From -omics to phenotyping for crop improvement

Proteomics to study flower and fruit phenotypes characterized by different colours and quality properties

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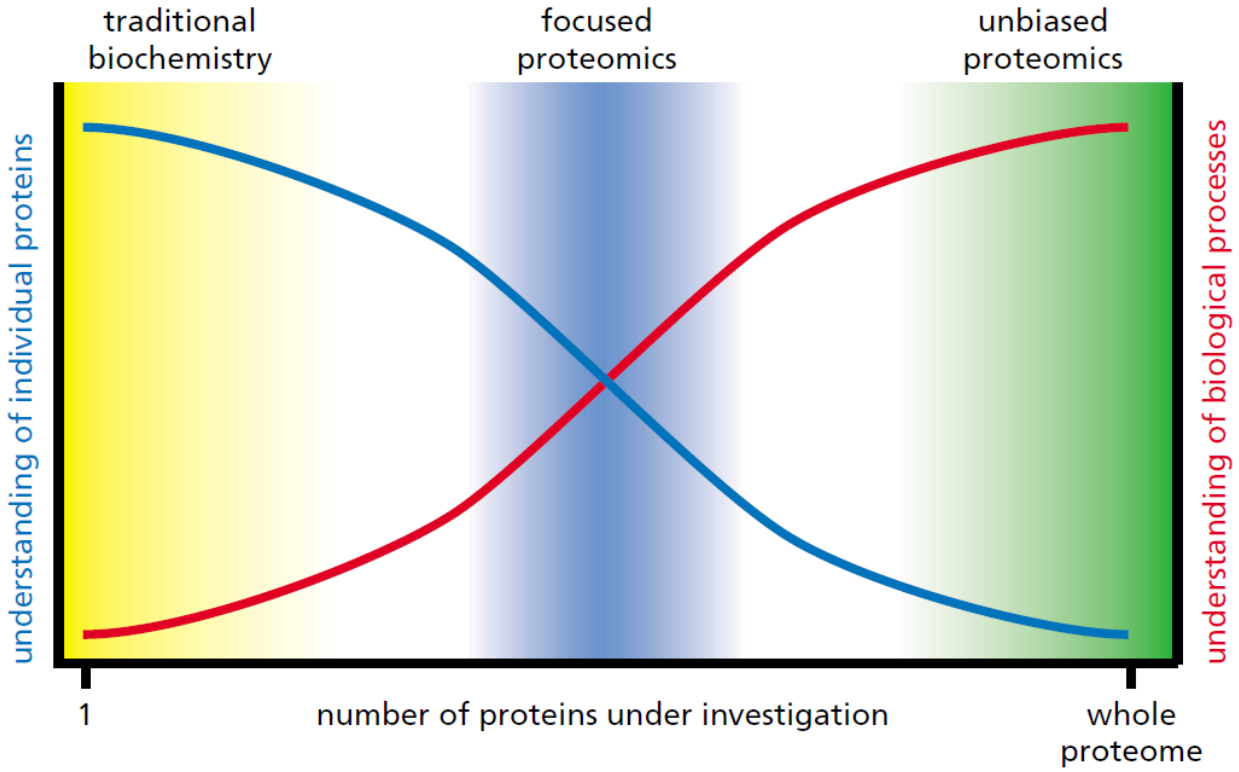
WHAT IS PROTEOMICS?

Proteomics was defined as the large-scale characterization of the entire protein complement of a cell line, tissue, or organism (Wilkins et al 1995, Genet Eng Rev 13:19)



(MacBeath, 2002 Nat Genet, doi:doi:10.1038/ng1037)

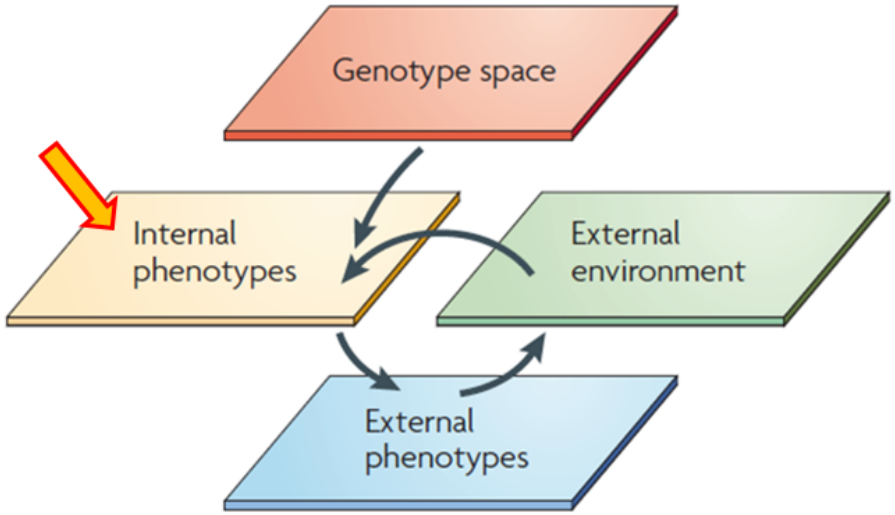
- Proteomic is a **system-wide approach**: study **of biological processes.**
  - Proteomics is **unbiased**: holistic approaches
- **Proteomics is discovery oriented:** is aimed at characterizing novel relations among proteins**.**

WHY PROTEOMICS?

Proteomics provides information about the internal **phenotypes:**

THE GENOTYPE–PHENOTYPE MAP

Phenotype: Genotype X Environment



(Houle et al., 2010 Nat Genet, doi:10.1038/nrg2897)

" Internal phenotypes are the levels in which the environmental factors and crop management are integrated into appropriate cellular reactions that result in the actual phenotype ".

(Großkinsky et al, 2015, J Exp Bot doi:10.1093/jxb/erv345)

Cellular reactions Include biochemical and physiological responses.

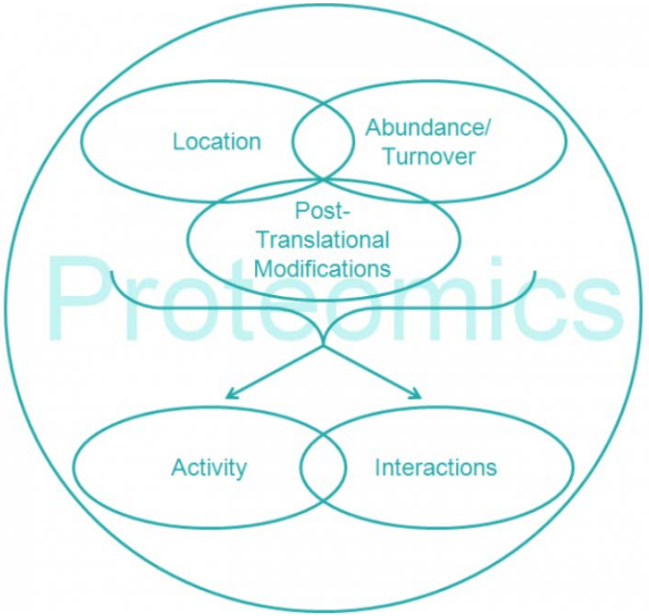
Proteomics provides information about:

- The protein variants affecting phenotypes.
- Relations among metabolic pathways and regulatory networks.
- Pleiotropy.

THE ROLES OF PROTEOMICS IN SYSTEM BIOLOGY

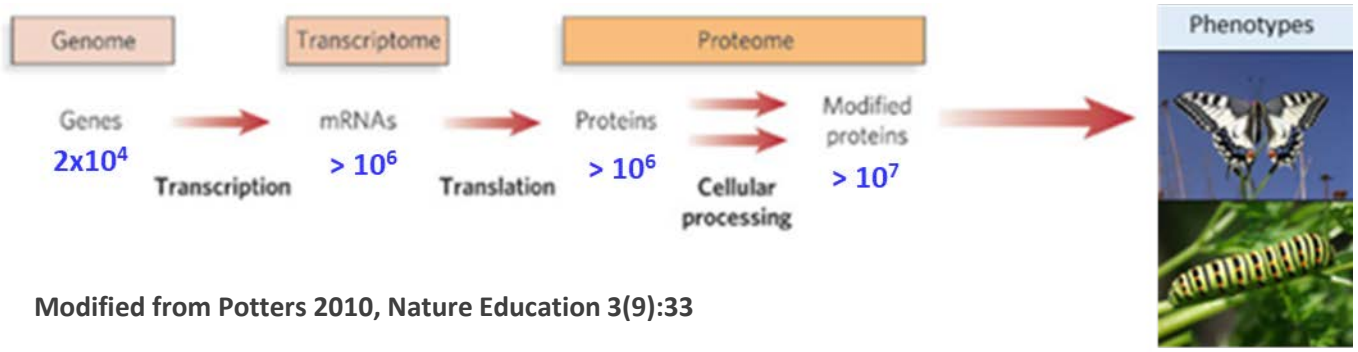
Proteomics provides distinct and complementary information to the other "omics"

- Study of the **metabolic interlinks** among biochemical pathways
- **Integration of the data obtained by other approaches**: transcriptomics often does not correlate with changes at protein level (Gigy et al 1999, Mol Cell Biol 19:1720); many proteins are not enzymes and their functionality is not reflected at metabolite level
- **Post-translational modifications (PTM)**: turnover, maturation by proteolysis, amino acid modifications (>200 amino acid variants by Mann and Jensen 2003, Nat Biotechnol 21:255), subcellular localization, isoforms, interactions with other proteins…
- **Quality and Safety of Food**: organoleptic properties, allergens, protein toxins.



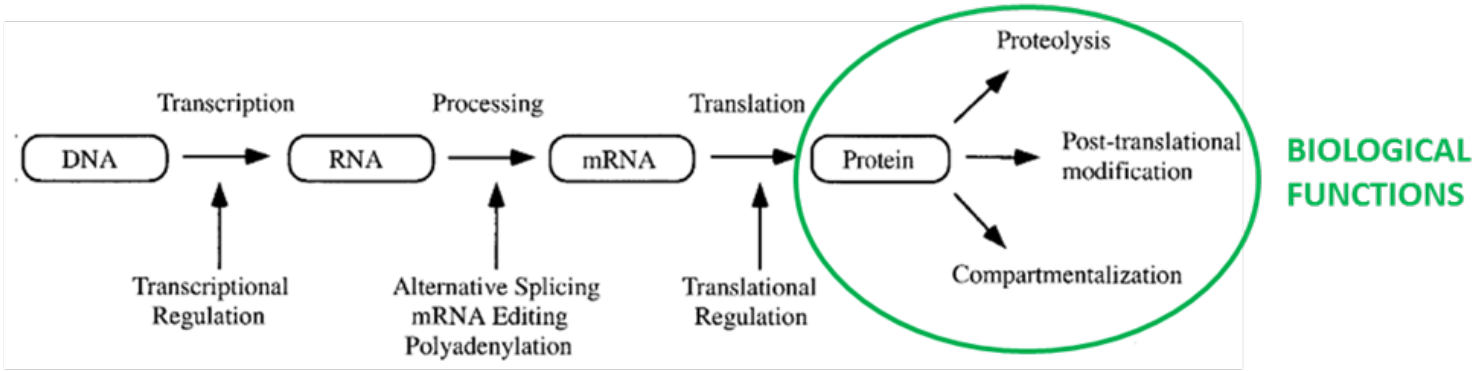
THE PROPERTIES OF PROTEOME: 1. HIGH COMPLEXITY

- **Every living organism is characterized by many different proteomes (organelle, cell and tissue specific) that are extremely dynamics.**



Modified from Potters 2010, Nature Education 3(9):33

- **Every single gene encodes for many proteins derived from post-translational modifications.**



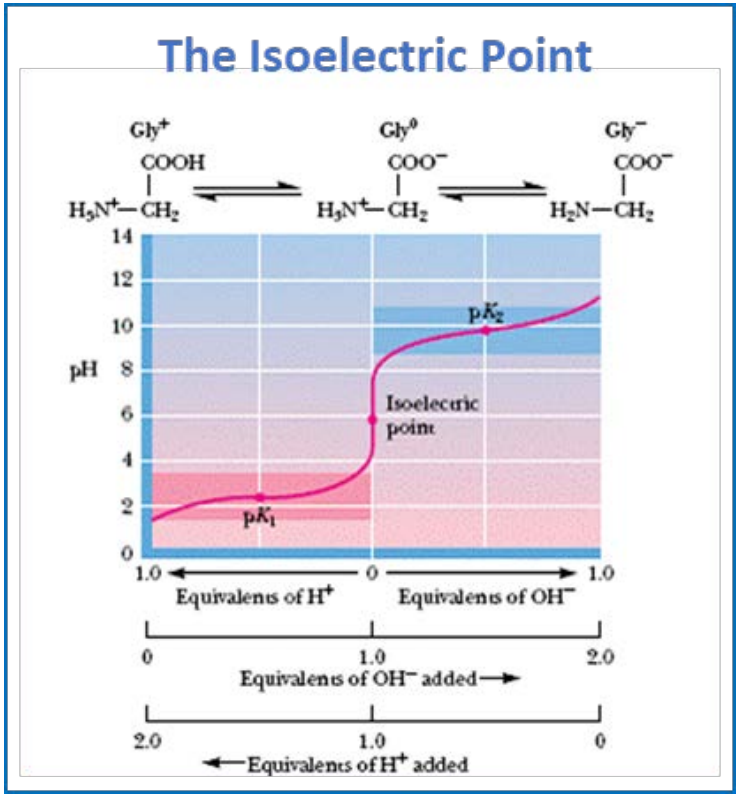
Mechanisms by which a single gene can give rise to multiple gene products. Multiple protein isoforms can be generated by RNA processing when RNA is alternatively spliced or edited to form mature mRNA. mRNA, in turn, can be regulated by stability and efficiency of translation. Proteins can be regulated by additional mechanisms, including posttranslational modification, proteolysis, or compartmentalization.

A cell can contain up to 20.000-50.000 unique proteins

THE PROPERTIES OF PROTEOME: 2. HIGH HETEROGENEITY

The proteins are macromolecules characterized by many and different chemo-physical properties:

- **Charge**: is variable and is linked to the isoelectric point **(p*I*=0-14)**.
- **Molecular Weight**: from 50 to 2000 amino acids (5.000-200.000 Da)
- **Solubility**: depends on charge, molecular weight and on the hydrophobicity of the primary sequence.

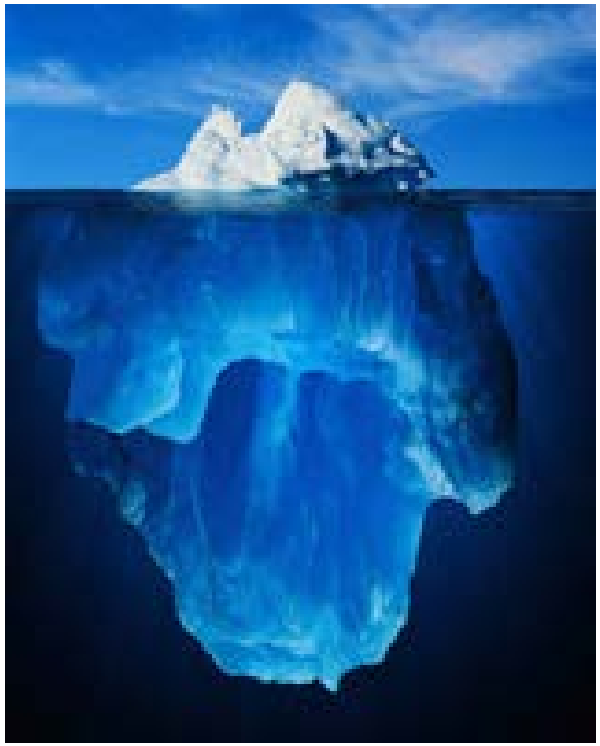


The proteomic studies need of extraction procedures which allow to collect and preserve a very heterogeneous set of polypeptides.

THE PROPERTIES OF PROTEOME: 3. HIGH DYNAMIC RANGE

The proteome comprises molecules with very different abundances:
from few 10s copy/cell (like transcription factors or transporters) to a lot of 10.000s copy/cell (like structural proteins).

