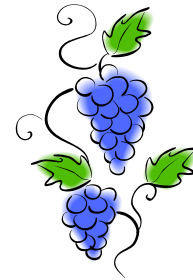




être française
et apprendre
à faire du vin
aux USA !



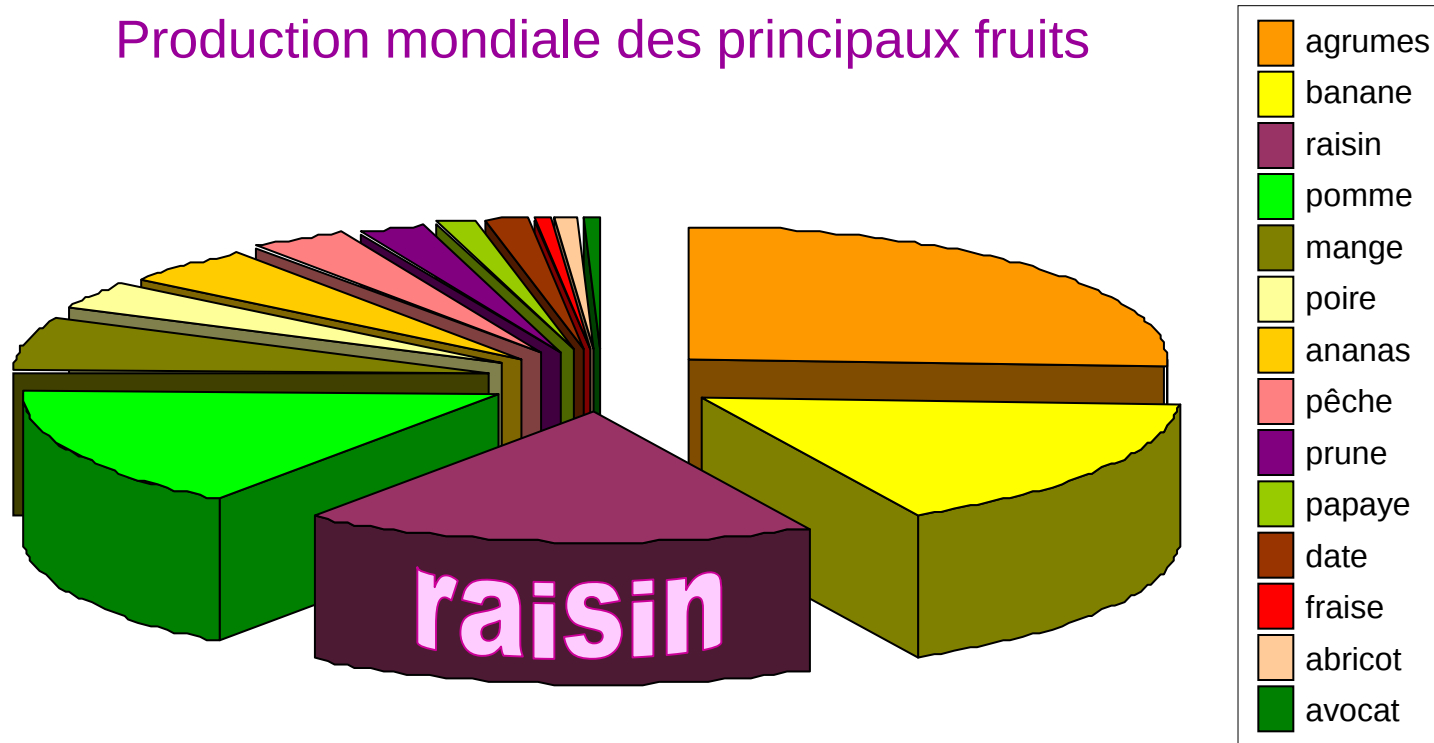
Trahison !

Dr Delphine Vincent

27 Mars 2023

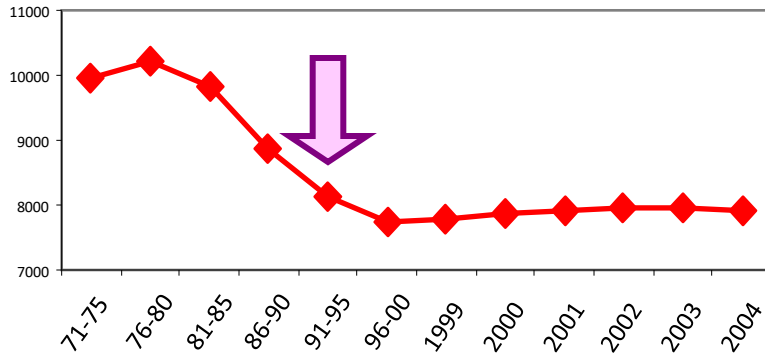
PLACE DE LA VIGNE DANS LE MONDE

Production mondiale des principaux fruits

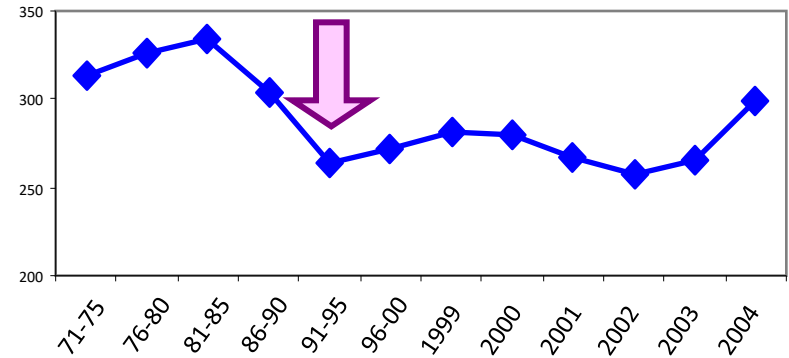


QUELQUES CHIFFRES...

