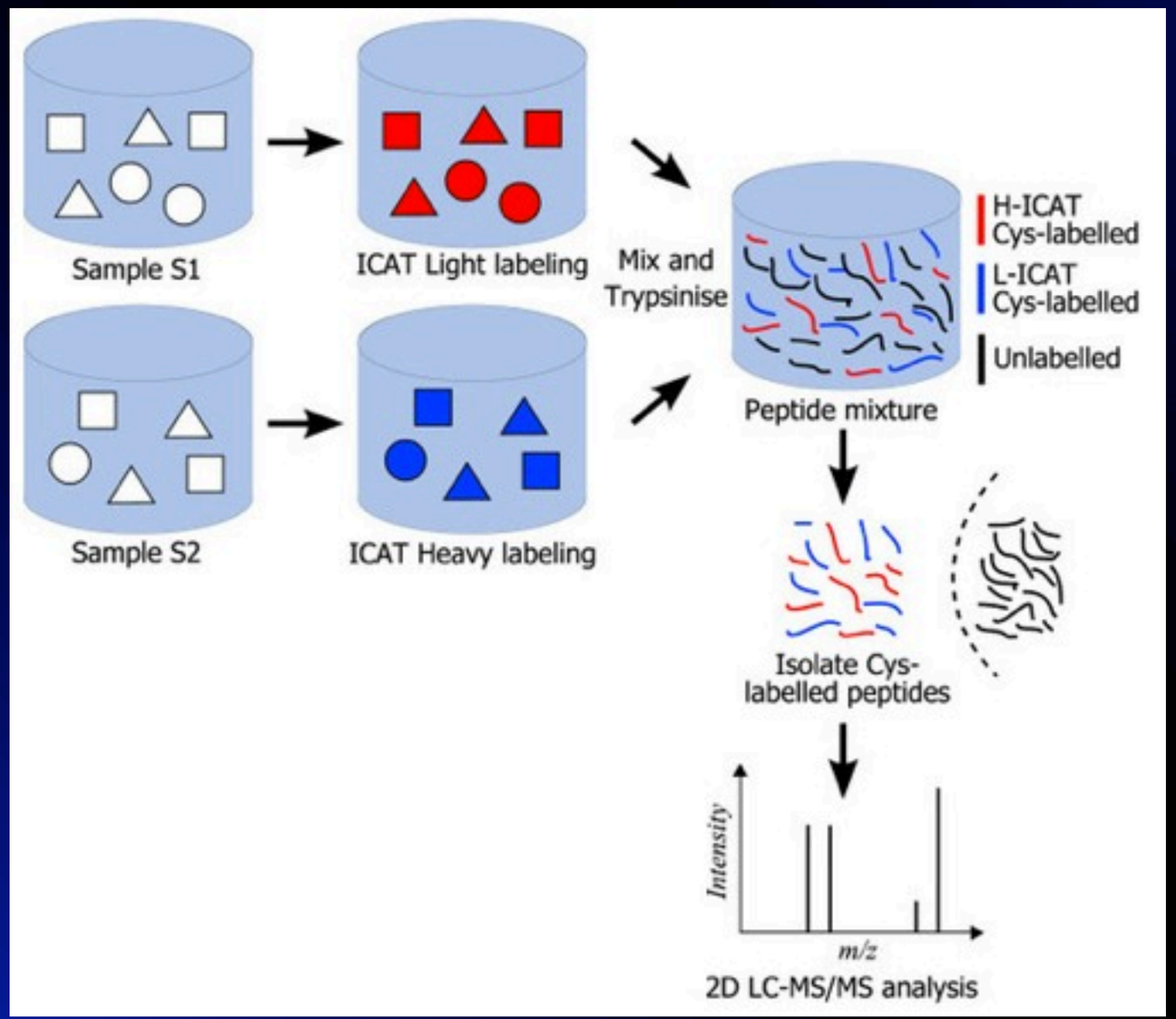


# ICAT (marcaje isotópico)

iodoacetamida-marcador ligero-biotina

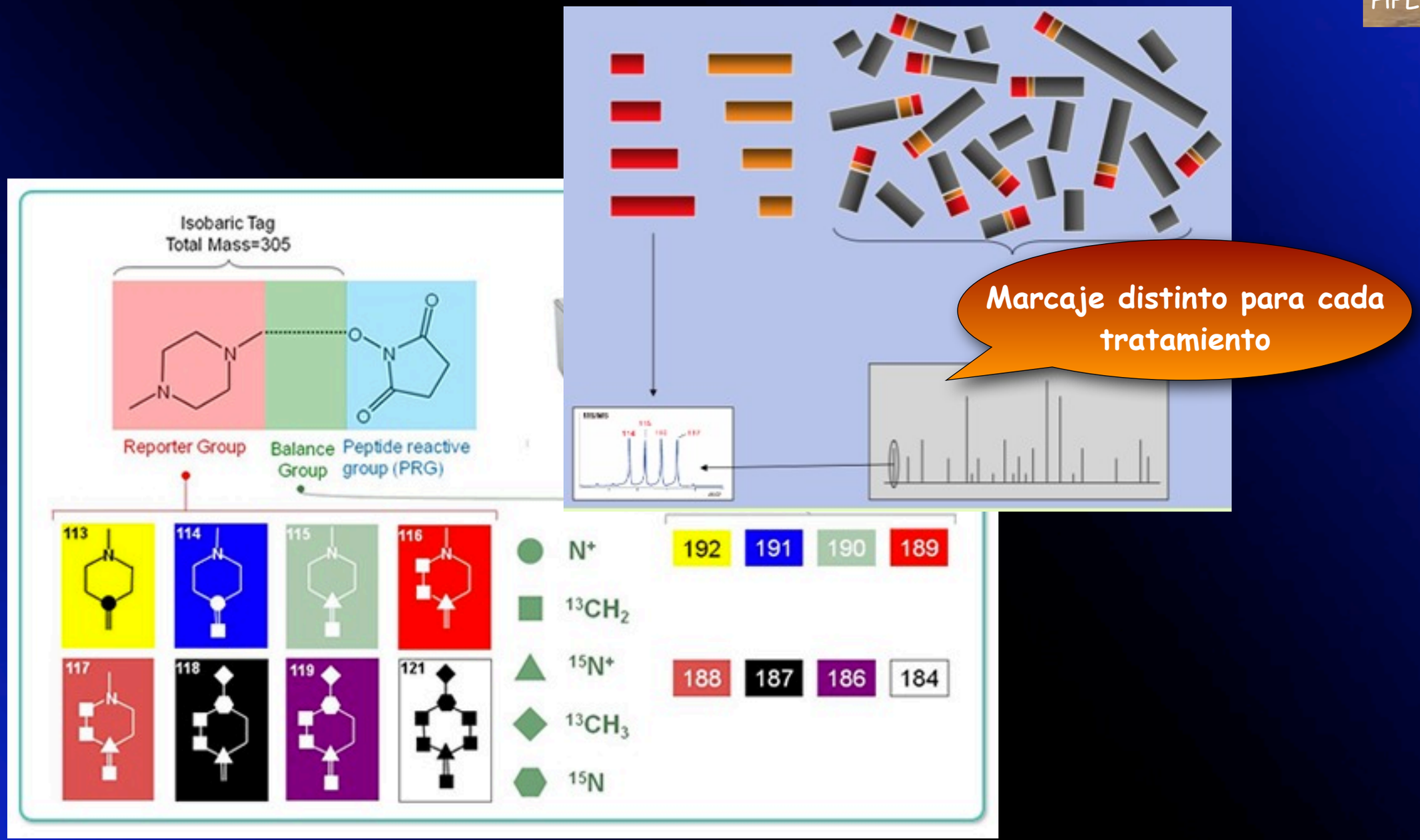
iodoacetamida-marcador pesado-biotina

**Marcaje distinto para cada tratamiento**



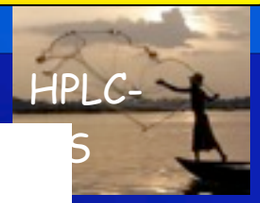
# iTRAQ (marcaje isobárico)