- **The estimated Dynamic Range is about 10^7 order of magnitude**
- **The proteomic researcher works with protein in NATURAL ABUNDANCE**



GENERAL WORKFLOW IN PLANT PROTEOMICS

EXPERIMENTAL DESIGN:

- **Functional proteomics: comparative analysis of proteomes in control vs perturbed sample**
- **Descriptive proteomics: proteome profiles to obtained electrophoretic reference maps**

SAMPLE MANIPULATION:

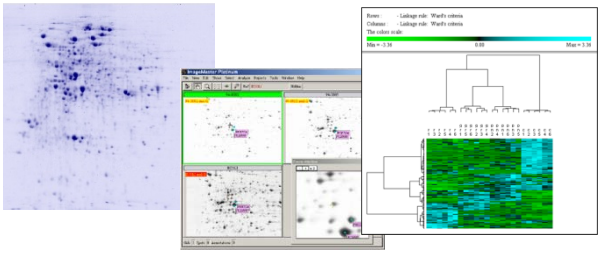
purification of the protein fraction from sample. This is a very crucial step in plant proteomics

SAMPLE FRACTIONATION:

GEL-BASED PROTEOMIC

2DE:

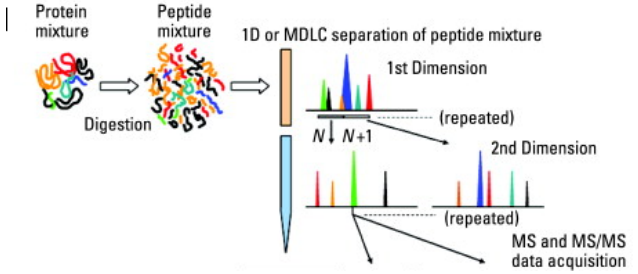
IEF, electrophoresis, detection, image statistical analysis to highlight proteins of interest



GEL-FREE PROTEOMIC

2D-HPLC:

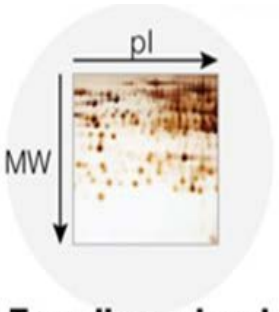
SCX/RP-chromatography of the peptides; coupled with isotope-labeling to get quantitative analysis



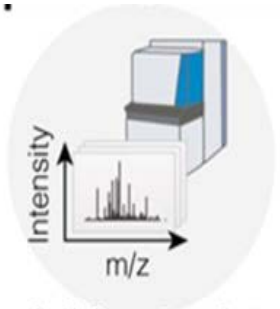
MASS SPECTROMETRY:

protein identification and characterization

GENERAL WORKFLOW IN PLANT PROTEOMICS



Two-dimensional gel electrophoresis



Gel-free tandem mass spectrometry

		untargeted	targeted
Scope (# proteins)	~10^2	10^3 - 10^4	~10^2
Throughput (# samples)	~10^2	~10^2	10^2 - 10^3
Dynamic Range	~10^3	~10^7	~10^5
Sensitivity	µg/ml	ng/ml	pg/ml

Platforms for protein biomarker discovery. The scope, throughput, sensitivity, and dynamic range of gel-based, gel-free mass spectrometry, and aptamer platforms are outlined.

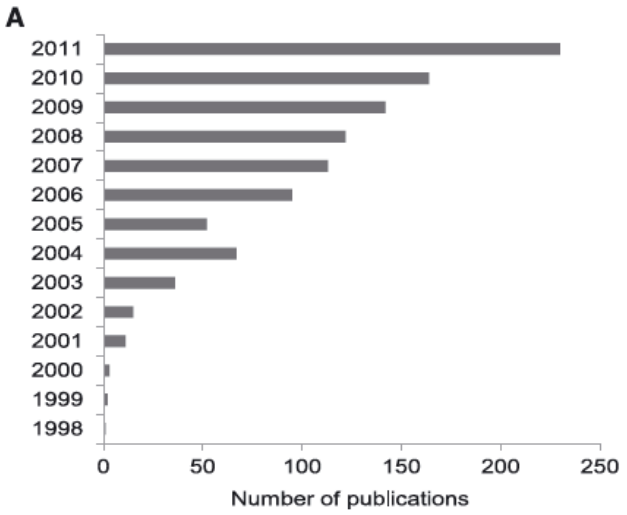
Gramolini et al. Circulation. 2016;134:286-289

PLANT PROTEOMICS RELIES ON GENOMICS AND TRANSCRIPTOMICS DATA

Protein identification depends on the availability of gene sequences:
the recent progresses in mass spectrometry and bioinformatics approaches allow to move from model plant species to non-model plant species

NCBI
National Center for Biotechnology Information

SIB ExPASy Bioinformatics Resource Portal



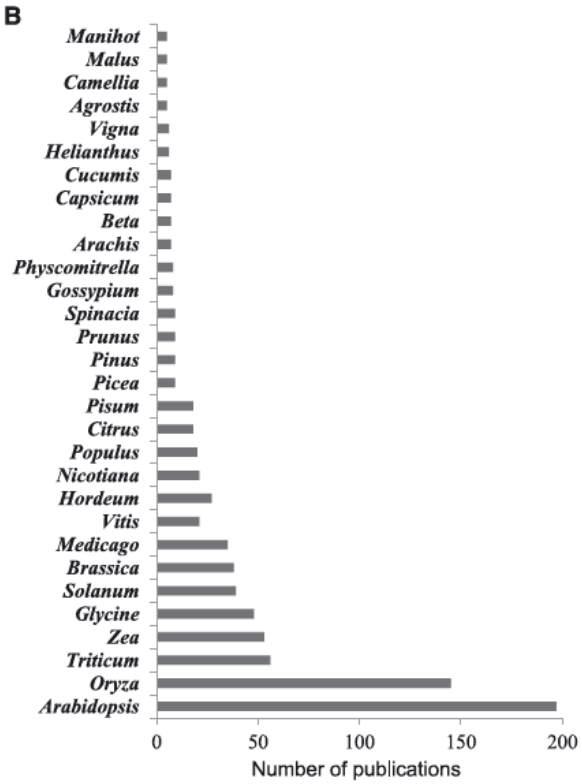


Figure 1. Number of publications per year related to plant proteomics. (A) Number of publications per year. The key words used in the search of the Scopus database were "proteom*" and "plant*." The raw data can be found in Supporting Information Table 1. (B) Number of publications for the 30 genera most often used over the period 1998–2011.

Champagne and Boutry 2013, Proteomics 13:663

GEL-BASED PROTEOMICS

Protein solubilization

a. PROTEIN SAMPLE EXTRACTION

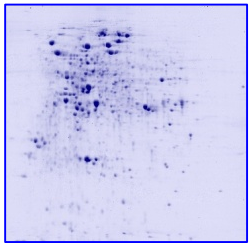
IEF

pH 3 pH 10

SDS-PAGE

pI

MW



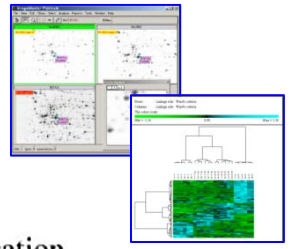
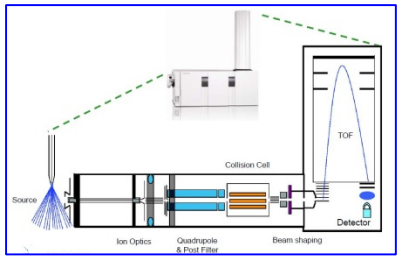


Image Analysis

Sample for protein identification

b. 2D-ELECTROPHORESIS

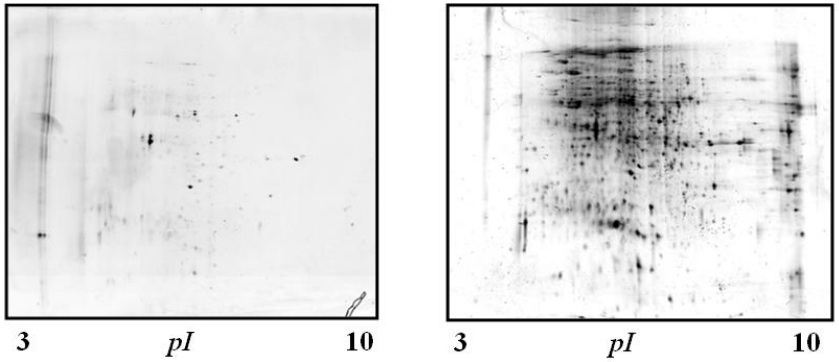
Mass spectrometry for protein characterization



c. LC-ESI-MS/MS

PROTEIN EXTRACTION

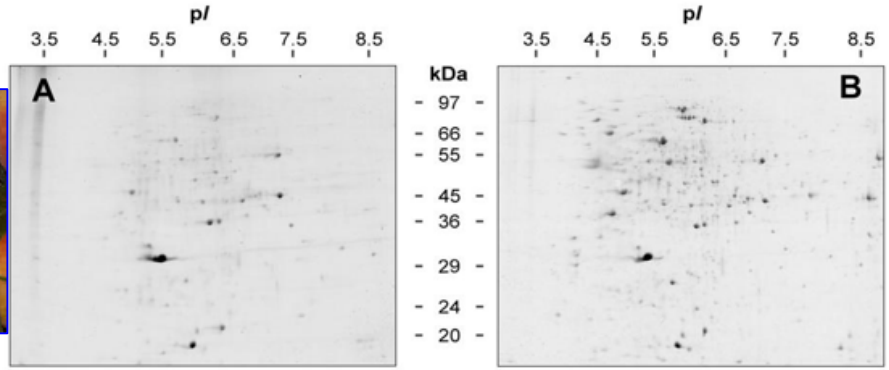
The optimization of extraction procedures depends on tissue/organ features



Grape cell wall proteome

acetone washing to remove phenolic compounds.

Negri et al 2007, J Plant Physiol 165:1379

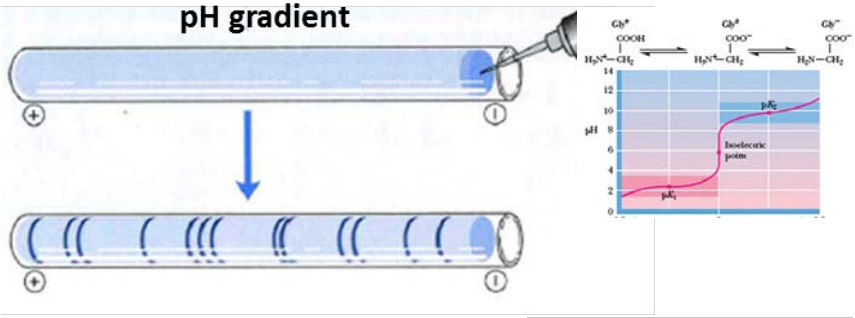


Peach mesocarp

lyophilized samples and direct solubilization in buffered phenol to avoid excess of water that provokes sugar-protein aggregation

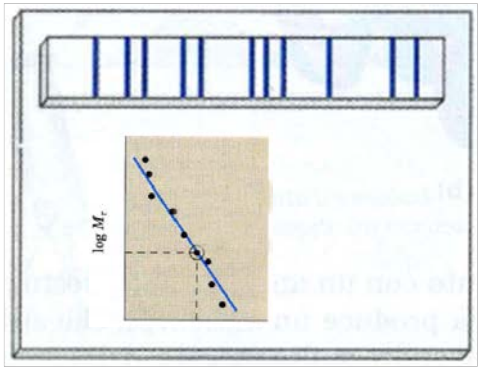
Prinsi et al 2011, Phytochemistry 72:1251

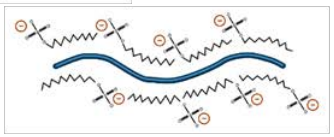
GEL-BASED PROTEOMICS: 2D-PAGE



1st Dimension: IsoElectroFocusing

Proteins migrate under an electric field on the basis of their isoelectric point

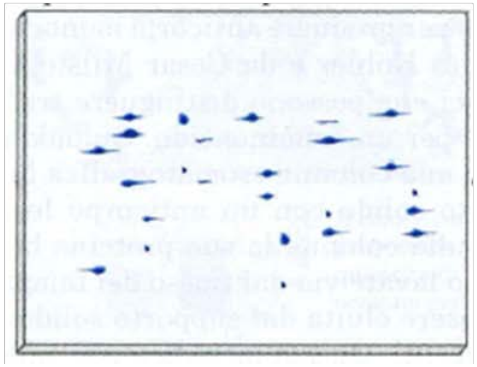




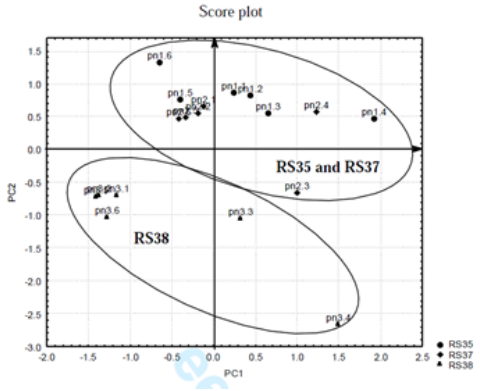
SDS-treatment and second electrophoretic run

2nd dimension: SDS-PAGE

The gel obtained by the IEF is treated with SDS. The proteins, under an electric field, migrate on the basis of their molecular weight, following a logarithmic relationship between mass and migration.



Spot visualization by staining



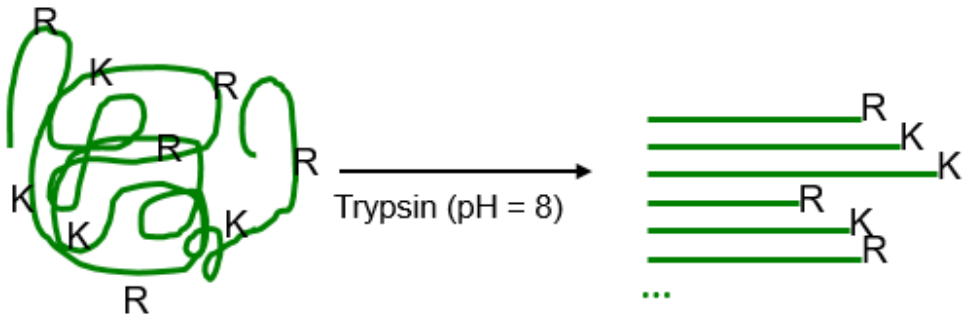
Statistical analysis

Image analysis and different statistical approaches can be applied to individuate the proteins of interest

PROTEIN IDENTIFICATION BY LC-ESI-MS/MS

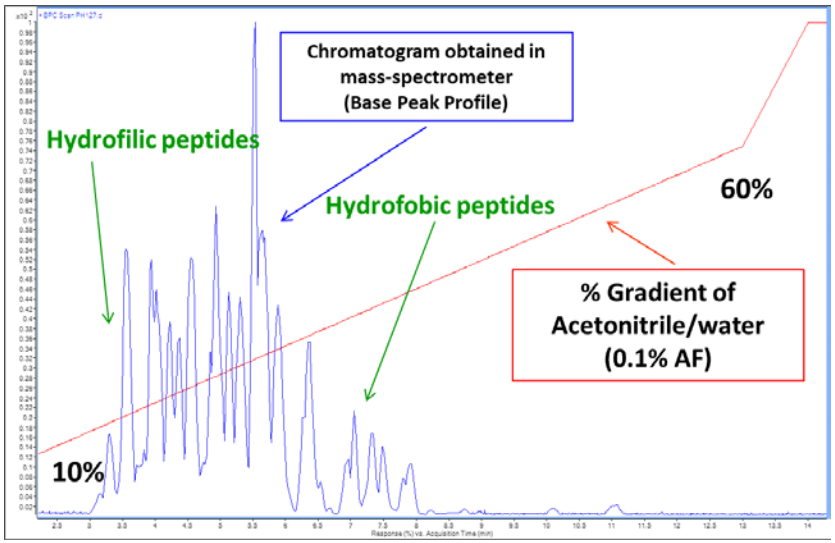
1. ENZYMATIC DIGESTION

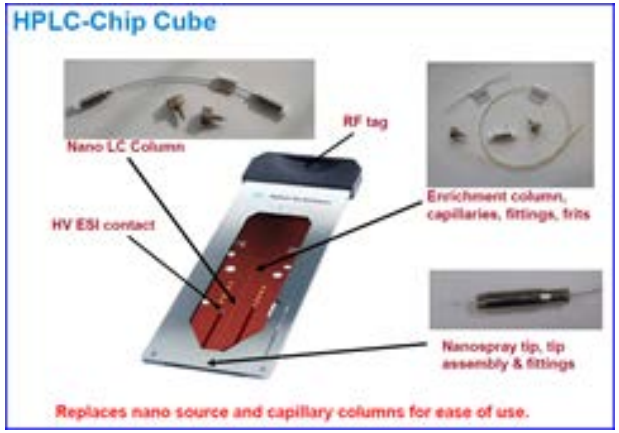
- Incubation of the protein sample in the presence of specific proteases