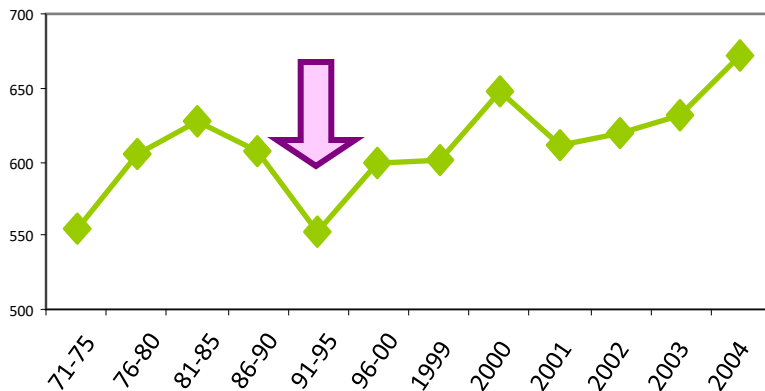
Superficie mondiale



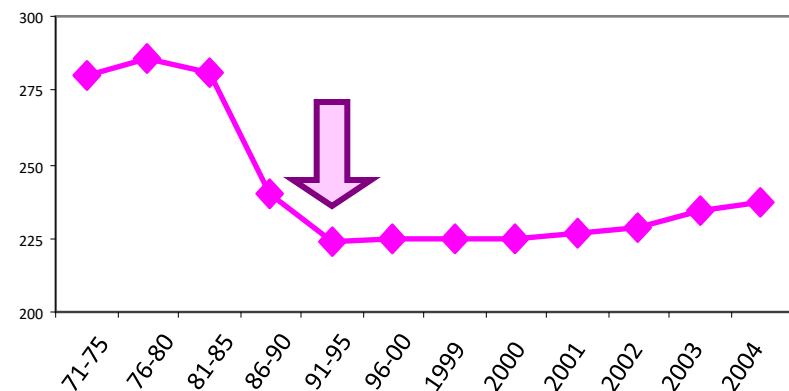
Production mondiale de vins



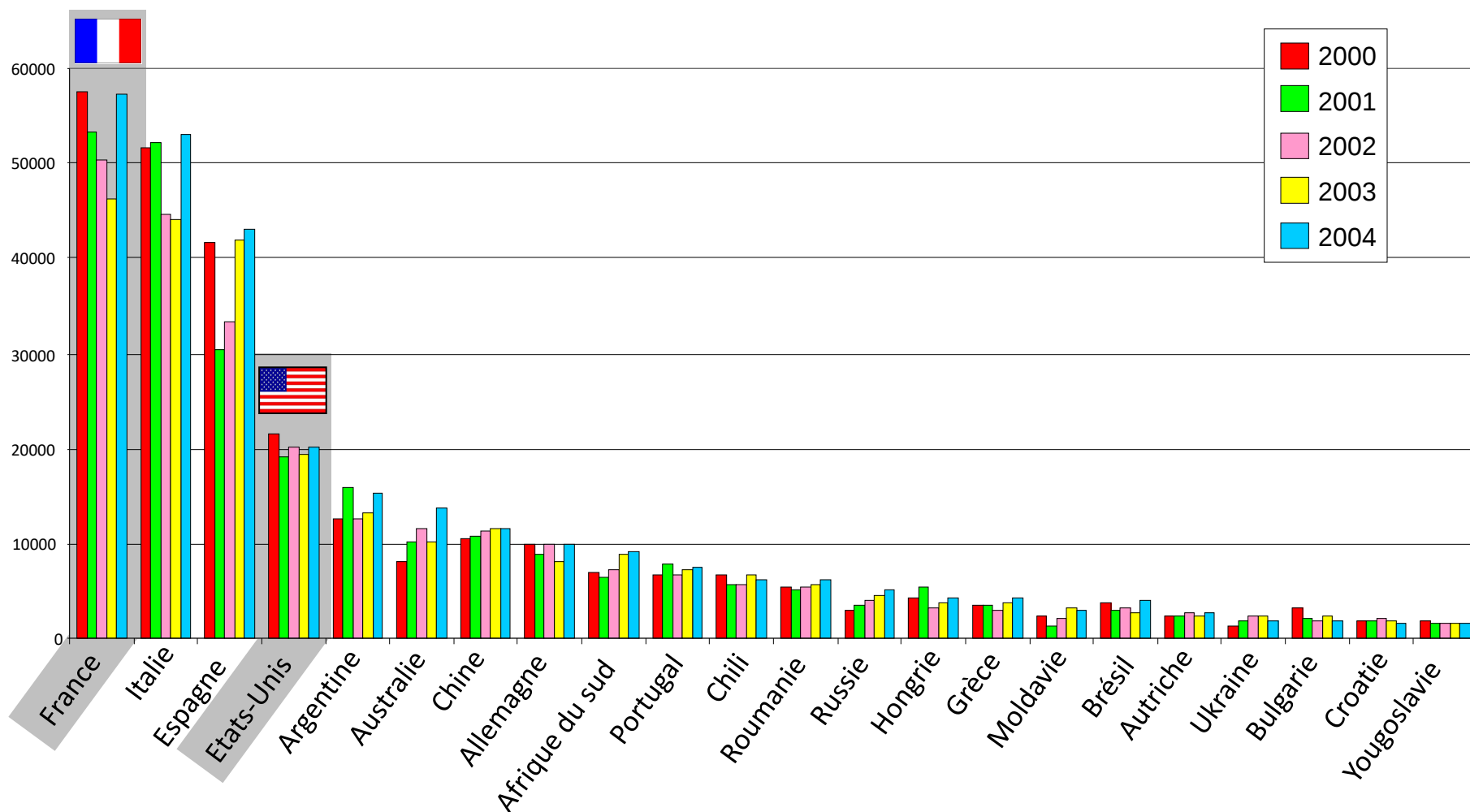
Production mondiale de raisins



Consommation mondiale de vins

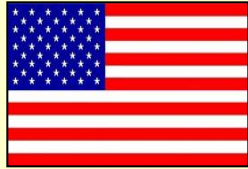


LES PRINCIPAUX PRODUCTEURS MONDIAUX

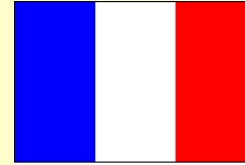


FRENCH PARADOX