# iTRAQ (marcaje isobárico)





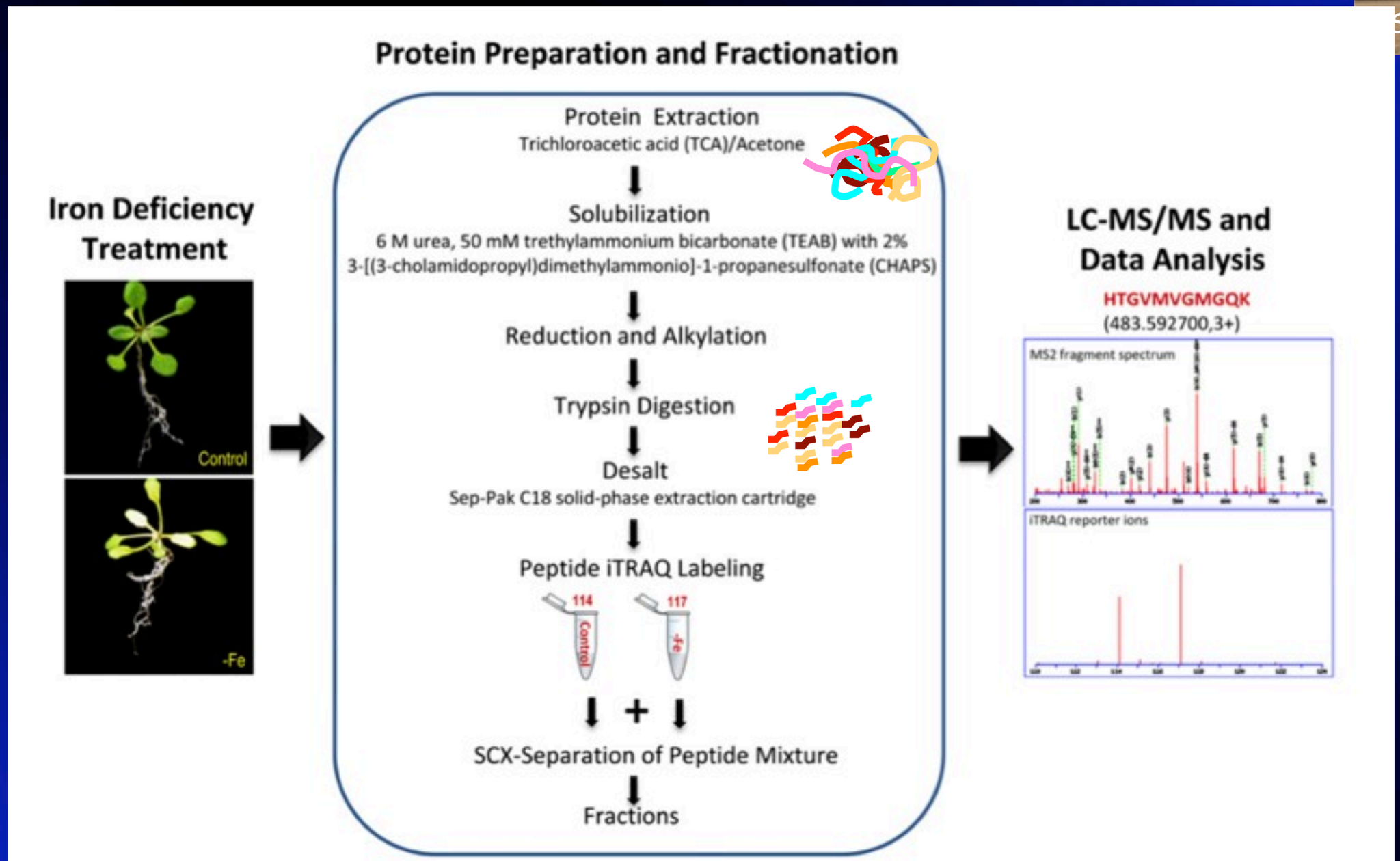



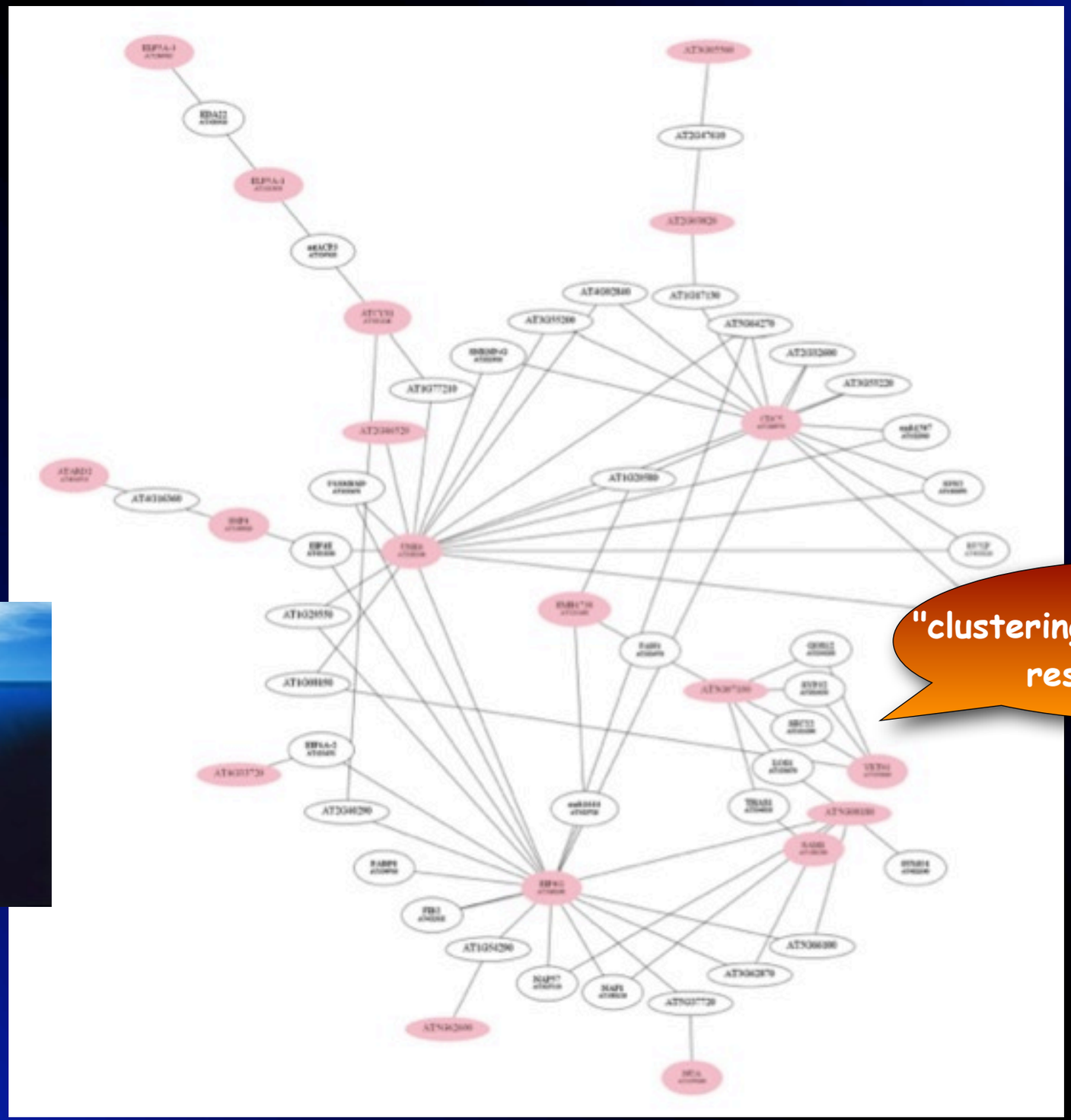

**Figure 1.** Experimental scheme of the iTRAQ analysis. [See online article for color version of this figure.]

Lan et al (2011) Plant Physiol

# iTRAQ (marcaje isobárico)

4.454
identificadas

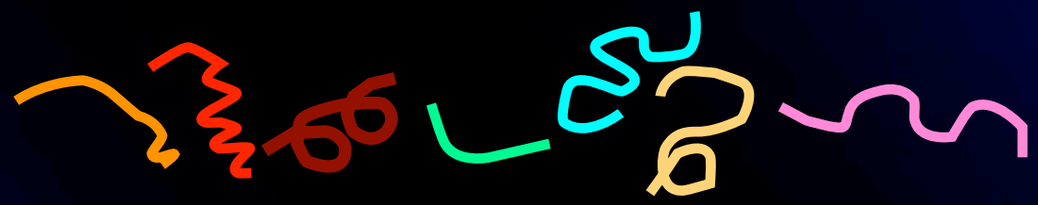
2.882
cuantificadas



"clustering" de proteínas que responden a Fe

Lan et al (2011)
Plant Physiol

# "Shotgun proteomics"



Digestión con tripsina

nano rp-LC-MS/MS

Fraccionamiento de proteínas
SDS-PAGE, IEF, rp-HPLC, FFE