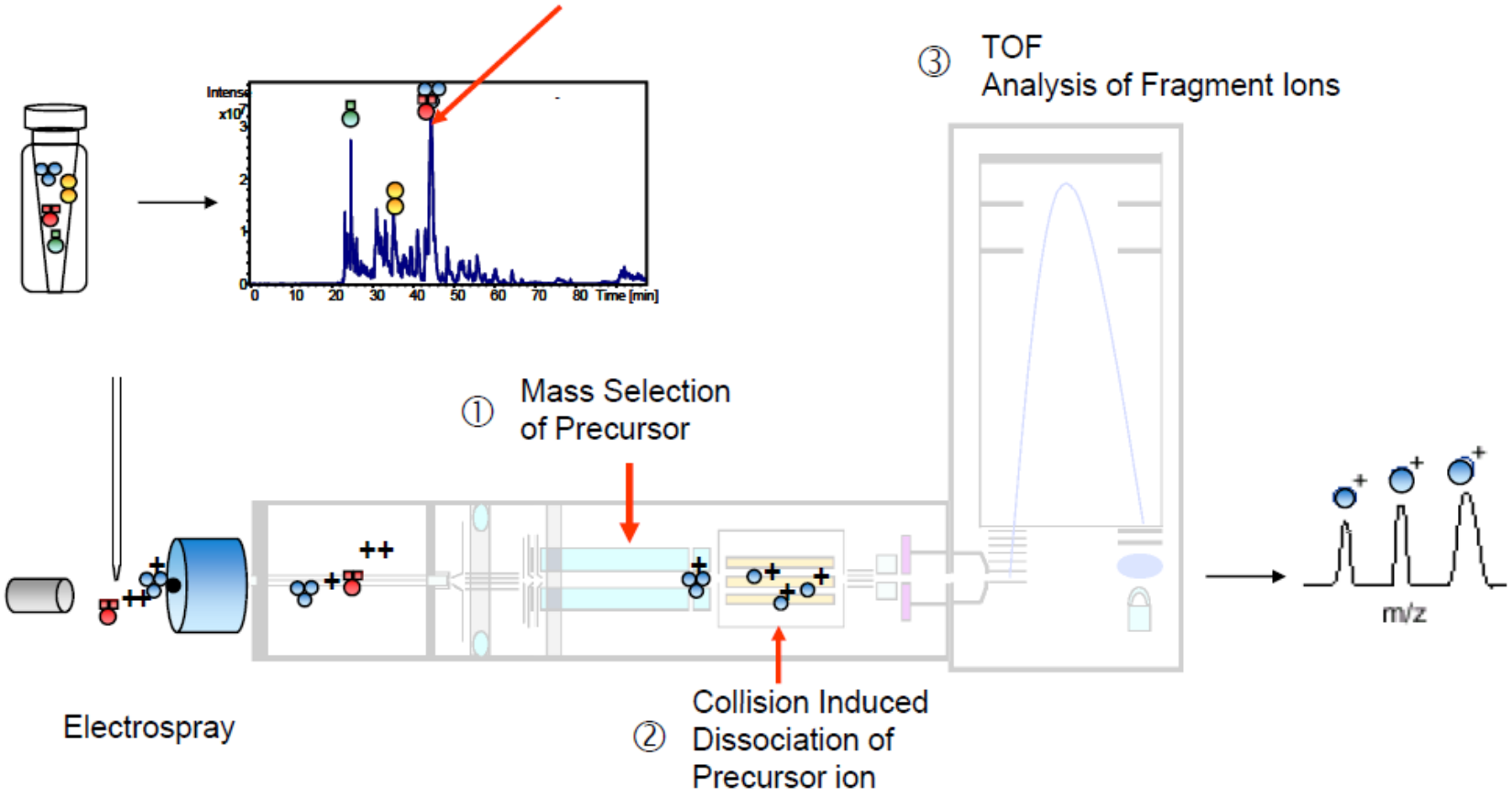
The trypsin hydrolyzes the peptide bond in Lysine (K) and Arginine (R) residues: releasing acidic peptides (generally from 15 to 30 aa)

2. HPLC REVERSE-PHASE CHROMATOGRAPHY



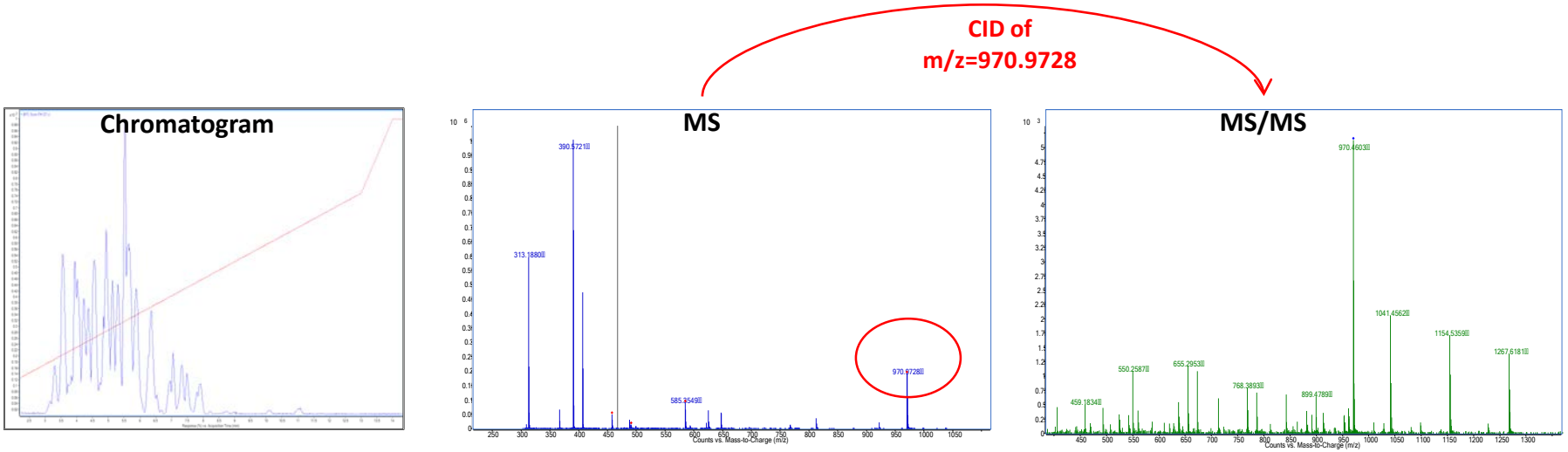


TANDEM MASS SPECTROMETRY
Quadrupole Time-Of-Flight mass spectrometer

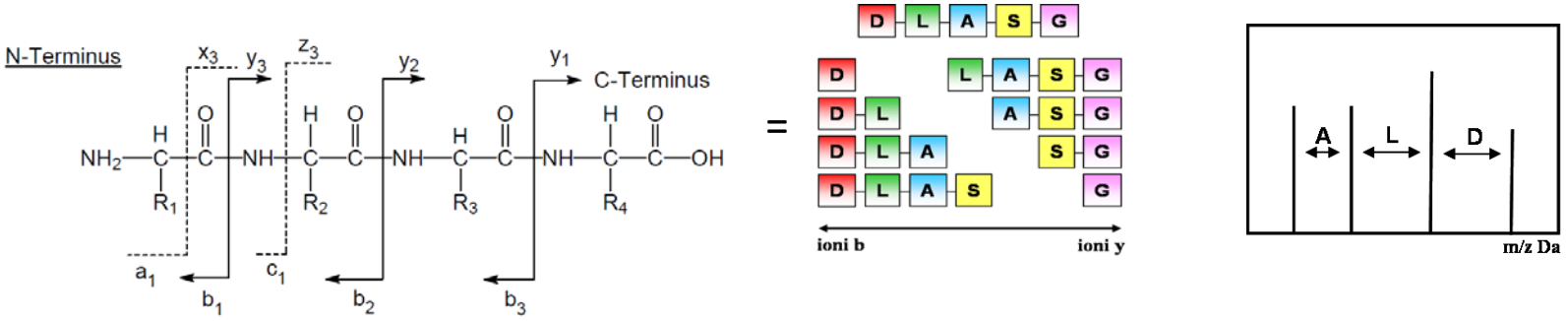


1. Measure of m/z petide (precursor)
2. Selection and Fragmentation of precursor ion (CID)

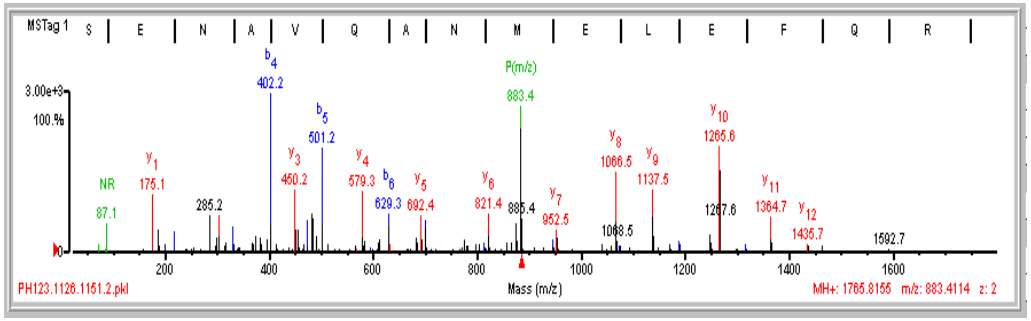
TANDEM MASS SPECTROMETRY
Quadrupole Time-Of-Flight mass spectrometer



PEPTIDE SEQUENCING BY COLLISION INDUCED DISSOCIATION



PROTEIN IDENTIFICATION: BY IDENTITY



Fragment-ion (m/z)	87.050	175.118	285.158	303.175	331.125	384.161	402.163	450.250	473.231	483.221	501.229	579.291	629.293	682.297	692.374	700.322	821.405	952.466	1066.499	1137.545	1248.560	1265.591	1364.673
Frac. Inten.(% of TIC)	0.05	3.26	2.10	3.05	1.95	1.67	11.02	4.81	2.32	4.65	8.13	4.61	3.66	2.11	3.38	3.58	3.76	2.81	7.12	6.72	2.41	13.35	3.45
Rel. Inten.(% of BP)	0.38	24.41	15.76	22.86	14.63	12.54	82.52	36.03	17.39	34.85	60.91	34.54	27.45	15.84	25.32	26.82	28.19	21.02	53.32	50.36	18.08	100.00	25.83
Score	0.33	1.50	-0.16	-0.23	-0.15	0.25	0.50	-0.36	0.50	0.25	0.50	-0.35	0.50	-0.16	-0.25	0.50	-0.28	-0.21	-0.53	1.50	0.50	1.50	1.50
Ion-type	NR	y_1				$b_4\text{-}H_2O$	b_5		a_6	$b_6\text{-}H_2O$	b_6		b_8			PWGYRG-NH_3				y_9	$y_{11}\text{-}NH_3$	y_{11}	y_{12}
Delta ppm	35.7	-8.3				25.8	3.1		-10.2	1.7	-3.5		6.6			2.2				-15.3	-27.4	-23.9	-11.9

Protein identification is done by dedicated software which compare and match the experimental spectra with *virtual spectra* derived from the DNA/Protein sequences available in Database repository

Run #	Run Name	Group (#)	Spectra (#)	Distinct Peptides (#)	Distinct Summed MS/MS Search Score	% AA Coverage	Mean Peptide Spectral Intensity	Database Accession #	Protein Name
1	PH123	1	29	19	338.05	21	1.96e+006	30407206	aconitase

Validation category	#	Filename	z	Score	Fwd-Rev Score	SPI (%)	Un-matched Ions	Spectrum Intensity	Sequence Map	MH+ Matched (Da)	MH+ Mass Shift (Da)	MH+ Error (ppm)
○ V ○ R	1	PH123.1246.1272.2	2	27.76	27.76	97.4	1 / 25	1.01e+005	(K) F V E F Y G/D G m/S E/L/S L/A/D/R(A)	1935.879	15.9944	-0.3
○ V ○ R	2	PH123.0933.0958.0	2	24.25	24.25	100.0	0 / 25	1.42e+005	(R) S E/N A V Q A N m/E/L/E/F/Q/R(N)	1765.817	15.9947	-0.1
○ V ○ R	3	PH123.0818.0875.2	2	23.50	23.50	100.0	0 / 25	4.44e+006	(R) A G E D A D T L G L T G/Q/E/R(Y)	1532.719	0.0018	1.2
○ V ○ R	4	PH123.1306.1335.2	2	23.34	23.34	91.8	4 / 25	3.57e+005	(R) S D E T V A m/I/E/A/Y/L/R(A)	1497.725	15.9966	1.1
○ V ○ R	5	PH123.1345.1370.0	2	23.27	23.27	88.0	4 / 24	2.57e+004	(K) F V E F Y G D/G H/S/E L/S/L/A/D R(A)	1935.879	0.0000	0.0
○ V ○ R	6	PH123.1126.1151.2	2	23.08	16.69	100.0	0 / 25	3.09e+005	(R) S E M A V Q A M/H E/L/E/F/Q/R(N)	1765.817	-0.0019	-1.0
○ V ○ R	7	PH123.1811.1828.0	2	22.80	22.80	92.2	3 / 25	3.97e+004	(R) N G V T A/T/D/L/V L T V/T/Q/H/L/R(K)	1831.995	-0.0033	-1.8
○ V ○ R	8	PH123.0906.0996.2	2	22.66	7.38	97.5	3 / 25	6.65e+006	(K) I/I D W E N/S/A P K(L)	1172.595	0.0009	0.8
○ V ○ R	9	PH123.1337.1362.2	2	22.23	18.27	94.0	4 / 25	6.11e+004	(R) S N L V G/m/G/I V P L I C/F K(A)	1534.812	15.9933	-1.0
○ V ○ R	10	PH123.1172.1252.2	2	21.08	8.78	96.5	3 / 25	5.37e+006	(K) F Y S L P/A/L/N D P R(I)	1292.663	0.0024	1.8
○ V ○ R	11	PH123.0901.0966.2	2	21.03	7.20	96.3	3 / 25	8.09e+006	(K) Y/L L L Q/S/G L/Q K(Y)	1049.599	0.0010	0.9
○ V ○ R	12	PH123.1046.1106.2	2	20.58	16.96	94.1	3 / 25	3.19e+006	(K) S A/G Q D\T I I L A G A E/Y/G/S/G S S R(D)	1939.936	0.0013	0.7
○ V ○ R	13	PH123.1562.1562.0	2	19.65	19.65	80.4	6 / 24	2.39e+004	(R) N G V/T/A/T/D/L/V L/T/V/T Q/m/L/R(K)	1831.995	15.9949	0.0
○ V ○ R	14	PH123.0807.0810.2	2	19.27	11.19	86.4	7 / 24	1.41e+005	(K) G H/T/m/D P/P/G P H/G V R(D)	1323.618	15.9971	1.6
○ V ○ R	15	PH123.1541.1567.0	2	19.06	19.06	88.9	5 / 25	2.54e+004	(R) N G V T\A T/D L/V L/T/V/T/Q/m/L R(K)	1831.995	15.9949	0.0
○ V ○ R	16	PH123.1043.1108.0	2	19.00	5.05	96.2	4 / 25	7.43e+005	(K) L/V E I P/F K P A R(V)	1169.704	0.0012	1.0
○ V ○ R	17	PH123.0707.0770.2	2	18.88	5.35	97.1	3 / 25	9.52e+006	(K) T S L A P/G S G V V/T/K(T)	1116.626	0.0020	1.8
○ V ○ R	18	PH123.0541.0600.2	2	18.59	5.21	83.0	5 / 25	4.40e+006	(K) S/T V/E/A/I/T/K(G)	912.467	0.0020	2.2
○ V ○ R	19	PH123.1422.1451.2	2	18.41	18.41	98.2	1 / 22	5.03e+005	(R) S D\E T V I A M I/E/A/Y L/R(A)	1497.725	0.0018	1.2
○ V ○ R	20	PH123.0772.0808.0	2	18.38	12.60	89.4	7 / 25	1.83e+005	(K) G H T H/D P/P/G P H/G V R(D)	1323.618	0.0013	1.0
○ V ○ R	21	PH123.0745.0811.2	2	17.68	5.15	97.8	4 / 21	2.07e+006	(K) L/L N G E/V/G/P/R(T)	926.531	0.0012	1.3
○ V ○ R	22	PH123.0613.0686.2	2	17.26	6.16	92.9	5 / 25	1.34e+006	(K) T/V H I/P T/G E K(L)	981.536	0.0040	4.0
○ V ○ R	23	PH123.0520.0605.2	2	16.95	16.95	76.2	3 / 23	1.11e+006	(R) D A H N N L/G/S/D/S D/K(I)	1266.527	0.0005	0.4
○ V ○ R	24	PH123.1078.1156.2	2	16.62	6.41	93.3	8 / 25	6.77e+005	(K) L/S V F/D/A/A/H/K(Y)	981.507	0.0017	1.8
○ V ○ R	25	PH123.0928.0960.2	2	16.59	7.72	89.3	8 / 24	1.03e+006	(K) L/S V F/D/A/A/m/K(Y)	981.507	15.9985	3.6