une autre raison d'être jaloux !



Gras 72 g/j
Graisses saturées +
Cholesterol +
Attaque cardiaque: 230/100000



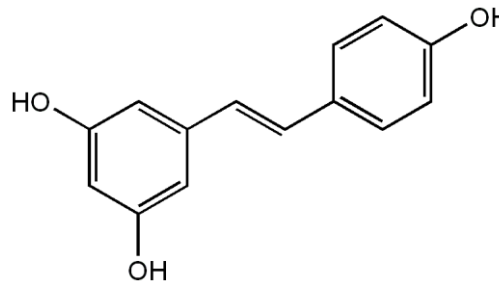
Gras 108 g/j
Graisses saturées + + +
Cholesterol + + +
Attaque cardiaque: 83/100000



Associé à la forte consommation en vin rouge.
Faut boire pour être en bonne santé !

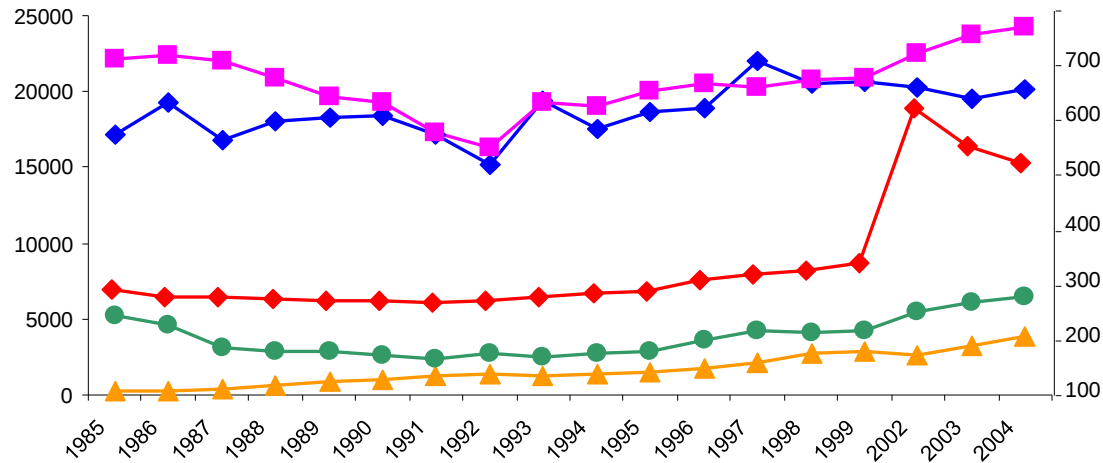
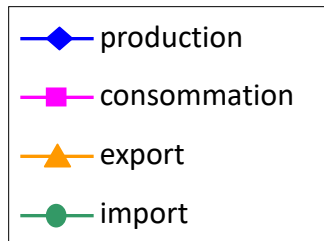