Mezcla compleja

Identificación y
cuantificación de proteínas

Digestión con tripsina

Fraccionamiento de péptidos
IEF, SCX, rp-HPLC, FFE, m/z in MS

# "Shotgun proteomics"

Problema: falta de software adecuado

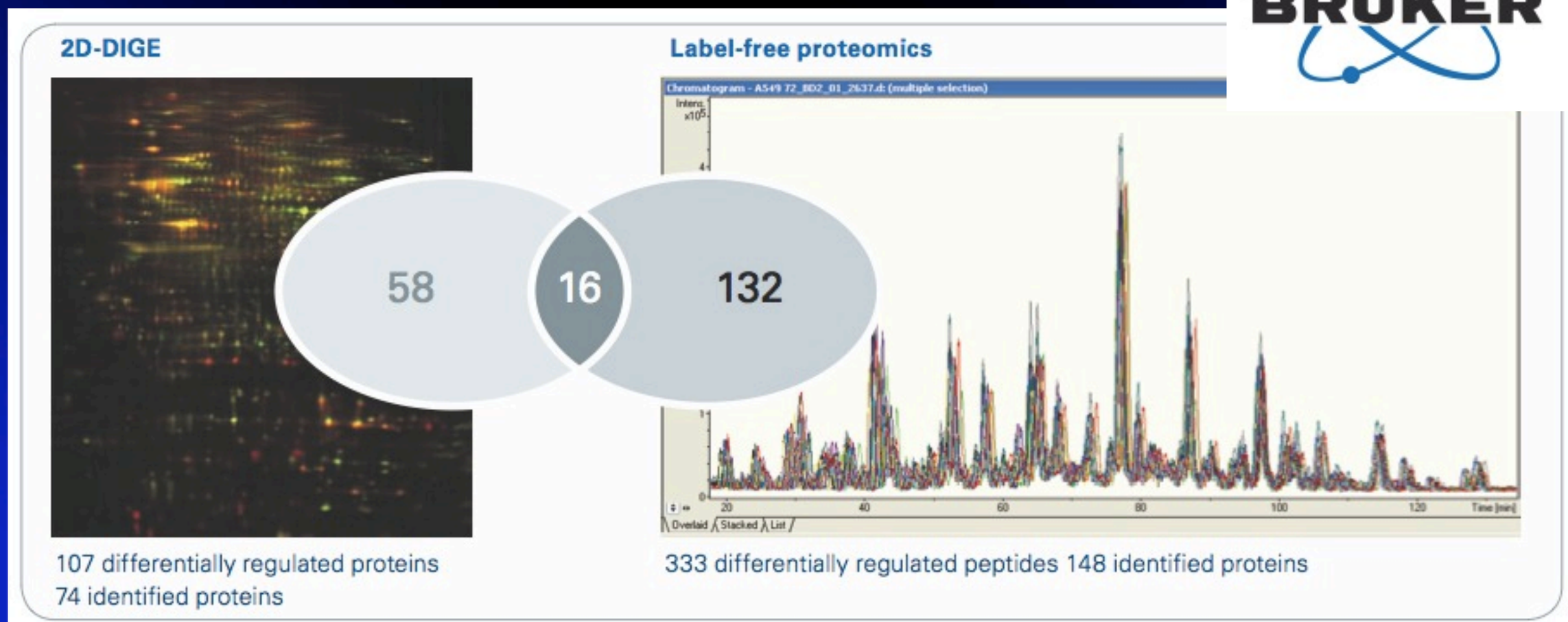
| Condition | No of identified proteins | | | Redundant count of peptides | | |
|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R1 | R2 | R3 |
| Control | 2323 | 2353 | 2446 | 48056 | 45031 | 46629 |
| Moderate | 1900 | 1859 | 1888 | 46499 | [illegible] | [illegible] |
| Extreme | 2225 | 2400 | 2317 | 40385 | [illegible] | [illegible] |