Cytosolic aconitase
(*Nicotiana tabacum*)
(gi11066033;gbAAG28426.1)

```
1   MAAENPFKGI LTVLPKPGGG EFGKFYSLPA LNDPRIDKLP YSIRILLESS IRNCDNFQVK KEDVEKIIDW ENSAPKLVEI  80
81  PFKPARVLLQ DFTGVPAVVD LACMRDAMNN LGSDSDKINP LVPVDLVIDH SVQVDVTRSE NAVQANMELE FQRNKERFAF 160
161 LKWGSNAFQN MLVVPPGSGI VHQVNLEYLG RVVFNREGLL YPDSVVGTDS HTTMIDGLGV AGWGVGGIEA EAAMLGQPMS 240
241 MVLPGVVGFK LSGNLRNGVT ATDLVLTVTQ MLRKHGVVGK FVEFYGEGMS GLSLADRATI ANMAPEYGAT MGFFPVDHVT 320
321 LQYLKLTGRS DETVGMVESY LRANNMFVDY KEPQQEKVYS SYLNLDLADV EPCLSGPKRP HDRVPLKEMK SDWHACLDNK 400
401 VGFKGFAVPK EVQDKVAEFS FHGQPAELKH GSVVIAAITS CTNTSNPSVM LGAALVAKKA SELGLHVKPW VKTSLAPGSG 480
481 VVTKYLLKSG LQKYLNQQGF NIVGYGCTTC IGNSGDLDES VASAISENDI VAAAVLSGNR NFEGRVHALT RANYLASPPL 560
561 VVAYALAGTV DIDFEKDPIG VGKDGKDVYF RDIWPSTEEI AEVVQSSVLP DMFKSTYEAI TKGNTMWNEL SVPTTKLYQW 640
641 DPKSTYIHEP PYFKGMTMDP PGPHGVKDAY CLLNFGDSIT TDHISPAGSI HKDSPAARYL MERGVDRRDF NSYGSRRGND 720
721 EIMARGTFAN IRLVNKLLNG EVGPKTVHIP SGEKLSVFDA AMKYKSAGQS TIILAGAEYG SGSSRDWAAK GPMLLGVKAV 800
801 IAKSFERIHR SNLVGMGIVP LCFKAGEDAD TLGLTGQERY TIDLPENISE IRPGQDVTVQ TDTGKSFTCV VRFDTEVELA 880
881 YFNHGGILQY VIRQLTKH                                                                    898
```

PROTEIN IDENTIFICATION: BY HOMOLOGY

Identification of the peptide sequence in large protein/EST database from distant species *(i.e. Viridiplantae kingdom)*

1. **Sequencing: peptide can identified in homologous proteins across plant species -> high redundancy -> Selection of the peptide pool (unique peptides)**
2. **Alignment of unordered unique peptides to individuate the most similar sequence in other species**

z	Score	Fwd-Rev Score	SPI (%)	Spectrum Intensity	Sequence Map	m/z measured (Da)	MH+ matched (da)	MH+ mass shift (Da)	MH+ Error (ppm)	Hom. Protein
3	**22,65**	13,58	**95,9**	2,16E+06	(R)I/I|G|V/S/V/D/S/S/G K/P A L R(M)	500,292	1498,86	0,0036	2,4	3334199
2	**22,28**	10,49	**92,5**	8,61E+05	(K)I/A|I|L|N/A/N Y/M/A/K(R)	611,337	1221,67	-0,0003	-0,2	46576630
2	**21,52**	9,79	**96,2**	6,24E+05	(K)A/D|V|N/N/N/V|L|K(V)	493,767	986,527	0,0002	0,2	115000000
2	**20,91**	3,07	**98,7**	1,60E+06	(K)I/V|A|V|G T/D/A/K(G)	437,256	873,504	0,0011	1,3	12229797
2	**17,29**	3,54	**93,7**	2,48E+06	(R)V/D|N|V|Y/G D/R(H)	469,223	937,437	0,0011	1,2	307000000
2	**17,07**	7,04	**65,2**	3,05E+05	(K)I/A|I|L|N/A/N Y/m A K(R)	619,334	1221,67	15,9943	-0,5	46576630
2	**16,94**	3,11	**94,8**	1,13E+06	(R)E E|I/A/Q/I/E/K(G)	480,257	959,504	0,0021	2,2	46576630
2	**15,75**	15,75	**80,3**	4,01E+05	(R)F/C|D|A|L|I/S I/R(E)	547,787	1094,57	0,0002	0,2	46576630
2	**15,67**	7,53	**91,9**	1,02E+06	(K)N T|A|G|I|E|P E D V/A K(R)	622,313	1243,62	0,0023	1,8	46576630
2	**15,49**	4,75	**88,2**	1,42E+06	(K)G N|I|N|I/E/E/L/R(N)	529,287	1057,56	0,0027	2,5	12229797
2	**15,29**	3,80	**89,5**	1,30E+06	(R)V/D|N|V|Y/G D/R(H)	469,223	937,437	0,0015	1,6	307000000
2	**15,19**	6,28	**91,2**	3,15E+05	(R)E Y|A|A|F|P/A S W L R(V)	655,83	1310,65	0,0007	0,6	304000000
2	**13,46**	7,94	**79,2**	2,18E+05	(R)E Y|A|A|F|P A S\W/L R(V)	655,83	1310,65	0,0007	0,6	304000000
2	**12,88**	12,88	**85,1**	2,82E+04	(K)L G T V|E/V|Q|D|L|P F F D T V/K(V)	904,475	1807,95	-0,0043	-2,4	3334200
2	**12,22**	5,07	**88,0**	2,61E+05	(R)I/I|G|V|S/V D/S S G K(Q)	531,296	1061,58	0,0006	0,6	12229797
2	**10,86**	3,25	**72,4**	8,73E+05	(R)A/D|G|F E/L/K(V)	390,2	779,393	0,0001	0,2	121083
3	**10,48**	10,48	**58,7**	2,38E+04	(K)I V\A|V|G/T D A K/G N/I/N I E E L R(N)	638,019	1912,05	-0,0068	-3,5	12229797

Peptide alignment:
The importance of bioinformatics tools (Blast, MS blast, FASTS)

It is the best approach for non model species

It is NOT quantitative (the spot quantification is done on 2D-E maps)

APPLICATIONS OF PLANT PROTEOMICS: … SOME EXAMPLES

JOURNAL OF PROTEOMICS 74 (2011) 1301–1322

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Review

Plant proteome changes under abiotic stress — Contribution of proteomics studies to understanding plant stress response

Klára Kosová[a,*], Pavel Vítámvás[a], Ilja Tom Prášil[a], Jenny Renaut[b]

[a] Department of Genetics and Plant Breeding, Crop Research Institute, Drnovská Street 507, 161 06 Prague 6 – Ruzyně, the Czech Republic
[b] Centre de Recherche Public, Gabriel Lippmann, Rue du Brill, 4422 Belvaux, Luxembourg

624 DOI 10.1002/pmic.201200263 *Proteomics* 2013, *13*, 624–636

REVIEW

Proteomics dissection of plant responses to mineral nutrient deficiency

Cuiyue Liang, Jiang Tian and Hong Liao

State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Root Biology Center, South China Agricultural University, Guangzhou, P. R. China

2976 DOI 10.1002/pmic.200700181 *Proteomics* 2007, *7*, 2976–2996

REVIEW

Crop proteomics: Aim at sustainable agriculture of tomorrow

Ghasem Hosseini Salekdeh[1] and Setsuko Komatsu[2]*

[1] Agricultural Biotechnology Research Institute of Iran, Karaj, Iran
[2] National Institute of Crop Science, Tsukuba, Japan

JOURNAL OF PROTEOMICS 74 (2011) 1230–1245

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ELSEVIER

Review

Proteomics as an approach to the understanding of the molecular physiology of fruit development and ripening

José M. Palma, Francisco J. Corpas, Luís A. del Río*

Departmento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín, CSIC, Apartado 419, 18080 Granada, Spain

Journal of proteome research

dx.doi.org/10.1021/pr2008829 | *J. Proteome Res.* 2012, 11, 26–36

REVIEWS

pubs.acs.org/jpr

We Are What We Eat: Food Safety and Proteomics

Angelo D'Alessandro and Lello Zolla*

Department of Ecological and Biological Sciences, Tuscia University, Largo dell'Università snc, 01100 - Viterbo, Italy

Proteomics 2012, *12*, 673–690 DOI 10.1002/pmic.201100359 673

REVIEW

Proteomics and plant disease: Advances in combating a major threat to the global food supply

Christof Rampitsch[1] and Natalia V. Bykova[2]

[1] Agriculture and Agrifood Canada, Winnipeg, MB, Canada
[2] Department of Biology, Memorial University of Newfoundland, St. John's, NL, Canada

Update on Evaluation of Genetically Engineered Crops

Plant Physiology®, April 2011, Vol. 155, pp. 1752–1761,

Evaluation of Genetically Engineered Crops Using Transcriptomic, Proteomic, and Metabolomic Profiling Techniques[W]

Agnès E. Ricroch, Jean B. Bergé, and Marcel Kuntz*

Université Paris-Sud, Centre National de la Recherche Scientifique, AgroParisTech, Laboratoire Ecologie, Systématique et Evolution (Unité Mixte de Recherche 8079), F–91405 Orsay, France (A.E.R.); Institut National de la Recherche Agronomique, F–06606 Antibes, France (J.B.B.); Institut de Recherches en Technologies et Sciences pour le Vivant, Laboratoire de Physiologie Cellulaire Végétale, Commissariat à l'Energie Atomique/Centre National de la Recherche Scientifique/Institut National de la Recherche Agronomique/Université Joseph Fourier (Unité Mixte de Recherche 5158), F–38054 Grenoble cedex 9, France (M.K.)

JOURNAL OF PROTEOMICS 93 (2013) 234–244

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ELSEVIER

Review

Biofuels as a sustainable energy source: An update of the applications of proteomics in bioenergy crops and algae☆

Bongani Kaiser Ndimba[a,b], Roya Janeen Ndimba[c], T. Sudhakar Johnson[d], Rungaroon Waditee-Sirisattha[e], Masato Baba[f,g], Sophon Sirisattha[h], Yoshihiro Shiraiwa[f,g], Ganesh Kumar Agrawal[i,j], Randeep Rakwal[i,j,k,l,m,]*

PROTEOMICS FOR THE STUDYING MUTANTS GENOTYPES WITH ALTERATIONS IN ANTHOCYAN IN ACCUMULATION IN FLOWERS AND FRUITS

Proteomics can be a useful approach in order to obtain a large-scale characterization of biochemical traits in mutant genotypes of crop species, revealing unexpected pleiotropic effects.

GEL-BASED COMPARATIVE PROTEOMICS OF MUTANT *VS* WILD-TYPE

1. FLOWER: red vs white flower in petunia (*Petunia x hibrida)*

Journal of Proteomics 131 (2016) 38–47

Proteomics of red and white corolla limbs in petunia reveals a novel function of the anthocyanin regulator ANTHOCYANIN1 in determining flower longevity

Bhakti Prinsi [a,*], Alfredo S. Negri [a], Francesca M. Quattrocchio [b], Ronald E. Koes [b], Luca Espen [a]

2. FRUIT: fruit ripening in somaclonal variant of sweet cherry (*Prunus avium* L.)

DOI: 10.1021/acs.jafc.6b01039
J. Agric. Food Chem. 2016, 64, 4171–4181

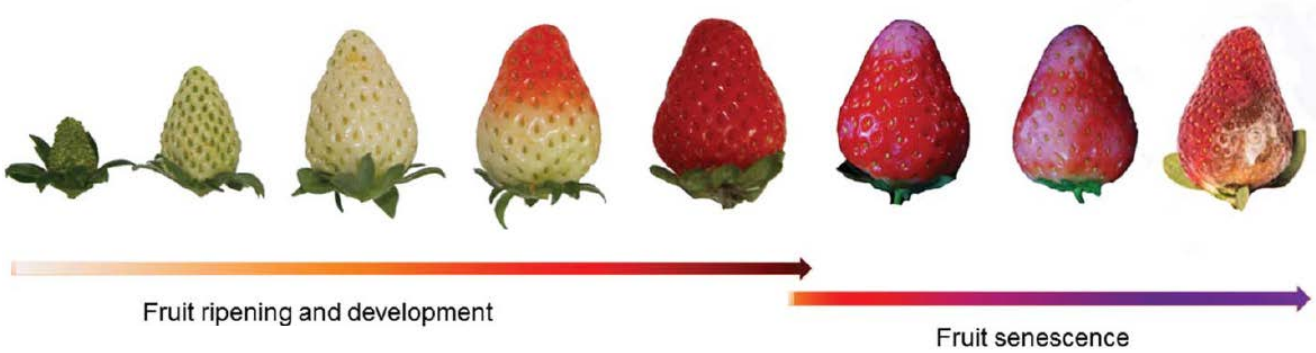
Proteomic Comparison of Fruit Ripening between 'Hedelfinger' Sweet Cherry (*Prunus avium* L.) and Its Somaclonal Variant 'HS'

Bhakti Prinsi,* Alfredo S. Negri, Luca Espen, and M. Claudia Piagnani

FLOWERING AND FRUIT RIPENING

(Van Doorn et al, 2003 Plant Mol Biol, doi:10.1023/B:PLAN.00000 23670.61059.1d)

Development of flowers) of *Iris hollandica* cv. Blue Magic. Bud development and flower opening from day –3 to day 2.



A specific example of strawberry fruit ripening and senescence.

PIGMENTS
- **Biosynthesis**
- **Accumulation**

SENESCENCE
- **Climacteric / non-climacteric fruits**
- **Ethylene sensitive / non-sensitive flowers**

Proteomic insights into floral biology☆

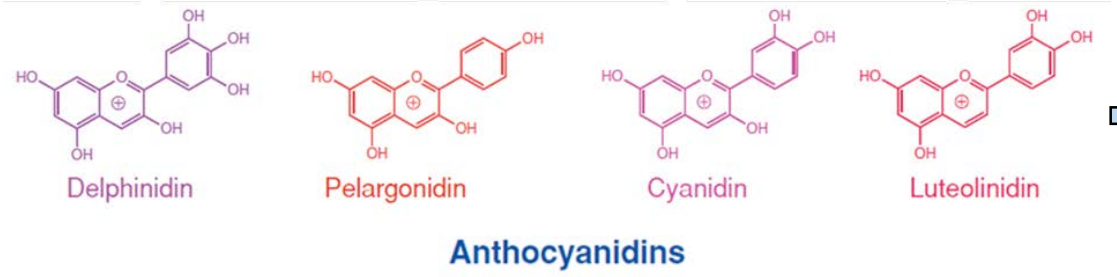
Biochimica et Biophysica Acta 1864 (2016) 1050–1060

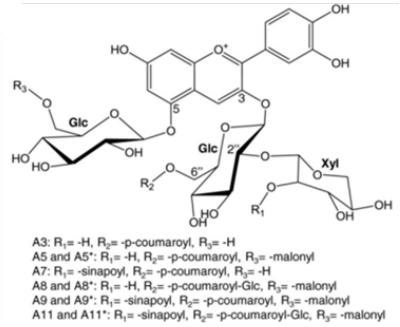
Xiaobai Li [a,b,*], Aaron Jackson [c], Ming Xie [a,*], Dianxing Wu [b], Wen-Chieh Tsai [d], Sheng Zhang [e]

Table 1
Overview of the latest proteomic studies on flower biology. The table lists the implemented proteomic approaches including separating and mass spectrometer methods, plant species, tissue, and references according to the floral organs.