Resveratrol (phytoalexin)
antioxydant



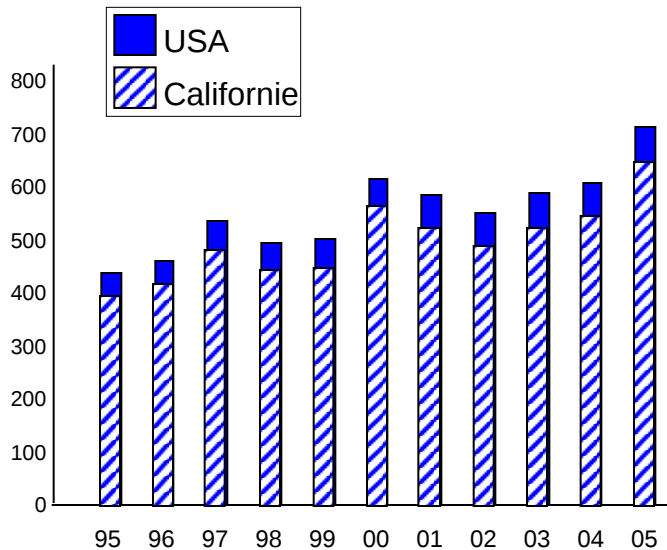
Présent dans divers fruits (myrtilles, fraises, cacahouètes, pignons !)
particulièrement concentré dans la peau des raisins rouges (donc dans les vins rouges)

LA VIGNE AUX USA



superficie

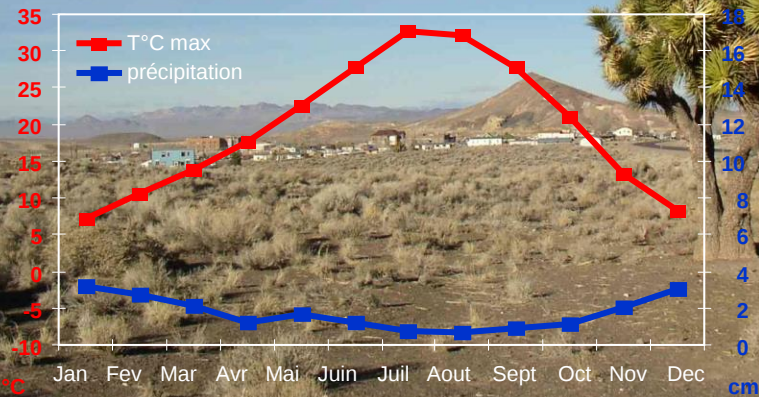
Production de vin aux USA



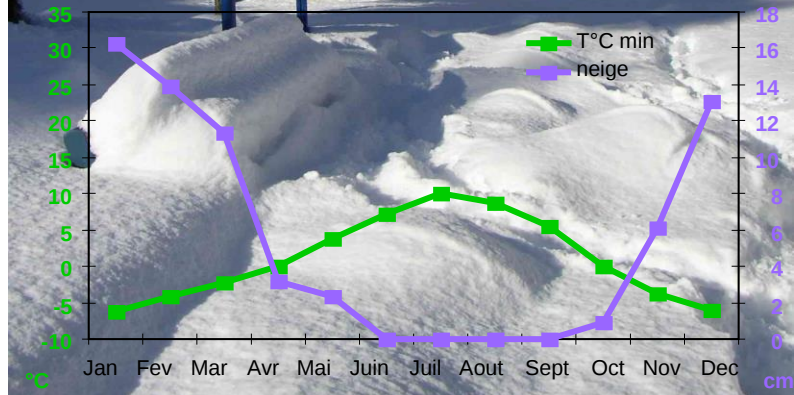
LA VIGNE DANS LE NEVADA

A priori, c'est pas gagné !