> 2.000 proteínas

> 40.000 péptidos

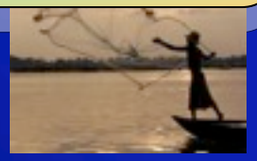
MACQUARIE UNIVERSITY FACULTY OF SCIENCE

# Complementariedad de métodos proteómicos



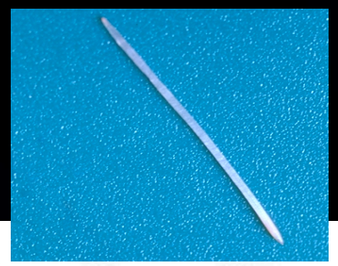
# "-ómicas" combinadas

**Deficiencia de Fe**

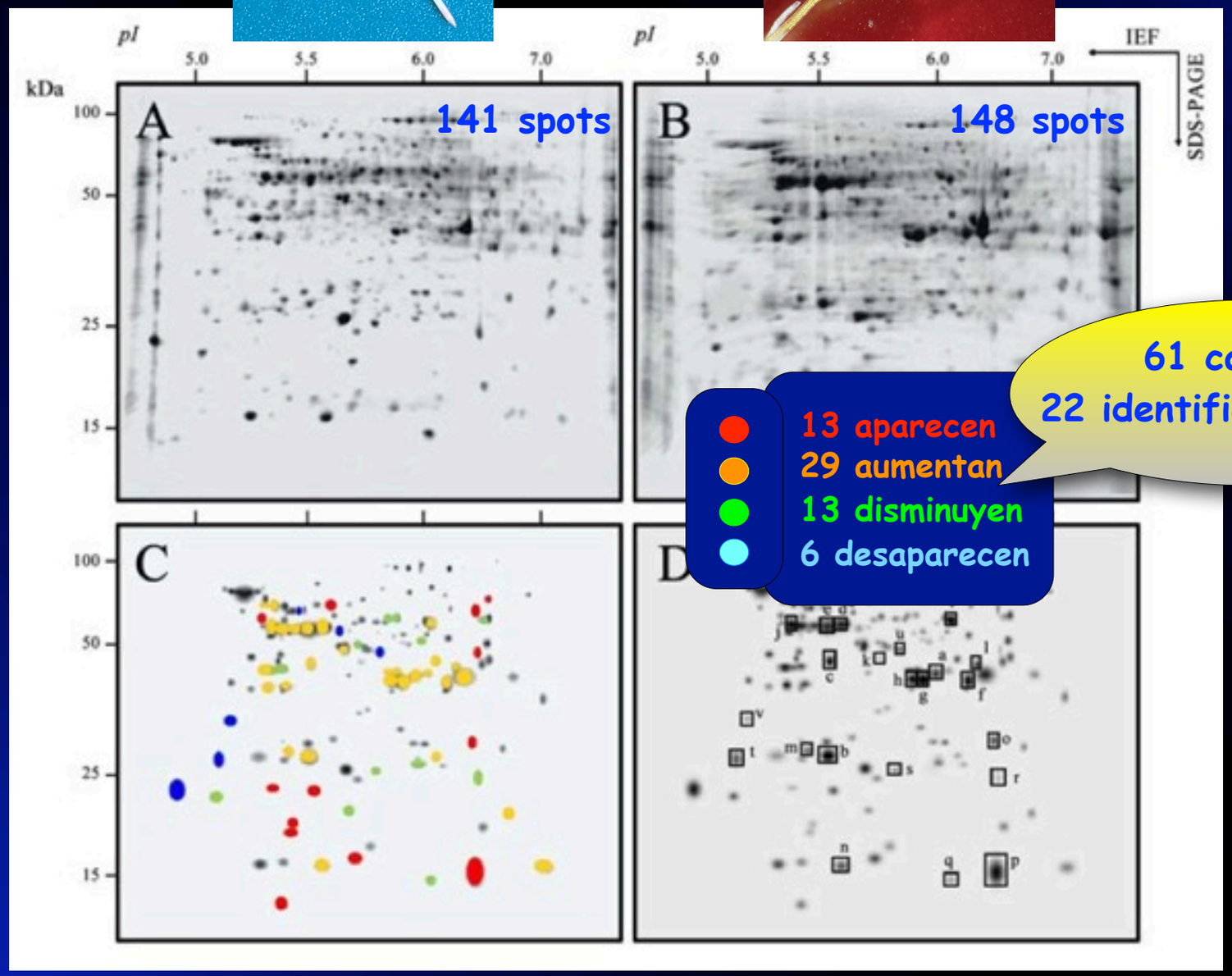


**Beta vulgaris puntas de raíz**

+Fe



-Fe




- 13 aparecen
- 29 aumentan
- 13 disminuyen
- 6 desaparecen

61 cambian
22 identificados (36%)