	Species	Organ/tissue	Protein separation	MS analysis	Reference
Corolla	Rose (*Rosa hybrida*)	Closed bud, mature flower and flower at anthesis	2DE	MALDI-TOF	[53]
	Petunia (*Petunia hybrida*)	Non-senescing (unpollinated) and senescing (pollinated) corollas	2DE	LC–MS/MS	[54]
	Soybean (*Glycine max* L)	Flower buds, and flowers	2DE	MALDI-TOF; N-terminal amino acid sequencing	[91]
	Cymbidium ensifolium	Labellum and the inner lateral petals	2DE	MALDI-TOF/TOF MS	[55]
	Lotus (*Nelumbo nucifera*)	Red and white petals	2DE	MALDI-TOF/TOF	[56]
	Watermelon, tomato, carrot, orange cauliflower, red papaya, and red bell pepper	Chromoplasts	1DE	LC–MS/MS	[107]
	Three *Ophrys* species (*O. exaltata*, *O. sphegodes* and *O. garganica*)	Labellum tissue of an unpollinated flower	SDS-PAGE	LC–MS/MS	[32]
	Arabidopsis	Flower from wild-type and ferritin mutant plants	2DE	nanoLC–MS/MS	[88]
Pedicel	Tomato	Floral pedicel treated with ethylene or 1-methylcyclopropene	iTRAQ	nanoLC–MS/MS	[136]
Nectar	*Nicotiana attenuata*	Nectary	1DE, 2DE	LC–MS/MS	[87]
	Chestnut, acacia, sunflower, eucalyptus and orange	Honey	1DE, 2DE	Orbitrap	[145]
Male reproductive organ	Rice (*Oryza sativa*)	Anthers	2DE	MALDI-TOF MS	[37]
	Arabidopsis thaliana	mature pollen	2DE	ESI–MS/MS.	[39]
	Arabidopsis thaliana	Pollen coat protein	SDS-PAGE	Edman sequencing	[35]
	Rice (*Oryza sativa*)	Mature pollen grains	1DE, 2DE	MALDI-TOF MS, ESI Q-TOF MS/MS	[36]
	Maize (*Zea mays*)	Pollen coat protein	SDS-PAGE	Not mentioned	[38]
	Canola (*Brassica napus*)	Mature pollen and the	2DE using DIGE	MALDI-TOF/TOF MS	[42]
Female reproductive organ	Maize (Zea mays)	Egg	1DE, 2DE	LC–MS/MS	[48]
	Rice (*Oryza sativa*)	Egg	1DE	LC–MS/MS	[49]
	Soybean (*Glycine max*)	Un-pollinated and pollinated pistils	2DE	MALDI-TOF–MS	[47]
	Juniperus communis (common *juniper*), *Juniperus oxycedrus* (prickly *juniper*), *Chamaecyparis lawsoniana* (Port Orford *cedar*), and *Welwitschia mirabilis*.	Pollination drop	1DE	LC–MS/MS	[51]
	Douglas Fir	Ovular secretions (pollination drop)	1DE, 2DE	MALDI-TOF MS	[50]
	Lilium longiflorum and *Olea europaea*	Stigmatic exudate (pollination drop)	1DE	LC–MS/MS	[52]
	Chamaecyparis lawsoniana	Pollination drop	1DE, reversed-phase	Q-TOF MS/MS	[155]

ANTHOCYANINS IN FLOWERS AND FRUITS





Grotewold, Annu Rev Plant Biol 2006, 57:761–80; Kovinich et al, Planta 2014, 240: 931-941.

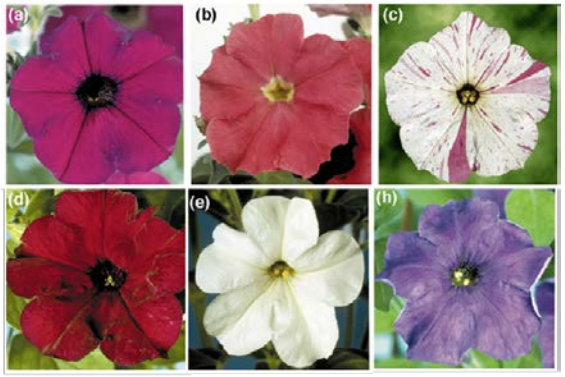
- Anthocyanins are **water-soluble pigments** that are responsible for the **orange, red, purple, and blue colors** of flowers and fruits.
- Anthocyanins are derived from a branch of the **flavonoid pathway**.
- Anthocyanins are **modified** at one or several positions by methylation, acylation, or glycosylation.
- Anthocyanins are accumulated in the **vacuole of epidermal cells**.
- The main biological functions of anthocyanins are is to **attract** pollinators and animals for seed dispersal and **antioxidant activity** in epidermis.

Pietta J. Nat. Prod. 2000, 63, 1035-1042 .

Table 3. Trolox Equivalent Antioxidant Capacity (mM), E_7 (V), and $E_p/2$ (V) of Flavonoids (the Hydroxylation Pattern Is Shown in Parentheses for Each Component)

Flavonols	TEAC (mM)	E_7 (V)	$E_p/2$ (V)
quercetin (3, 5, 7, 3′, 4′)	4.7	0.33	0.06
quercetin 3-rutinoside	2.42	0.6	0.18
kaempferol (3, 5, 7, 4′)	1.34	0.75	0.12
myricetin (3, 5, 7, 3′, 4′, 5′)	3.10	0.36	
galangin (3, 5, 7)	1.49	0.62	0.32
Flavanonols			
	TEAC (mM)	E_7 (V)	$E_p/2$ (V)
taxifolin (3, 5, 7, 3′, 4′)	1.9	0.5	0.15
dihydrokaempferol (3, 5, 7, 3′)	1.39		
Flavanones			
	TEAC (mM)	E_7 (V)	$E_p/2$ (V)
eriodictyol (5, 7, 3′, 4′)	1.8		
hesperetin [5, 7, 3′, 4′(och$_3$)]	1.4		0.4
naringenin (5, 7, 4′)	1.5		0.6
naringenin 7-rutinoside	0.8		
Catechins and Catechin Gallates			
	TEAC (mM)	E_7 (V)	$E_p/2$ (V)
catechin (3, 5, 7, 3′, 4′)	2.4	0.57	0.16
epicatechin (3, 5, 7, 3′, 4′)	2.5		
epigallocatechin (3, 5, 7, 3′, 4′, 5′)	3.8	0.42	
epicatechin gallate	4.93		
epigallocatechin gallate	4.75	0.43	
Anthocyanidins			
	TEAC (mM)	E_7 (V)	$E_p/2$ (V)
cyanidin (3, 5, 7, 3′, 4′)	4.4		−0.23
cyanidin 3-rutinoside	3.2		
pelargonidin (3, 5, 7, 4′)	1.3		

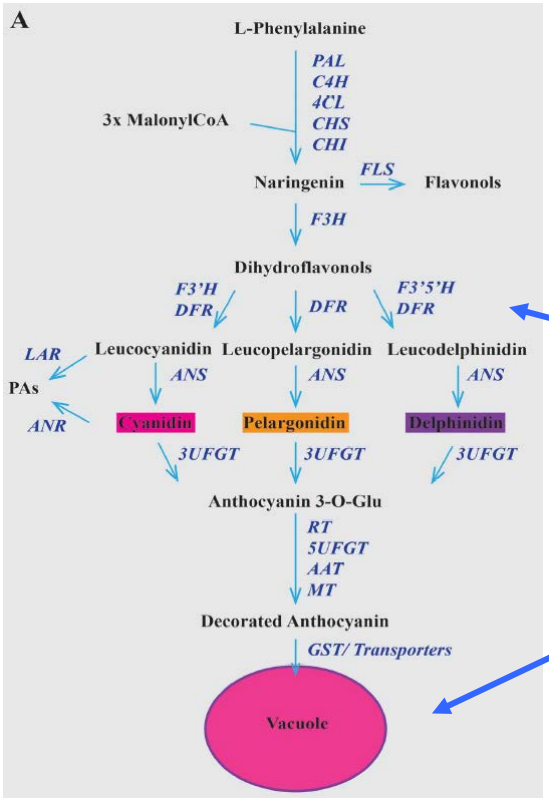
PIGMENTATION IN *PETUNIA HYBRIDA* FLOWERS



Petunia was chosen as a model species to study several aspects of flower pigmentation:

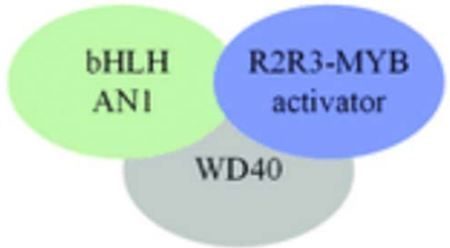
- **Large collections of spontaneous mutants.**
- **Large flowers and continuous flowering in greenhouse.**
- **Wide-world cultivation and high economic relevance.**

Fig. 1. Phenotypes of pigmentation mutants in *Petunia hybrida* flowers. (Mol et al, 1998, Trends Plant Sci, 3, 212-217)



A transcription factor complex (WMB) is involved in the control of:

- **Biosynthesis**
- **Transport into the vacuole**
- **Vacuolar acidification**



Scheme of the biosynthetic pathway for different flavonoid pigments among which anthocyanins. The main enzymes catalysing there actions in the pathway are reported in blue. (Pesseri et al, 2016, Front Plant Sci, 7:153)

SENESCENCE IN *PETUNIA HYBRIDA* FLOWERS

Petal senescence (wilting, withering, abscission) is developmental-regulated and comprises ordered events:

- **Protein degradation in mitochondria and cytoplasm, and fatty acid breakdown in peroxisomes**
- **Degradation of macromolecules due to autophagic processes in the vacuole**
- **Transport of the mobile compounds out of the petal**
- **Tonoplast rupture, degradation of nucleic acids in the nuclei → Programmed Cell Death (PCD)**

In petunia:

- **Corolla senescence is induced after pollination.**
- **Corolla senescence occurs in unpollinated flowers within 3 days after anthesis.**
- **Corolla senescence is highly ethylene sensitive and regulated by ethylene**

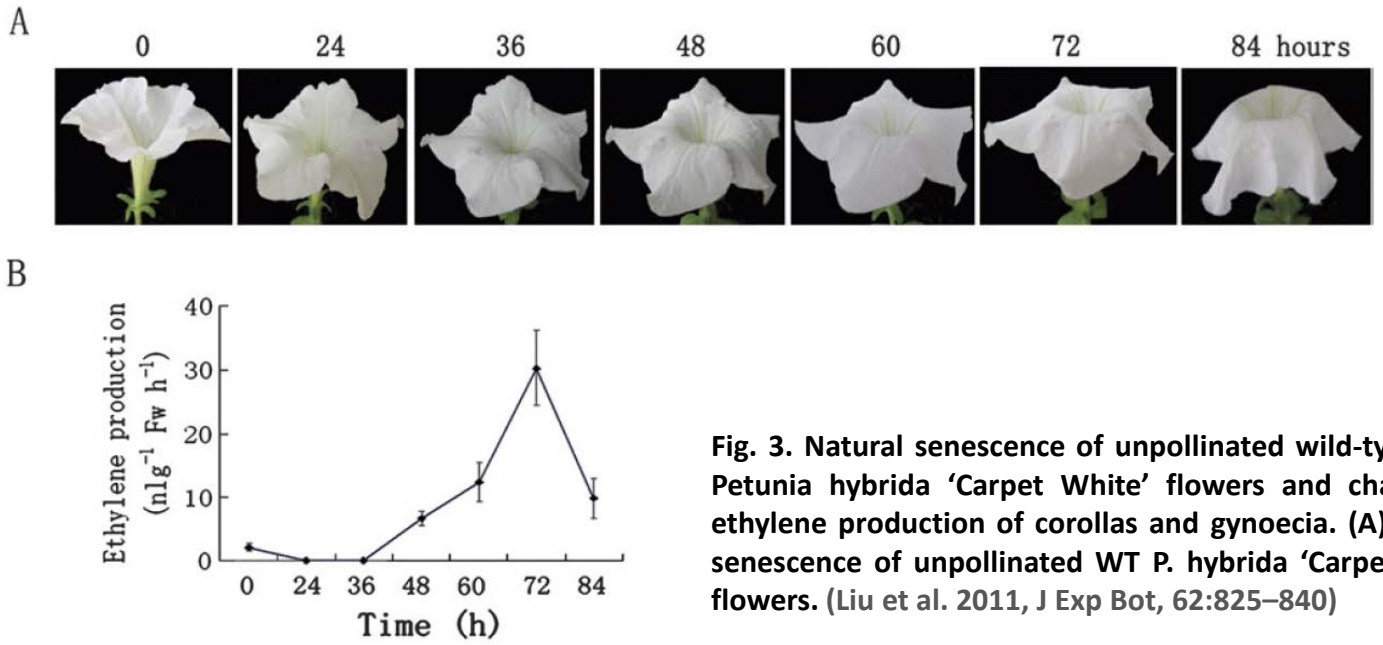


Fig. 3. Natural senescence of unpollinated wild-type (WT) Petunia hybrida 'Carpet White' flowers and changes in ethylene production of corollas and gynoecia. (A) Natural senescence of unpollinated WT P. hybrida 'Carpet White' flowers. (Liu et al. 2011, J Exp Bot, 62:825–840)

PROTEOMIC COMPARISON OF RED *vs* WHITE FLOWER IN PETUNIA

EXPERIMENTAL DESIGN

- **R27: bright red coloured flowers accumulating cyanidin derivatives**
- **W225: isogenic line with stable recessive null allele *an1,* white flowers**
- **Plants grown in pots in growth chamber (16/8 h; 24/20 °C; 65% UR)**
- **Corolla limbs were collected from flowers at 1 day after anthesis (DAA).**

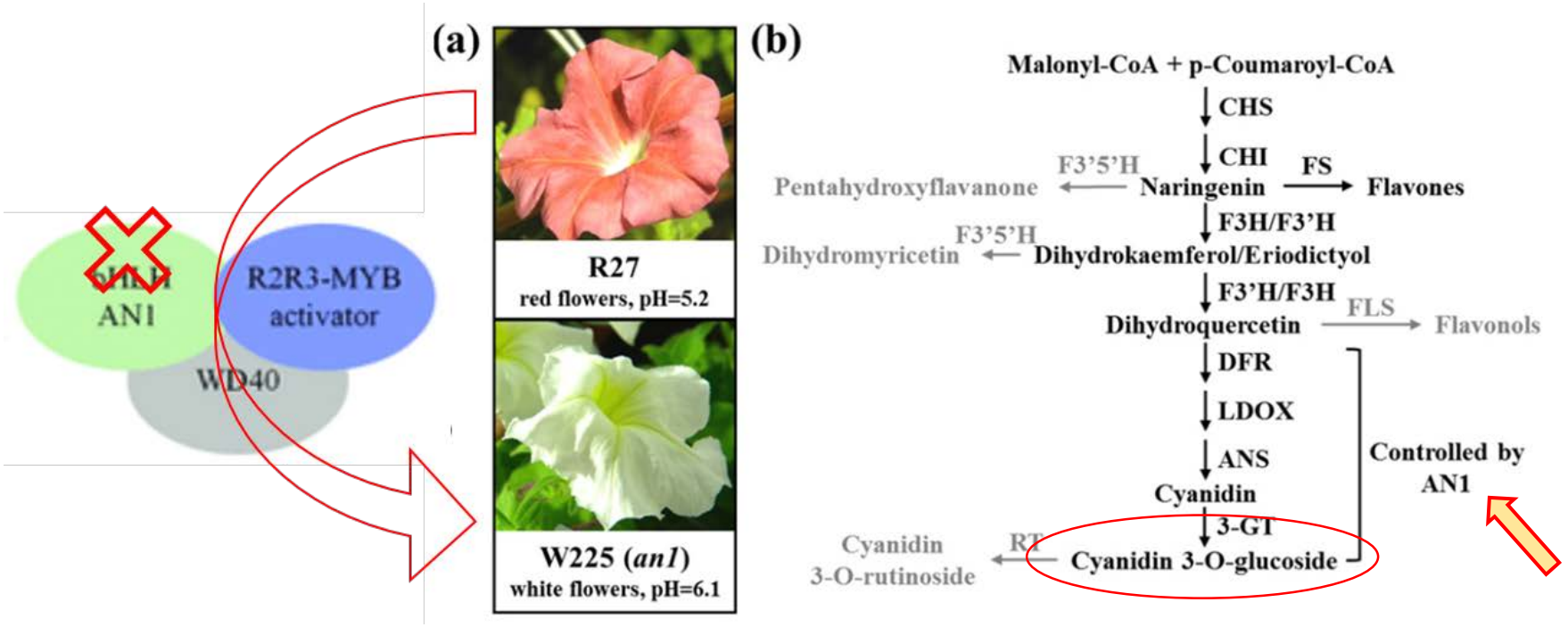


Fig. 1. Phenotypic and genetic characters of the *Petunia hybrida* lines. (a) Flower feature and pH of petal cell sap in R27 and W225 lines. (b) Simplified scheme of the flavonoid pathway in the R27 genetic background. The enzymes (and relative products) encoded by mutated gene are reported in grey. W225 harbours a mutation in the *AN1* gene, encoding a transcription factor that controls Late Biosynthetic Genes (indicated by bracket) and vacuolar acidification. CHS: chalcone synthase; CHI: chalcone isomerase; F3′5′H: flavonoid 3′5′ hydroxylase; FS: flavone synthase; F3H: flavonoid 3-hydroxylase; F3′H: flavanone 3′-hydroxylase; FLS: flavonol synthase; DFR: dihydroflavonol 4-reductase; LDOX: leucoanthocyanidin dioxygenase; ANS: anthocyanidin synthase; 3-GT: 3-glucosyltransferase; RT: anthocyanin rhamnosyl transferase.