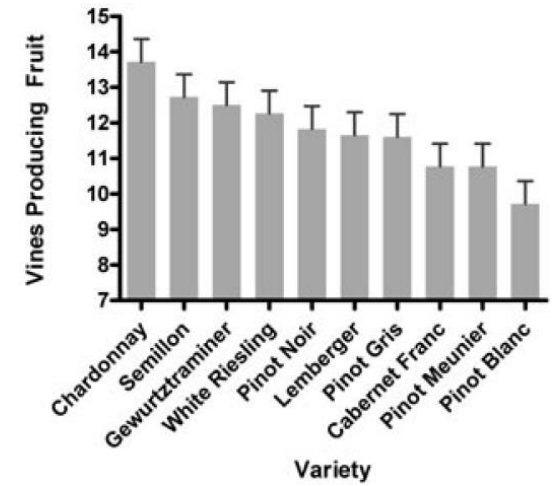
Chaud l'été
Sec toute l'année



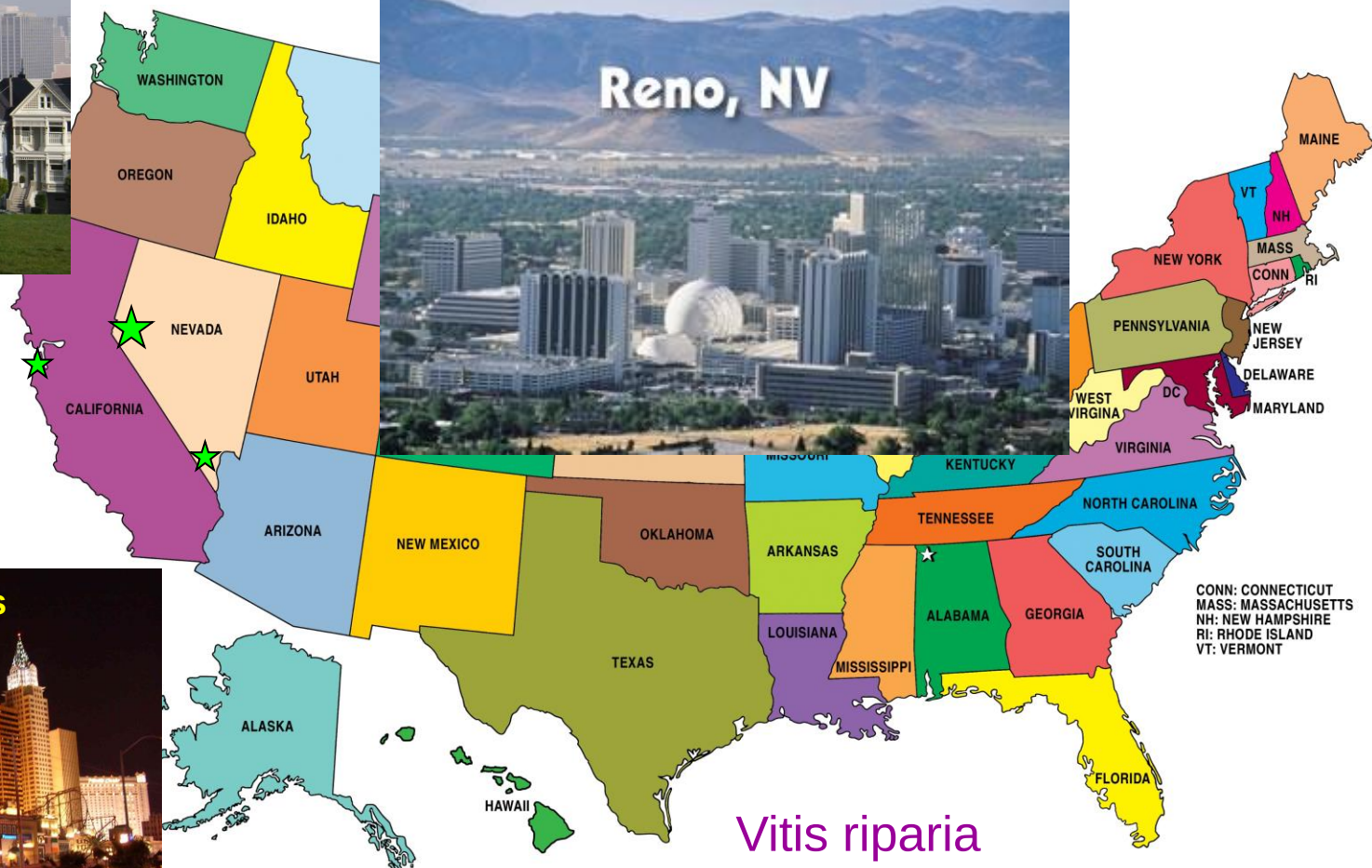
Froid l'hiver



LA VIGNE DANS LE NEVADA en effet...