Rellán-Álvarez et al., (2010) BMC-Plant Biol

# "-ómicas" combinadas

Deficiencia de Fe

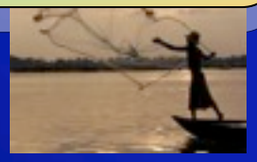


**Table 2: Identified metabolite response ratios of the different treatments *vs.* Fe-sufficient controls (+Fe)**

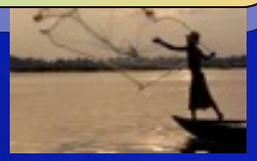
| # | metabolites | -Fe | 24h | 72hYZ | 72hWZ | *A. thaliana* KEGG pathway number |
|---|---|---|---|---|---|---|
| | | | | Aminoacid and Nitrogen Metabolism | | |
| 1 | nicotianamine | **7.9** | **11.8** | **2.9** | **2.1** | |
| 2 | 2-aminoadipic acid | **6.4** | **3.5** | **12.3** | 11.2 | 0300, 1100, 0310 |
| 3 | 2-hydroxyglutaric acid | **3.8** | **4.1** | **2.3** | 1.3 | 0650 |
| 4 | citrulline | **3.6** | 1.4 | **2.9** | 1.7 | 0330, 1100 |
| 5 | lysine | **3.0** | **2.2** | 2.5 | **-1.2** | 0300, 1100, 0310, 0780, 0960, 0310 |
| 6 | serine | **2.4** | **1.7** | **1.8** | **-1.8** | 0970, 1100, 0260, 0600, 0460, 027 |
| | | | | Carbohydrate metabolism | | |
| 23 | galactinol | **33.7** | **86.2** | **23.2** | 7.6 | 0052 |
| 24 | raffinose | **16.3** | **59.7** | **9.7** | 9.9 | 0052 |
| 25 | sucrose | **4.7** | **2.3** | **1.9** | 2.2 | 0052, 1100, 0500 |
| 26 | lactobionic acid | **3.3** | **10.9** | **4.8** | **3.2** | |
| 27 | N-acetyl-D-mannosamine | **2.1** | **1.5** | **2.0** | **1.6** | 1100, 0530 |