PROTEOMICS COMPARISON OF RED *vs* WHITE FLOWER IN PETUNIA

2-DE COMPARATIVE PROFILE

- The analysis visualized an average of 1600 spots.
- 62 spots showed statistical significant differences in the accumulation levels.
- 56 spots were identified by homology search.
- 21 proteins were more abundant in red flowers, 35 proteins were more abundant in white flowers

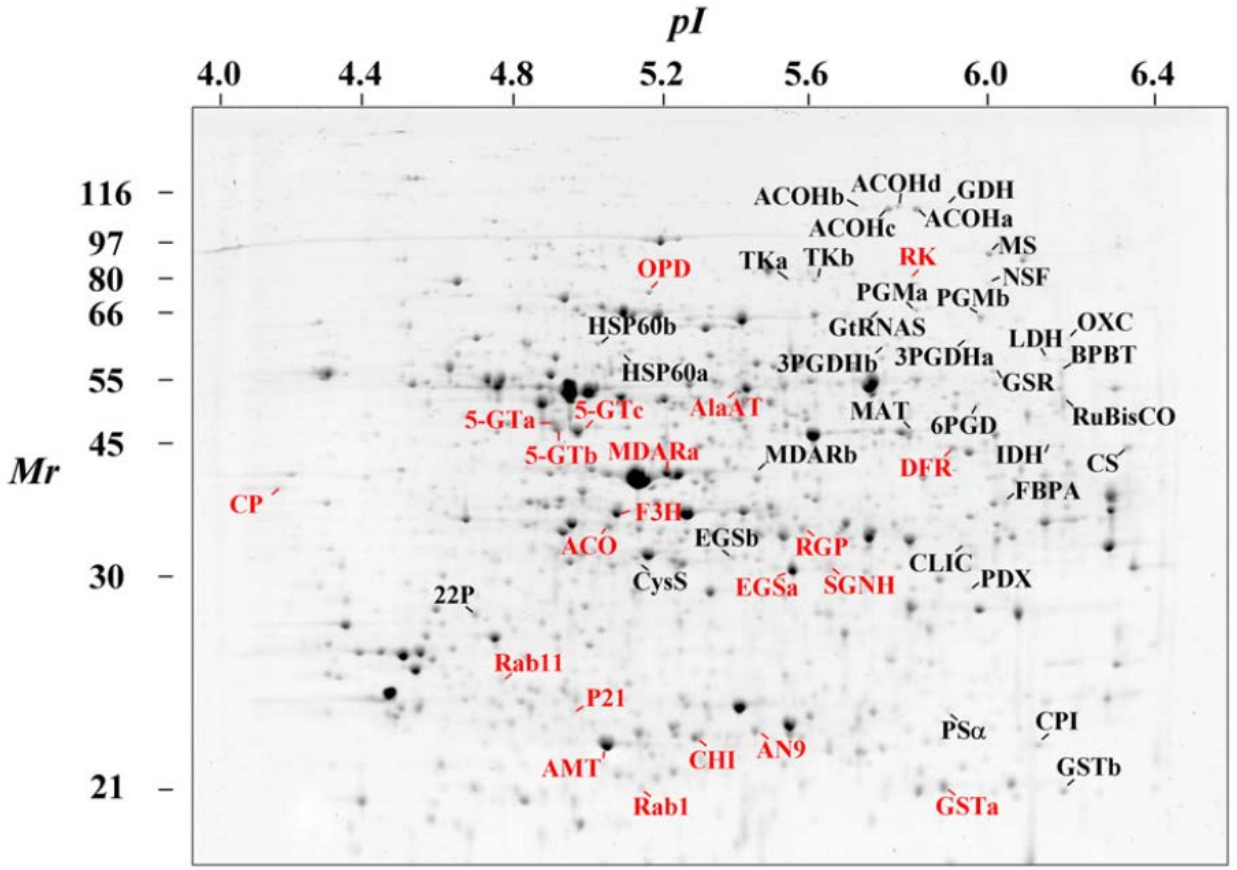


Fig. 2. 2-DE profile of proteins differentially accumulated in corolla limbs of *Petunia hybrida AN1* (R27) and *an1* (W225) lines at 1 day after anthesis. The figure reports one of the electrophoretic maps of the R27 corolla limbs. Total proteins (400 μg) were analysed by IEF at pH 4–7, followed by 10% SDS-PAGE and visualized by cCBB staining. Acronyms refer to Table 1. Protein with higher accumulation level in R27 red flowers are reported in red, those more abundant in W225 white flowers are reported in black. Standard molecular mass range in kDa (Mr) and p*I* range are reported on the left and above, respectively.

PROTEOMICS COMPARISON OF RED *vs* WHITE FLOWER IN PETUNIA

FUNCTIONAL CLASSIFICATION OF DIFFERENTIALLY ACCUMULATED PROTEINS

- **Nine functional classes**
- **The enzyme involved in anthocyanin pathway were more abundant in R27.**
- **White flowers were characterized by higher levels of enzymes related to primary cell functions.**

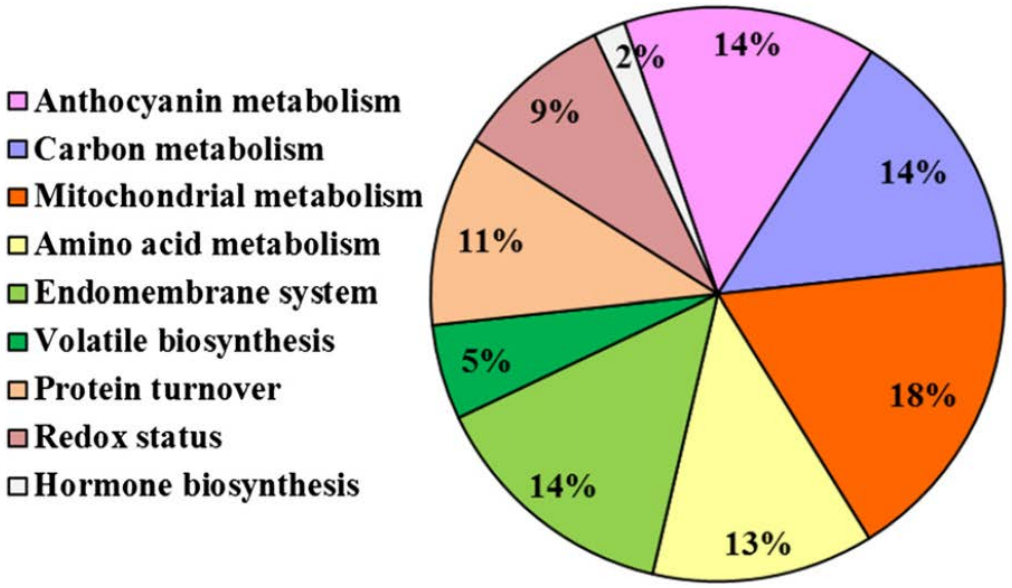


Fig. 3. Functional distribution of the characterized proteins in corolla limbs of *Petunia hybrida* flowers. The proteins differentially accumulated in corolla limbs of petunia *AN1* (R27) and *an1* (W225) lines are classified in nine distinct functional classes, according to function assignment in literature and GeneBank. Functional distribution indicates the percentage of each metabolic class as compared to the total number of identified proteins (56, see Table 1 and Fig. 2).

The analysis reveals that the mutation in the *AN1* gene in W225 has ample pleiotropic effects on flower metabolism

PROTEOMICS COMPARISON OF RED *vs* WHITE FLOWER IN PETUNIA

DIFFERENCES IN FLAVONOID METABOLISM

Protein abundance in W225 (white flower) relative to R27 (red flower)

N[a]	Acronym[b]	Accession species	Protein description	Δ W225/R27[d]
Anthocyanin metabolism				
1596	CHI	P11650 *P. hybrida*	Chalcone isomerase A	0.24
1024	F3H	Q07353 *P. hybrida*	Flavanone 3-hydroxylase	0.50
809	DFR	P14720 *P. hybrida*	Dihydroflavonol 4-reductase	0.50
744	5-GTa	BAA89009 *P. hybrida*	Anthocyanin 5-O-glucosyltransferase	0.48
761	5-GTb	BAA89009 *P. hybrida*	Anthocyanin 5-O-glucosyltransferase	0.23
776	5-GTc	BAA89009 *P. hybrida*	Anthocyanin 5-O-glucosyltransferase	0.09
1610	AMT	AIE77046 *P. hybrida*	Anthocyanin methyltransferase	0.19
1582	AN9	CAA68993 *P. hybrida*	Glutathione S-transferase	0.26

Early biosynthetic genes

Anthocyanin decoration and transport

Table 2
Levels of flavonoids in corolla limbs of *Petunia hybrida AN1* (R27) and *an1* (W225) lines at 1 day after anthesis.

	Molecular ion – Fragment ions[a] (M^+ m/z)	µmol g^{-1}FW[c]		
		R27	W225	
Flavanones		**0.48 ± 0.05**	**0.84 ± 0.04**	>
Eriodictyol[b]	289.07	0.48 ± 0.05	0.38 ± 0.02	
Eriodictyol glucoside[b]	451.12 – 289.07		0.45 ± 0.02	
Dihydroflavonols		**1.08 ± 0.10**	**3.06 ± 0.30**	>
Dihydroquercetin	305.07	0.62 ± 0.07	2.09 ± 0.24	
Dihydroquercetin glucoside	467.12 – 305.07	0.46 ± 0.03	0.97 ± 0.06	
Flavonols		**3.20 ± 0.40**	**1.71 ± 0.05**	<
Quercetin	303.05	0.36 ± 0.01		
Quercetin glucoside[b]	465.10	0.54 ± 0.03	0.34 ± 0.01	
Quercetin diglucoside	627.16 – 465.10 – 303.05	0.96 ± 0.14	0.60 ± 0.07	
Quercetin triglucoside	789.21 – 627.16 – 465.10	1.34 ± 0.21	0.77 ± 0.02	
Anthocyanins		**2.95 ± 0.26**		
Cyanidin glucoside[b]	449.11 – 287.06	1.40 ± 0.11		
Cyanidin diglucoside[b]	611.16 – 449.11 – 287.06	0.89 ± 0.14		
Cyanidin triglucoside	773.21 – 611.16 – 449.11	0.27 ± 0.01		
Peonidin glucoside	463.12 – 301.07	0.40 ± 0.01		
TOTAL		**7.72 ± 0.80**	**5.61 ± 0.39**	<

- **Changes in the composition of flavonoids in corolla**
- **Overall decrease in the flavonoid content in corolla**

Proteomics reveals that AN1 mutation in W225 provokes secondary effects on flavonoid metabolism, probably driven by biochemical factors.

PROTEOMICS COMPARISON OF RED *vs* WHITE FLOWER IN PETUNIA

DIFFERENCES IN PRIMARY METABOLISM

Protein abundance in W225 (white flower) relative to R27 (red flower)

N[a]	Acronym[b]	Accession species	Protein description	Δ W225/R27[d]
Carbon metabolism				
624	RuBisCO	P04992 *P. hybrida*	RuBisCO large subunit	5.23
497	HSP60a	AAB39827 *S. tuberosum*	Chaperonin-60 beta subunit	3.09
655	6PGD	BAA22812 *G. max*	6-phosphogluconate dehydrogenase	8.34
284	TKa	CAA75777 *C. annum*	Transketolase 1	2.56
285	TKb	CAA75777 *C. annum*	Transketolase 1	3.44
348	PGMa	Q9M4G4 *S. tuberosum*	Phosphoglucomutase cytoplasmic	2.46
354	PGMb	Q9M4G4 *S. tuberosum*	Phosphoglucomutase cytoplasmic	2.83
957	FBPA	ABC01905 *S. tuberosum*	Fructose-bisphosphate aldolase	2.06
Mitochondrial metabolism				
780	CS	P20115 *Arabidopsis thaliana*	Citrate synthase 4, mitochondrial	2.80
123	ACOHa	BAG16527 *C. chinense*	Putative aconitase	3.26
124	ACOHb	BAG16527 *C. chinense*	Putative aconitase	3.37
127	ACOHc	BAG16527 *C. chinense*	Putative aconitase	2.92
122	ACOHd	AAG28426 *N. tabacum*	Cytosolic aconitase	3.09
785	IDH	P50218 *N. tabacum*	Isocitrate dehydrogenase [NADP]	4.65
457	HSP60b	P29197 *A. thaliana*	Chaperonin CPN60, mitochondrial	2.87
108	GDH	O49954 *S. tuberosum*	Glycine dehydrogenase, mitoc.	7.94
473	LDH	AAS47493 *C. annum*	Lipoamide dehydrogenase	2.78
395	OXC	CAN69570 *V. vinifera*	Oxalyl-CoA decarboxylase[e]	4.02
Amino acid metabolism				
1247	PDX	AAS92255 *N. tabacum*	Pyridoxine biosynthesis isoform A	2.50
425	3PGDHa	XP_002273552 *V. vinifera*	D-3-phosphoglycerate dehydrogenase	2.92
461	3PGDHb	XP_002300235 *P. trichocarpa*	D-3-phosphoglycerate dehydrogenase[f]	2.92
1204	CysS	CAJ32462 *N. tabacum*	Put. cytosolic cysteine synthase 7	3.97
207	MS	AAF74983 *S. tuberosum*	Methionine synthase	3.18
771	MAT	P43282 *S. lycopersicum*	S-adenosylmethionine synthase 3	3.00
642	AlaAT	AAR05449 *C. annum*	Alanine aminotransferase	0.18

The corolla in W225 line was characterized by higher levels of enzymes of primary metabolism

→ Organelle dismantlement in R27 ?? Remobilization of macromolecules ??

PROTEOMIC COMPARISON OF RED *vs* WHITE FLOWER IN PETUNIA

DIFFERENCES IN THE PROGRESSION OF FLOWER SENESCENCE

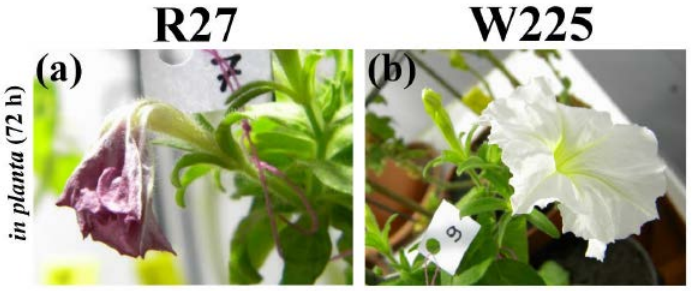
PROTEOMICS

Protein abundance in W225 (white flower) relative to R27 (red flower)

N[a]	Acronym [b]	Accession species	Protein description	Δ W225/R27 [d]
Protein turnover				
1548	P21	AAC49361 *P. hybrida*	P21	−∞
886	CP	AAU81589 *P. hybrida*	Cysteine proteinase	0.10
1766	CPI	AAU81597 *P. hybrida*	Cysteine proteinase inhibitor	+∞
1536	PSα	Q9XG77 *N. tabacum*	Proteasome subunit alpha type-6	2.90
362	GtRNAS	XP_002297878 *P. trichocarpa*	Glycyl-tRNA synthetase [f]	3.72
312	OPD	XP_002527223 *R. communis*	Oligopeptidase A, putative	0.15
Hormone biosynthesis				
1070	ACO	BAF33504 *O. minor*	ACC oxidase [g]	0.44

IN PLANTA ANALYSIS

Visual evaluation of flower longevity in (R27) and an1 (W225) lines.



BIOCHEMICAL EVALUATION AT 1 DAA

Table 3
Biochemical evaluation of senescence-related parameters in flowers of *Petunia hybrida AN1* (R27) and *an1* (W225) lines at 1 day after anthesis.

	R27	W225
Limb fresh weight (g) [a]	0.143 ± 0.002	0.144 ± 0.003
Limb water content (%) [a]	89 ± 1	90 ± 1
GSH + 2GSSG (nmol g^{-1}FW) [b]	34.76 ± 1.86	32.84 ± 2.53
GSH (%) of total glutathione [b]	89.2 ± 2.1	86.7 ± 2.0
Reducing sugars (μmol g^{-1}FW) [b]	119.36 ± 2.23[c]	77.11 ± 4.52[c]
Sucrose (μmol g^{-1}FW) [b]	6.88 ± 2.10	9.72 ± 4.01

The AN1 mutation in W225 line is associated with a prolonged longevity in flowers

PROTEOMIC COMPARISON OF RED *vs* WHITE FLOWER IN PETUNIA

THE SUGAR PARADOX IN FLOWER SENESCENCE

"… In some species, the petal sugar levels remain rather high, and thus do not seem to become limiting, but sugar feeding extended the life of these petals …

… **sugar signal is translated into an anti-ethylene signal** in sensitive species…"

(Van doorn and Woltering, 2008, J Exp Bot 59: 453-480)

IN VITRO EVALUATION OF SENSITIVITY TO ETHYLENE IN R27 AND W225

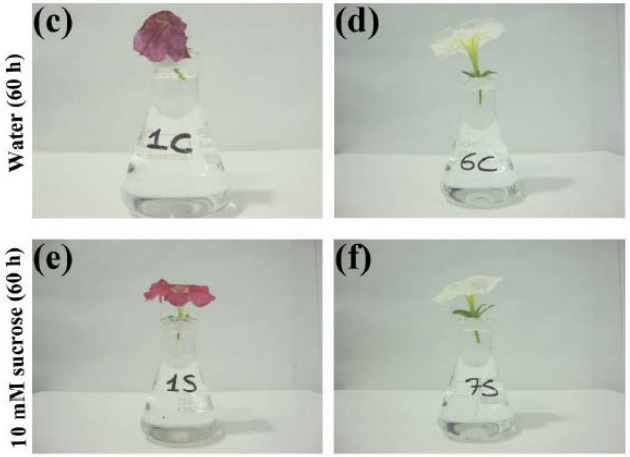


Table 4

Longevity of *in planta* and cut flowers of *AN1* (R27) and *an1* (W225) plants. The values reported are the hours from anthesis to the appearance of visible corolla wilting symptoms.