PROJET VIGNE A RENO, NEVADA



Vitis riparia
Vitis lambrusca
Vitis vinifera L
⇒ Chardonnay
⇒ Cabernet sauvignon

STRATEGIE: SYSTEMS BIOLOGY

espèce

Vitis vinifera

variétés

Chardonnay



Cabernet Sauvignon



stress abiotiques

sécheresse



salinité



froid



conditions de culture

champ



serre

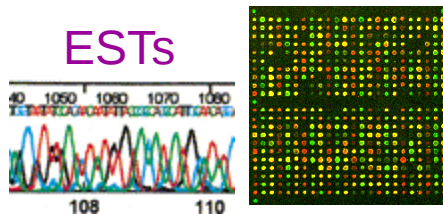


chambre de culture

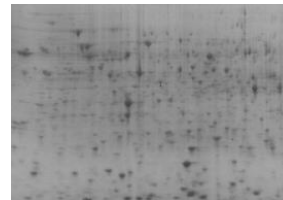


niveaux
d'expression
de gènes

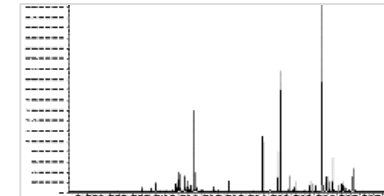
transcriptome



protéome



métabolome

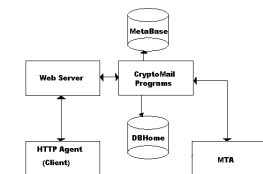


informatique

statistical analyses



database
integration



STRATEGIE: SYSTEMS BIOLOGY

espèce

Vitis vinifera

variétés

Chardonnay



Cabernet Sauvignon



stress abiotiques

sécheresse



salinité



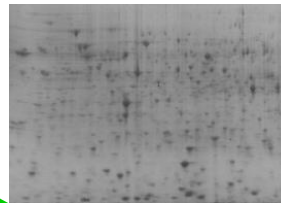
conditions de culture

serre



niveaux
d'expression
de gènes

protéome

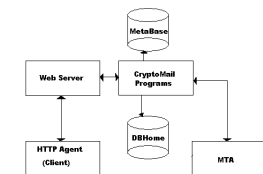


informatique

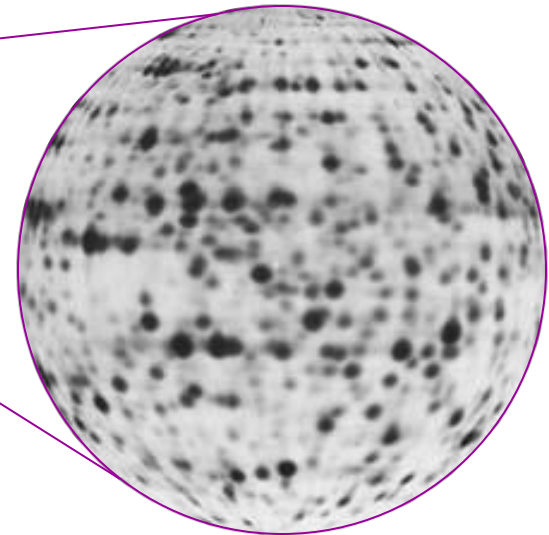
statistical analyses



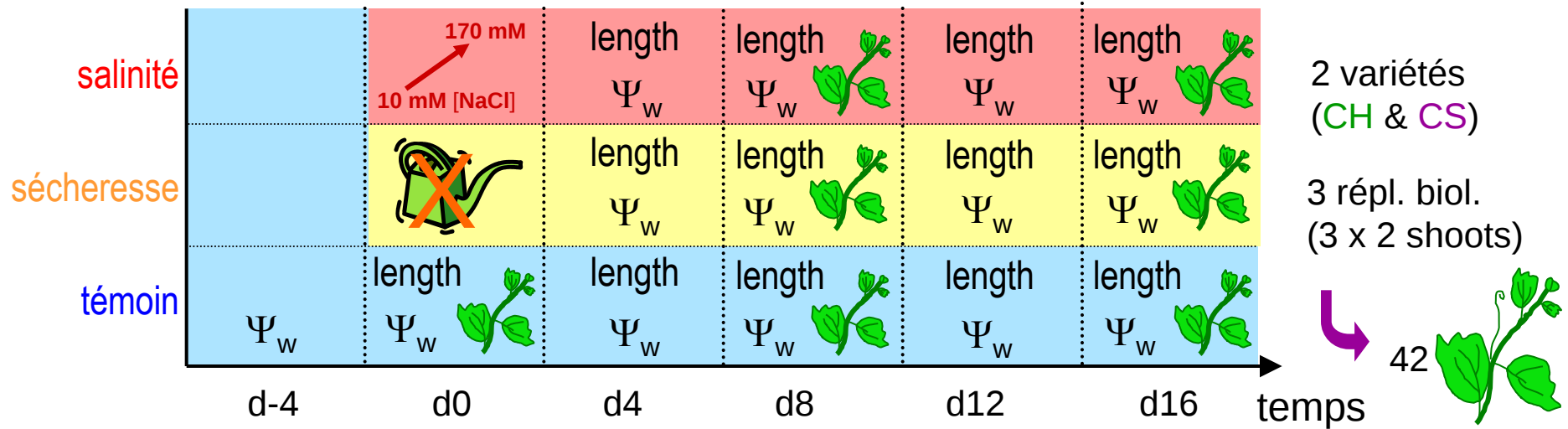
database
integration



Analyse protéomique des réponses de la vigne aux stress hydrique et salin



MATERIAL VEGETAL ET MESURES



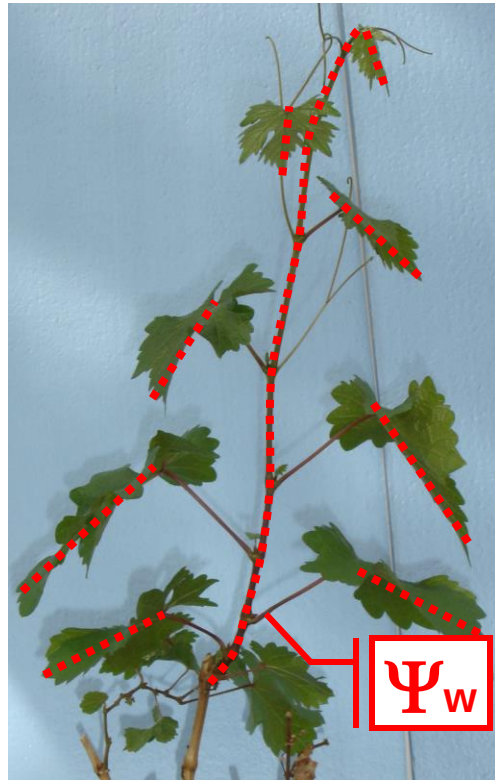
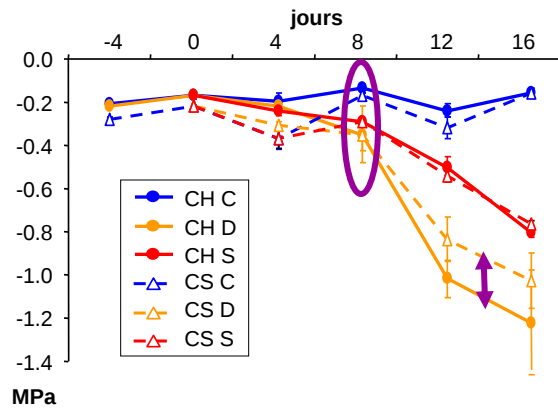
1 shoot =
1 bourgeon
4 feuilles
tige
tendrons