326 metabolitos
77 identificados
62 cambian

Rellán-Álvarez et al. (2010) BMC-Plant Biol

# "-ómicas" combinadas

## Deficiencia de Fe

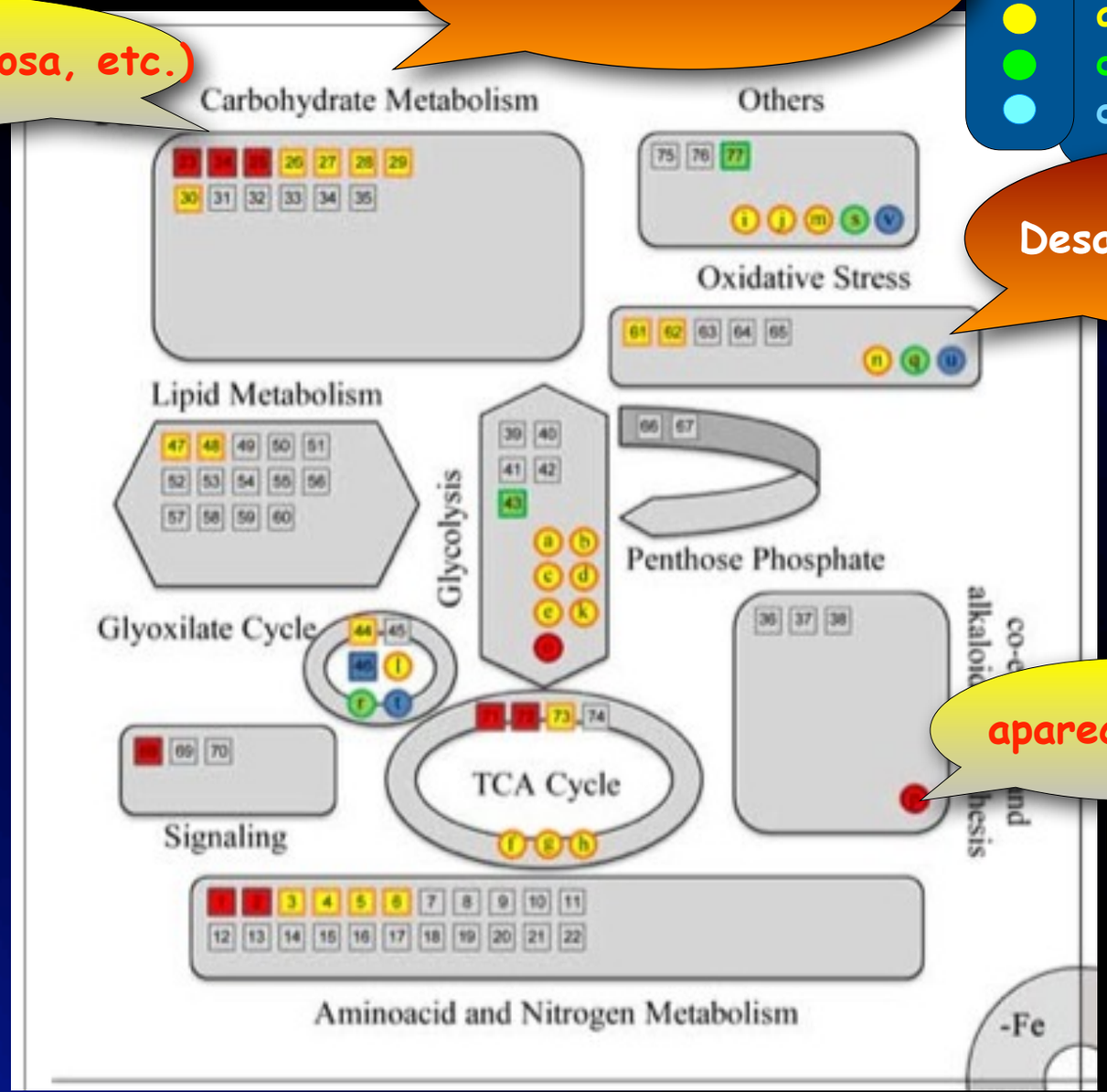


Activación de rutas

aparecen
aumentan
disminuyen
desaparecen

aumentan RFOs (rafinosa, etc.)

Desactivación de rutas



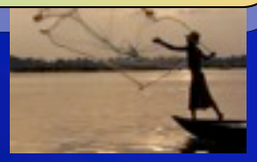

aparece DMRL

dimethyl-8-ribityllumazine (DMRL) synthase,

Rellán-Álvarez et al.
(2010) BMC-Plant Biol

# "-ómicas" combinadas

Deficiencia de Fe