	R27 (h)	W225 (h)
In planta	72.0 ± 1.1 (b)	123.3 ± 2.9 (c)
Cut flowers in water	55.3 ± 1.8 (a)	70.7 ± 1.3 (b)
Cut flowers in 10 mM sucrose	72.7 ± 0.7 (b)	73.3 ± 1.3 (b)

Values are the mean ± SE of six flowers analysed in triplicate (n = 3). The significance was assessed through factorial ANOVA test ($p < 0.01$, Tukey *post hoc* test).

- **Sucrose supply restores flower longevity of cut flowers in R27 → ethylene-sensitive**
- **Sucrose supply does NOT restore flower longevity of cut flowers in w225 → ethylene-insensitive**

The proteomic/biochemical analysis suggest that AN1 could be involved in ethylene perception

PROTEOMICS COMPARISON OF RED *vs* WHITE FLOWER IN PETUNIA

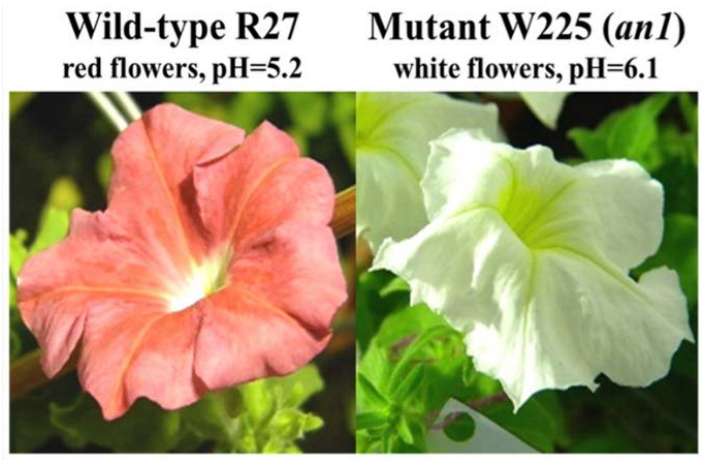
SUMMARY

The study on petunia mutant provides new information about

- **Biochemical factors affect the accumulation of enzymes involved in the anthocyanin biosynthesis that are independent from the AN1 genetic control**
- **Proteins/enzymes involved in flower senescence in *Petunia hybrida***
- **Novel roles for the AN1 transcription factor.**

⇩

THE PROTEOMIC INVESTIGATION COMBINED WITH BIOCHEMICAL AND PHYSIOLOGICAL APPROACHES WAS A VERY USEFUL TOOL FOR THE STUDY OF GENETIC PLEIOTROPY.



Proteomic Studies on Fruit Ripening and Senescence

L. Li[a], Z. Ban[b], Jarukitt Limwachiranon[a], and Z. Luo[a]

CRITICAL REVIEWS IN PLANT SCIENCES
2017, VOL. 36, NO. 2, 116–127
https://doi.org/10.1080/07352689.2017.1355173

Table 1. Proteomic studies of fruit ripening and development.

Fruit crops	Strategies	Proteomic approaches	References
Non-climacteric fruits			
Strawberry	Gel-based	2DE/2D-DIGE LC-ESI-IT-MS/MS	Bianco *et al.* 2009
	Gel-free	Peptides dimethylation labeling LC/MS qTOF	Li *et al.* 2013
	Gel-free	multiple reaction monitoring (MRM) LC-MS/MS	Song *et al.* 2015a; Song *et al.* 2015b
Grape	Gel-based	2-DE MALDI-TOF MS	Sarry *et al.* 2004
		On-line capillary HPLC nanospray ion trap MS/MS	Zhang *et al.* 2008
		LC-ESI-MS/MS	Deytieux *et al.* 2007
		MALDI-TOF MS	Negri *et al.* 2008
			Giribaldi *et al.* 2007
Pepper	Gel-based	SDS-PAGE MALDI-TOF/TOF	Camejo *et al.* 2015
Pomegranate	Gel-based	2-DE MALDI-TOF-TOF MS	Cao *et al.* 2014
Climacteric fruits			
Peach	Gel-based	SDS-PAGE MALDI-TOF/TOF & MS/MS	Camejo *et al.* 2010
	Gel-based	2-DE MALDI-TOF/TOF & MS/MS/LC-ESI-MS/MS	Zhang *et al.* 2012
			Hu *et al.* 2011
			Prinsi *et al.* 2011
			Nilo *et al.* 2012
			Wu *et al.* 2016
	Gel-based	2-DE LTQ Orbitrap XL LC-MS/MS	Karagiannis *et al.* 2016
Pear	Gel-based	2-DE MALDI TOF/TOFTM	Hu *et al.* 2012
	Gel-based	2D-DIGE MALDI-TOF/TOF	Gao *et al.* 2016
	Gel-free	iTRAQ	Li *et al.* 2015
	Gel-free	label-free (emPAI)	Reuscher *et al.* 2016
Apple	Gel-based	2-DE MALDI-TOF-MS and μLC-ESI-IT-MS/MS	Guarino *et al.* 2007
			Zheng *et al.* 2013
	Gel-free	tandem mass tag (TMT) nano liquid chromatography (LC)-MS/MS analysis in an LTQ-Orbitrap Velos	Li *et al.* 2016
Banana	Gel-based	2D-DIGE Q-TOF LC-MS/MS	Toledo *et al.* 2012
	Gel-based	SDS-PAGE nLC-MS/MS	Esteve *et al.* 2013
Prickly pear	Gel-based	2-DE ESI-Q-TOF MS/MS and MALDI-TOF MS	Rosas-Cárdenas *et al.* 2012
Mango	Gel-based	DIGE MALDI-MS/MS	Andrade *et al.* 2012
Apricot	Gel-based	2-DE MALDI-TOF-PMF nanoLC-ESI-LIT-MS/MS	D'Ambrosio *et al.* 2013
	Gel-free	Label-free LC-ESI-MS/MS	Zhang *et al.* 2017
Kiwifruit	Gel-based	SDS-PAGE Q-TOF LC-MS/MS	Minas *et al.* 2012
Papaya	Gel-based	2-DE nano-LC-ESI/MS/MS	Huerta-Ocampo *et al.* 2012
	Gel-based	DIGE Q-TOF LC-MS/MS	Nogueira *et al.* 2012
Olive	Gel-based	2-DE MALDI-TOF/TOF-MS	Wu *et al.* 2011
			Bianco *et al.* 2013
Sweet cherry	Gel-based	2-DE LC-ESI-MS/MS	Prinsi *et al.* 2016

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

THE SOMACLONE HS FROM SWEET CHERRY 'HEDELFINGER' (*Prunus avium* L.)

***Prunus avium* L**
cv Hedelfinger
(wild-type, H)

SOMACLONAL VARIATION
for crop improvement

Plant tissue *in-vitro* culture:
Regeneration from leaf explants

***Prunus avium* L**
Cv Hedelfinger
(somaclone, HS)

Commercial crop

Improvement of agronomic traits:

- **Reduction in vegetative vigour**
- **Reduction in tree size and canopy density**
- **High efficiency for light interception**
- **No differences in photosynthesis**
- **No differences in flowering time and flower morphology**

Morphological and physiological behaviour of sweet cherry 'somaclone' HS plants in field

Maria Claudia Piagnani · Dario Maffi · Mara Rossoni · Remo Chiozzotto

Euphytica (2008) 160:165–173
DOI 10.1007/s10681-007-9502-7

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

THE SOMACLONE HS FROM SWEET CHERRY 'HEDELFINGER' (*Prunus avium* L.): FRUIT QUALITY

- **No differences in fruit colour, shape, size and weight**
- **Differences in fruit titratable acidity.**
- **Differences in the dynamic of fruit growth.**

Table 7 Fruit skin colour as assessed by Minolta for wild type (H) and somaclone (HS) within the two ripening classes

	Genotype	Class	N	Min	Max	Average	Std. dev
a*	H	1	125	22.1	44.0	35.0	4.2
a*	H	2	124	5.7	30.7	18.0	5.8
a*	HS	1	67	19.5	44.3	34.4	5.7
a*	HS	2	58	6.1	28.2	16.0	4.8
b*	H	1	124	2.5	18.8	11.3	3.0
b*	H	2	124	0.9	14.5	3.9	2.6
b*	HS	1	67	1.1	18.8	11.0	4.2
b*	HS	2	58	0.9	15.8	3.3	2.6

a* and b* minimum, maximum and average value of the L*a*b* Minolta CIELAB scale

Morphological and physiological behaviour of sweet cherry 'somaclone' HS plants in field

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Table 8 Carpometric parameters (fruit weight, longitudinal and two equatorial poles diameter, pedicel length): effect of genotype × ripening class interaction

Genotype	Ripening class	Weight, g	long ∅, mm	Equat 1 ∅, mm	Equat 2 ∅, mm	Pedicel, mm
H	1	5.2 a	20.8 b	20.0 b	18.4 a	35.9 a
H	2	5.3 a	21.4 a	20.6 a	18.8 a	34.8 a
HS	1	4.6 b	19.3 d	19.7 b	18.0 b	31.9 b
HS	2	5.1 a	20.4 c	20.7 a	18.8 a	33.0 b

Means with the same letter are not different according to the Tukey's test; P = 0.05 (n = 20)

Table 9 Fruit stone/pulp ratio, flesh firmness, titratable acidity (TA, g malic acid 100 ml^{-1}), total soluble solids content (TSS) and pH as determined for H and HS ripening 'class 2'

Genotype	Stone/pulp ratio %	Flesh firmness N	TA°	TSS° (°Brix)	pH
H	7.5	4.2	17.2	16.9	3.8
HS	7.2	4.5	17.9*	16.1	3.8

The asterisks indicate a significant difference between means at 0.05 level (n = 20; π = 3°)

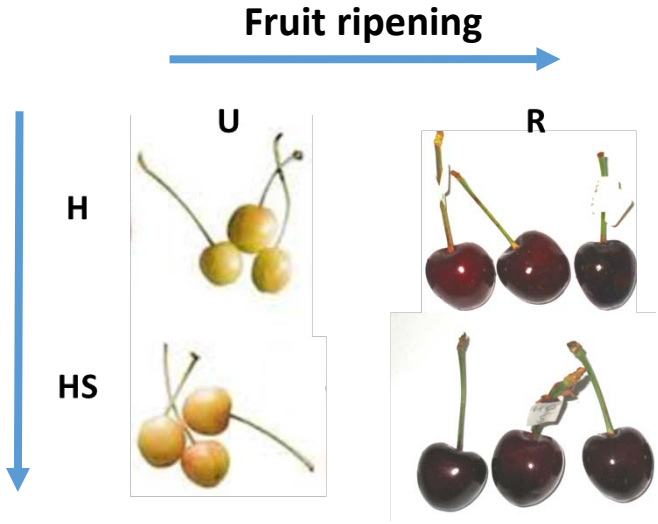
PROTEOMIC ANALISYS OF FRUIT RIPENING TO GAIN INFORMATION ABOUT BIOCHEMICAL FENOTYPIC TRAITS

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

EXPERIMENTAL DESIGN

- Comparison of Hedelfinger (H) and HS fruits
- Comparison of two stage of ripening:
  - (U, unripe): onset of ripening
  - (R, ripe): full ripeness

Genotypic differences



EVALUATION OF BIOCHEMICAL RIPENING-RELATED PARAMETERS

Table 1. Metabolic Profiles in Sweet Cherries of 'Hedelfinger' and Its Somaclonal Variant HS at Unripe and Ripe Stages[a]

	H unripe	HS unripe	H ripe	HS ripe
chlorophyll (μg/g FW)	11.75 ± 0.27 c	8.33 ± 0.34 b	1.88 ± 0.11 a	2.53 ± 0.19 a
reducing sugars (μmol glc/g FW)	432.3 ± 14.4 a	417.2 ± 7.7 a	1025.1 ± 11.9 b	1124.8 ± 49.6 b
sucrose (μmol glc/g FW)	26.27 ± 1.54 a	31.05 ± 1.23 a	33.14 ± 4.59 a	20.44 ± 3.73 a
amino acids (μmol leu/g FW)	73.57 ± 1.15 b	77.06 ± 4.66 b	32.86 ± 1.06 a	36.30 ± 0.97 a
TEAC (mM Trolox/100 g FW)	1.59 ± 0.01 b	1.79 ± 0.01 b	0.95 ± 0.08 a	1.03 ± 0.08 a
total anthocyanins (mg CGE/100 g FW)	0.33 ± 0.03 a	0.37 ± 0.02 a	11.47 ± 0.28 b	14.38 ± 0.76 c

- **Traits related to fruit ripening**: chlorophyll degradation, accumulation of reducing sugars, change in TEAC, anthocyanin accumulation.
- **Traits different in HS vs H**: HS accumulated a higher concentration of anthocyanins

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

THE PROTEOMIC MAP

- **40 spots was selected on the basis of discriminant power on PCA and PLS-DA**
- **39 spot were identified by homology search by LC-ESI-MS/MS**

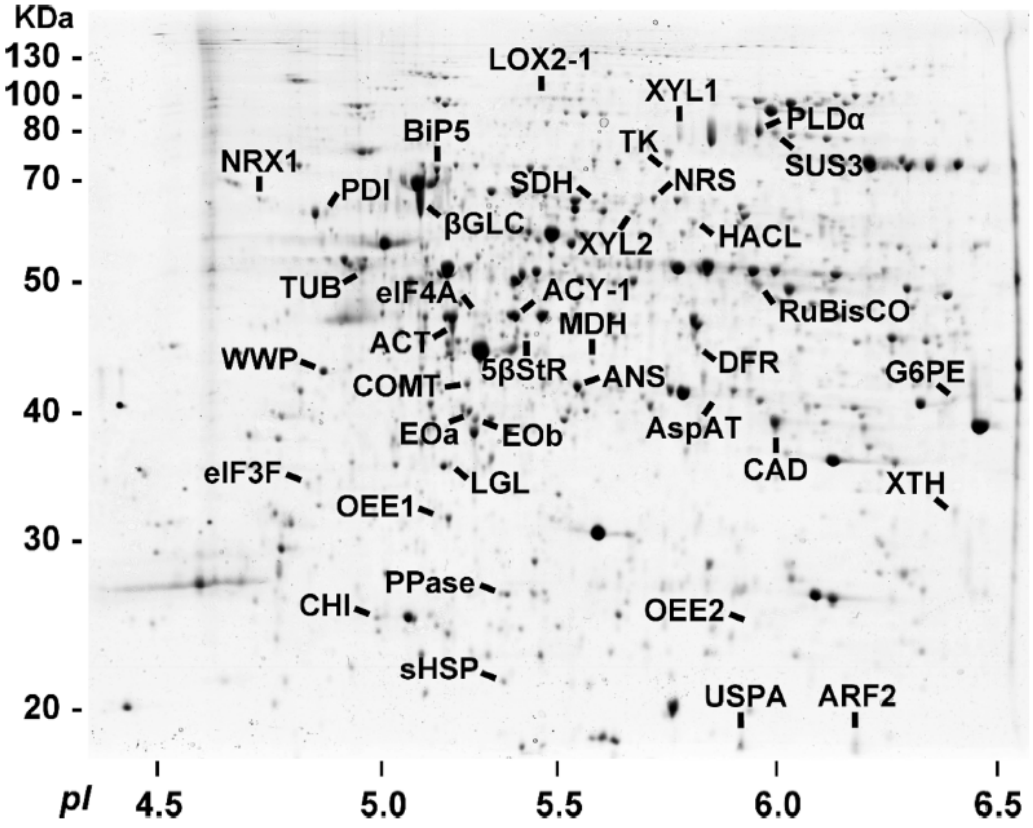


Figure 3. 2-DE profile of the proteins differentially accumulated in 'Hedelfinger' (H) and its somaclonal variant HS sweet cherries at unripe and ripe stages. The figure shows one of the electrophoretic maps of the ripe fruits of H. Total proteins were analyzed by IEF at pH 4–7, followed by SDS-PAGE and visualized by cCBB. Acronyms refer to Table 2 and Figure 4. Standard molecular mass range in kDa (M_r) and p*I* range are reported on the left and at the bottom, respectively.