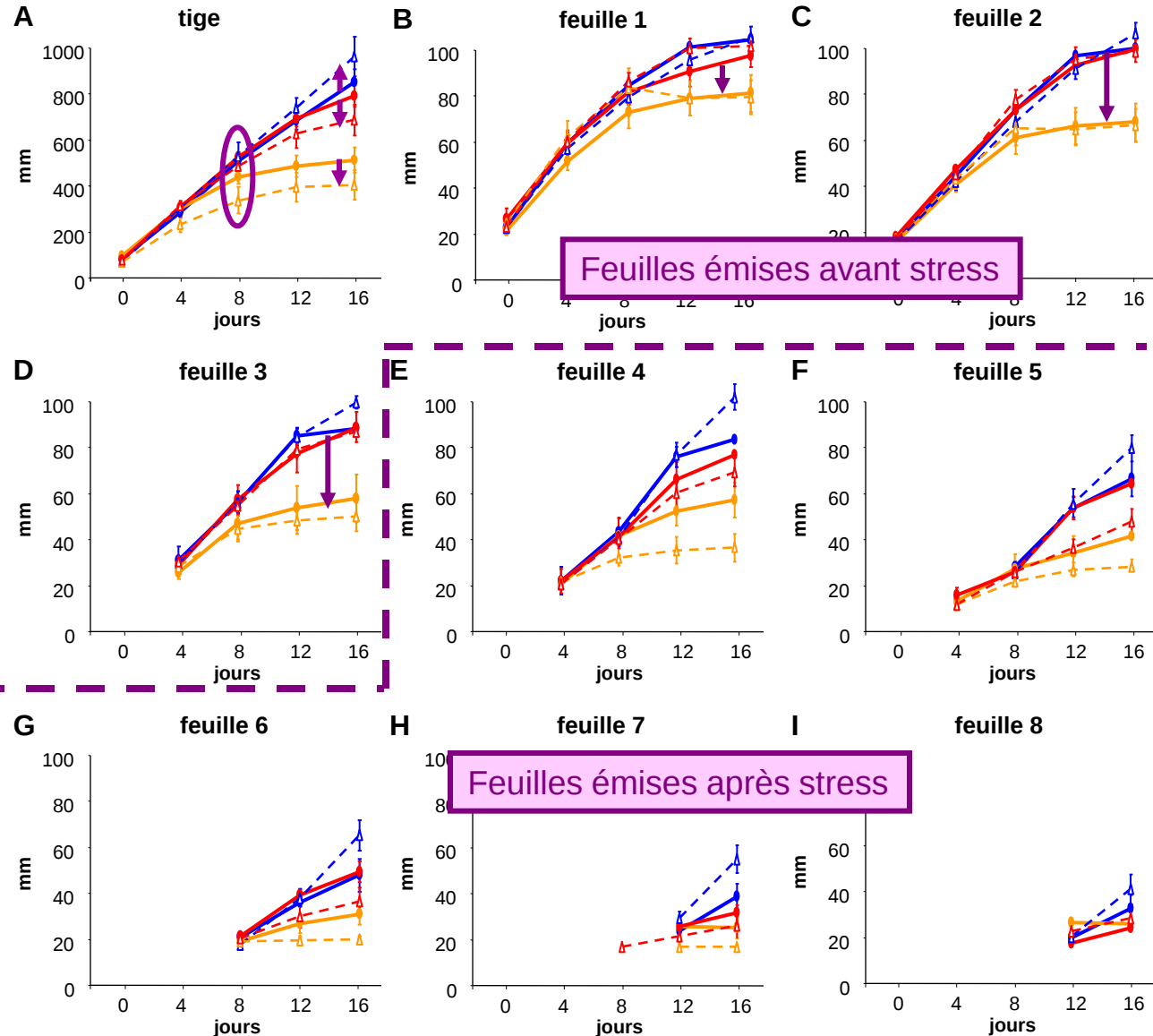
Flétrissement
dû à l'arrêt
de l'irrigation

DONNEES PHYSIOLOGIQUES

Potentiel hydrique



Cinétique de croissance des tiges/feuilles



DONNEES PHYSIOLOGIQUES

Au niveau physiologique...