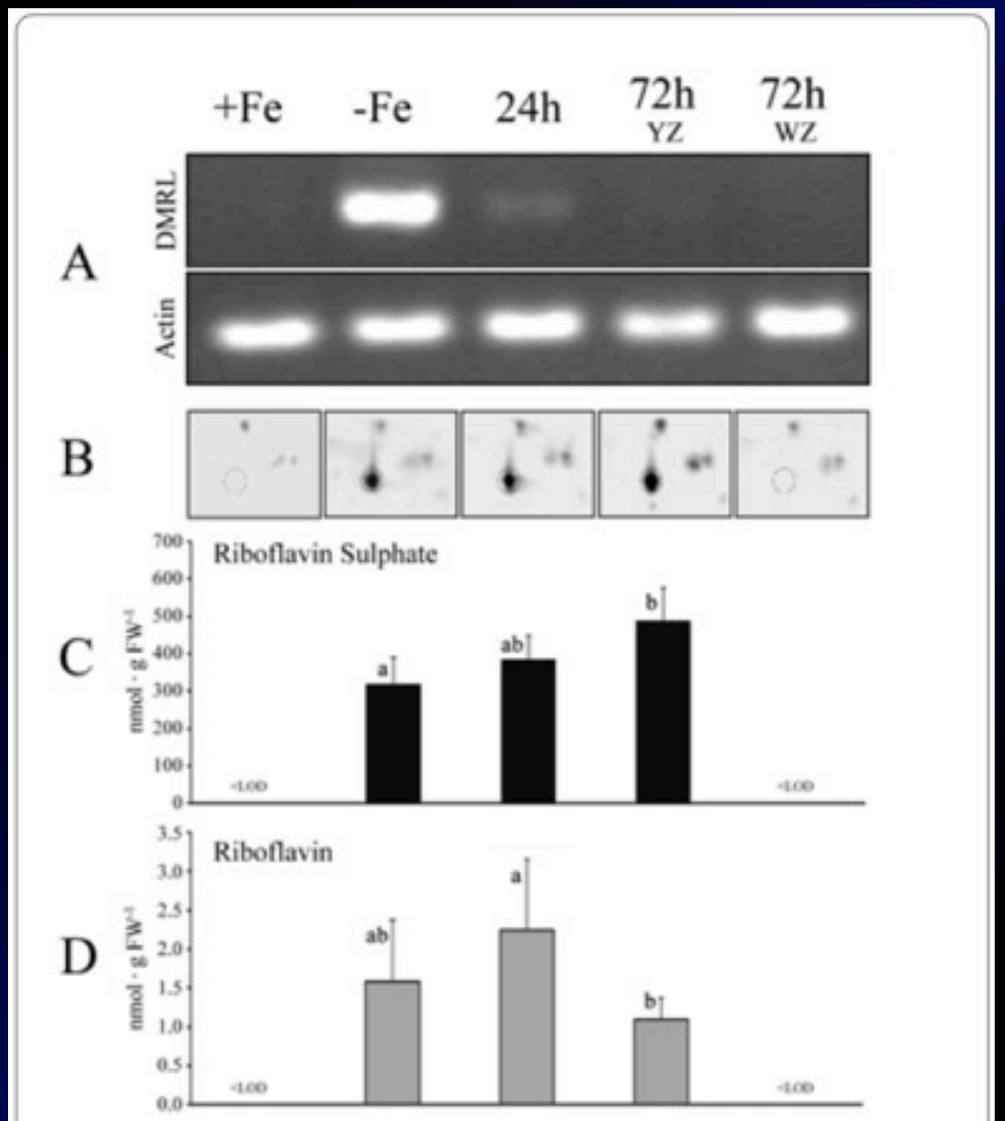



**Figure 2 DMRL and flavin analysis**. Semi-quantitative RT-PCR analysis of the *BvDMRL* and actin gene transcripts (A), zoom scans of typical gels containing the BvDMRL protein (B) and riboflavin sulphates (C) and riboflavin (D) concentrations in sugar beet root tips from Fe sufficient (+Fe), Fe-deficient (-Fe), 24 h Fe-resupplied, 72 h Fe-resupplied YZ and 72 h Fe-resupplied WZ. Letters in (C) and (D) indicate significant differences ($n = 6$, $p < 0.05$, Duncan test).