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

FUNCTIONAL CLASSIFICATION OF DIFFERENTIALLY ACCUMULATED PROTEINS

- **Eight functional classes, each of them embracing protein influenced by ripening or genotype**
- **The analysis suggests a large metabolic reprogramming during fruit ripening.**
- **The analysis suggests biochemical differences in H and HS fruits.**

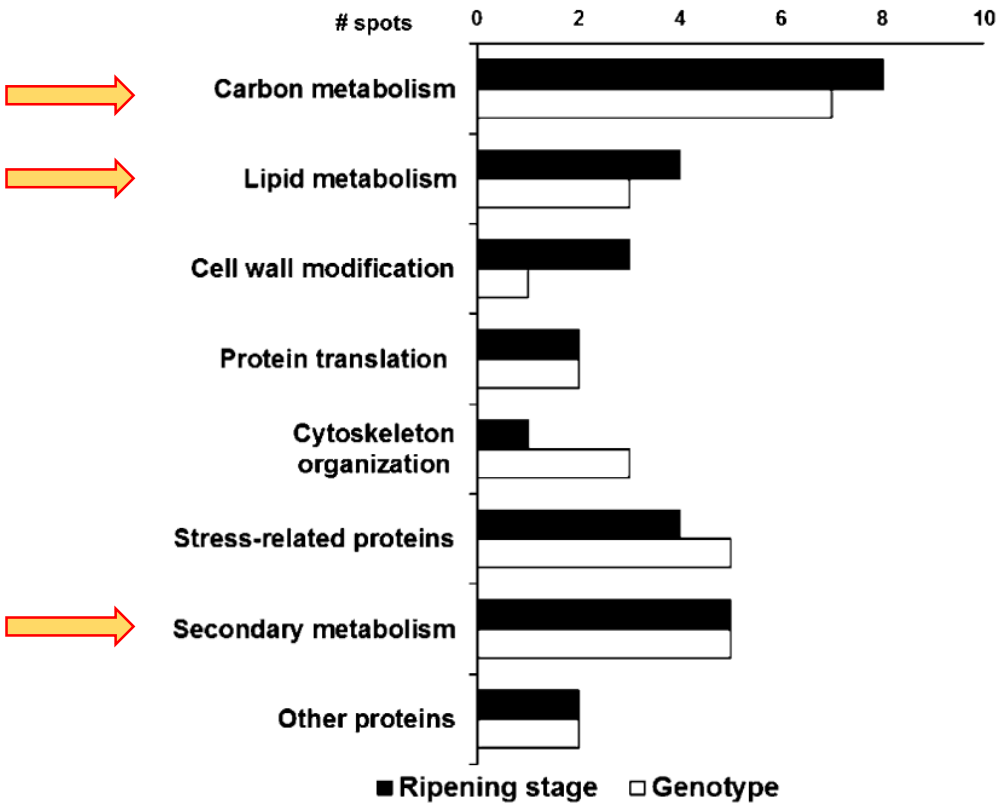
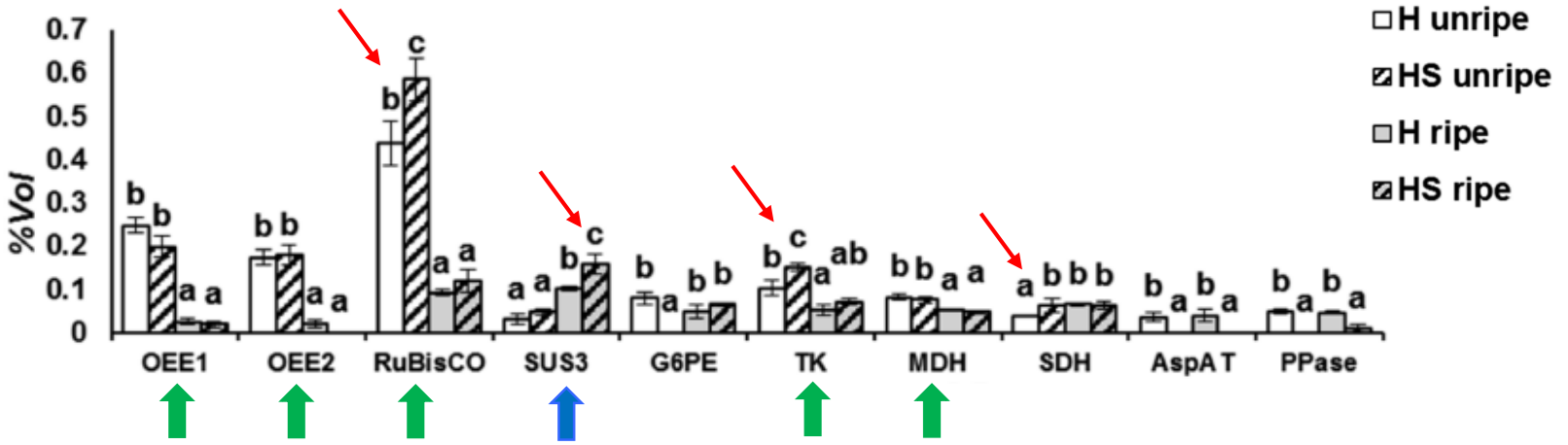


Figure 5. Number of proteins assigned to the different functional classes (Table 2) whose trends discriminated between ripening stages (black bars) or genotypes (white bars).

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

CARBON METABOLISM



OEE1 oxygen-evolving enhancer prot 1 chl; **OEE2** oxygen-evolving enhancer prot 2 chl; **RuBisCO** RuBisCO large subunit; **SUS3** sucrose synthase 3; **G6PE** glc-6-phosphate 1-epimerase; **TK** transketolase, chl; **MDH** malate dehydrogenase [NADP], chl, **SDH** succinate dehydrogenase [ubiq] flavoprotein sub 1, mit; **AspAT** aspartate aminotransferase, **PPase** soluble inorganic pyrophosphatase

Traits related to fruit ripening:

- **general decline of chloroplastic functionality, increase in SUS → lessening of gross photosynthesis and the increment in sink strength of fruits.**

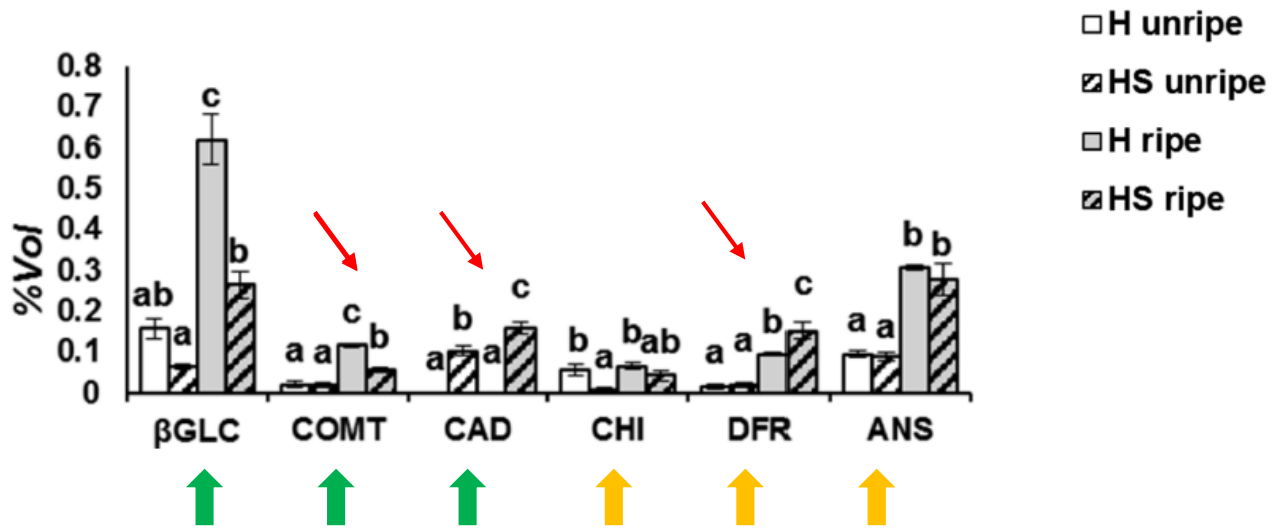
Traits different in HS vs H:

- **HS unripe fruits were characterized by higher levels of RuBisCO, TK, SDH.**
- **HS ripe sweet cherries were also characterized by higher abundance of SUS3.**

The proteomic analysis suggests that the higher exposure to light of leaves and/or fruits in HS trees supports an increment in fruit carbon metabolism.

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

METABOLISM OF PHENOLIC COMPOUNDS



βGLC AAA91166 avium β-glucosidase, partial; **COMT** caffeic acid 3-O methyltransferase; **CAD** cinnamyl alcohol dehydrogenase; **CHI** chalcone flavonone isomerase; **DFR** dihydroflavonol 4-reductase; **ANS** anthocyanidin synthase

Traits related to fruit ripening:

- **Increase in the levels of enzymes involved phenylpropanoid pathway.**

Traits different in HS vs H:

- **Differences in the levels of enzymes involved in phenolic metabolism**

The proteomic analysis suggests that the somaclonal variation provoked some effects on phenolic metabolism in HS fruits.

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

PROFILES OF COMPOSITION IN PHENOLIC COMPOUNDS

Table 3. Relative Percent Abundance of the Identified Phenolic Compounds in Sweet Cherries of 'Hedelfinger' and of Its Somaclonal Variant HS at Unripe and Ripe Stages[a]

	H unripe	HS unripe	H ripe	HS ripe
coumaroylquinic acid	148.8 ± 6.8 b	153.4 ± 6.1 b	46.3 ± 0.7 a	51.4 ± 1.4 a
neochlorogenic acid	139.8 ± 6.2 b	139.2 ± 4.9 b	59.4 ± 0.6 a	61.6 ± 1.9 a
chlorogenic acid	145.8 ± 9.3 b	141.6 ± 4.6 b	56.9 ± 0.8 a	55.7 ± 1.2 a
catechin	158.9 ± 5.7 b	152.5 ± 4.3 b	40.3 ± 0.7 a	48.3 ± 1.6 a
epicatechin	138.8 ± 8.3 b	154.0 ± 6.2 b	50.7 ± 0.3 a	56.6 ± 1.5 a
procyanidin B	90.3 ± 5.1 a	133.6 ± 7.0 b	81.2 ± 0.5 a	94.9 ± 2.4 a
quercetin-3-*O*-rutinoside	75.2 ± 2.8 a	85.0 ± 0.4 a	114.0 ± 3.9 b	125.8 ± 2.6 b
kaempferol-3-*O*-rutinoside[b]	52.3 ± 2.6 a	63.1 ± 1.2 b	127.5 ± 5.5 c	157.1 ± 2.1 d
cyanidin-3-*O*-glucoside	nd[c]	nd	108.3 ± 4.6 b	91.7 ± 1.0 a
cyanidin-3-*O*-rutinoside	nd	nd	89.9 ± 2.5 a	110.1 ± 2.8 b

Traits related to fruit ripening:

- **hydroxycinnamic acids and catechins sharply decreased while flavonols and anthocyanins increase during fruit ripening → these compounds contribute in green and red fruits, respectively, to protect tissues from oxidative stress.**

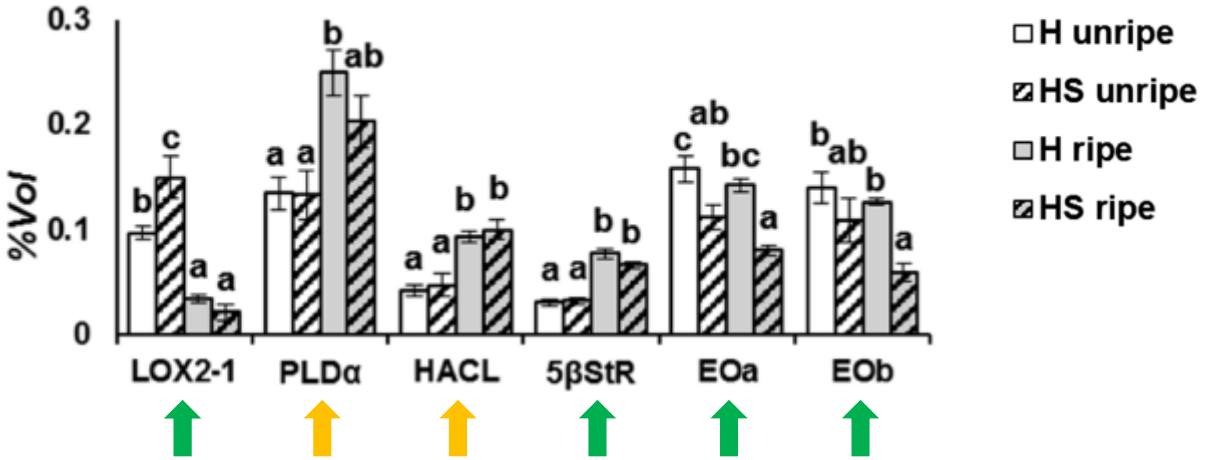
Traits different in HS vs H:

- **Procyanidin B was more abundant by about +40% in HS with respect to H.**
- **HS fruits contain higher amounts of flavonols and anthocyanins.**

The analysis suggests that the higher exposure to light of fruits in HS supports a higher accumulation of phenolic compounds and, in particular, anthocyanins at full ripening.

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

LIPID METABOLISM



LOX2 linoleate 13S-lipoxygenase 2-1, chl like; **PLDa** phospholipase D a1; **HACL** 2-hydroxyacyl-CoA lyase; **5ßStR** 3-oxo-d(4,5)-steroid 5-ß-reductase-like; **EOa** enone oxidoreductase; **EOb** enone oxidoreductase

Traits related to fruit ripening:

- **LOX2-1, Eoa, Eob, 5βStR markedly decreased to similar levels in ripe fruits of both genotypes → production of volatile component of sweet cherry aroma (hexanal, furaneol).**
- **PLDα and HACL increased during ripening → markers of phospholipid catabolism in the senescing systems.**

The proteomic analysis suggests that

- **fatty acid catabolism has valuable relevance during fruit development in sweet cherry.**
- **the somaclonal variation has had a lower impact on this metabolism in fruits.**

FRUIT RIPENING IN SOMACLONAL VARIANT OF SWEET CHERRY

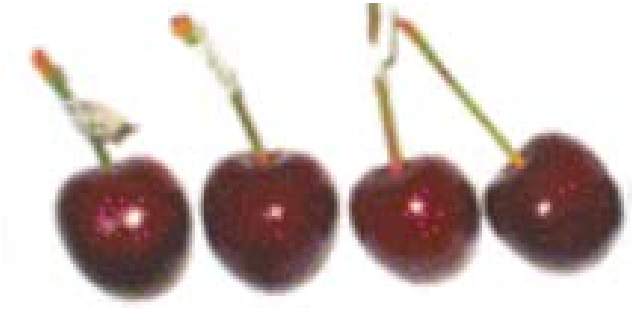
SUMMARY

The study on somaclonal variant in sweet cherry 'Hedelfinger' provides information about:

- **Protein markers discriminating genotype and fruit ripening stage.**
- **Modulation of pivotal enzymes of carbon metabolism in fruits influenced by tree morphological traits, such as the canopy light interception.**
- **Protein markers involved in production of volatile aroma and in senescence during fruit ripening.**
- **Biochemical changes in phenolic metabolism in fruits related to somaclonal variation**

⇩

THE PROTEOMIC INVESTIGATION PROVIDES NEW INFORMATION ABOUT FRUIT RIPENING AND FRUIT PHENOTYPE RELATED TO SOMACLONAL VARIATION IN SWEET CHERRY.



CONCLUSION

PROTEOMIC CAN BE VERY USEFUL FOR THE LARGE SCALE CHARACTERIZATION OF BIOCHEMICAL PHENOTYPES RELATED TO GENETIC VARIATION IN CROP SPECIES

PROTEOMIC CAN BE USED FOR VALIDATION OF PHYSIOLOGICAL / BIOCHEMICAL PARAMETERS EVALUATED BY NON-INVASIVE PHENOTYPING APPROACHES

PROTEOMIC CAN PROVIDE NOVEL INFORMATION ABOUT PUTATIVE MOLECULAR MARKERS OF PHYSIOLOGICAL / DEVELOPMENTAL PROCESSES RELATED TO CROP PRODUCTIVITY AND QUALITY

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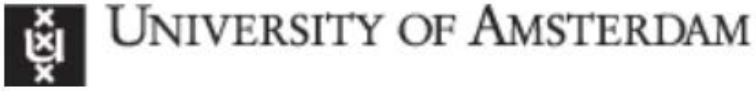

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THANK YOU FOR YOUR ATTENTION

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