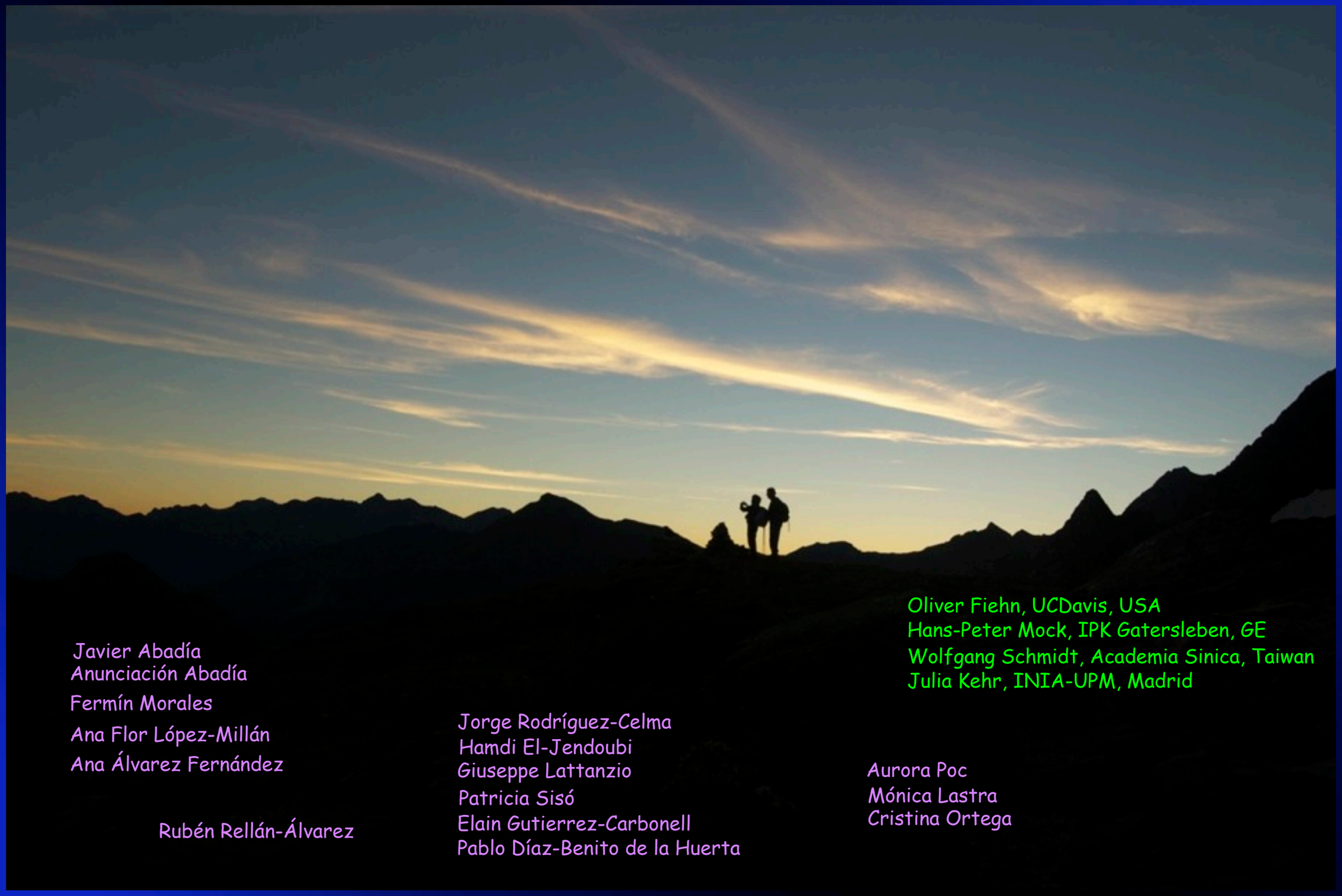
Genes

Proteínas

Metabolitos

Rellán-Álvarez et al. (2010) BMC-Plant Biol

http://www.stressphysiology.com



Oliver Fiehn, UCDavis, USA
Hans-Peter Mock, IPK Gatersleben, GE
Wolfgang Schmidt, Academia Sinica, Taiwan
Julia Kehr, INIA-UPM, Madrid

Javier Abadía
Anunciación Abadía
Fermín Morales
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Rubén Rellán-Álvarez

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Patricia Sisó
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Pablo Díaz-Benito de la Huerta

Aurora Poc
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Cristina Ortega

Plant Stress Physiology

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