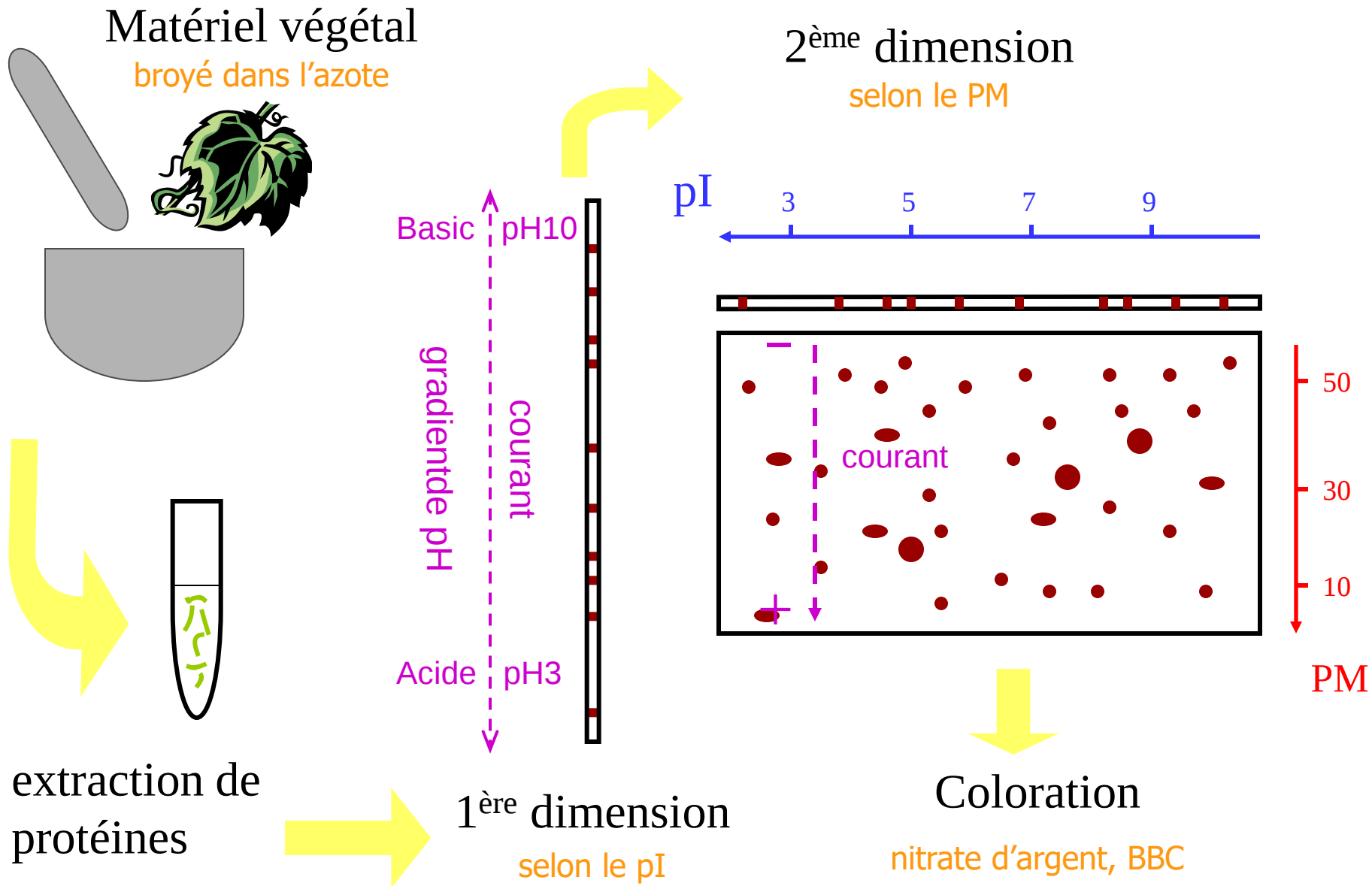
	Salinité	Sécheresse
CH	++	+++
CS	+	++

Feuilles présentes avant stress moins affectées

Quant est-il au niveau moléculaire ?

Réponses avec les protéines...

ELECTROPHORESE BIDIMENSIONNELLE



ANALYSES STATISTIQUES

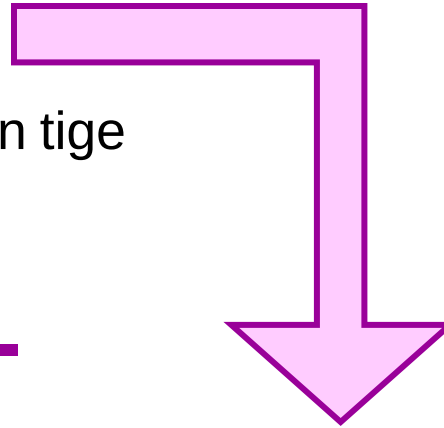
~1500 spots détectés $\Rightarrow$ 758 spots reproductibles (2/3)

$\Rightarrow$ Régressions pas-à-pas

2 modèles: Ψ_w + élongation tige

$\Rightarrow$ ANOVA

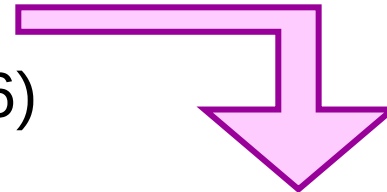
~~$\Rightarrow$ corrélations, ACP, ACH~~



IDENTIFICATION DE PROTEINES

$\Rightarrow$ Régressions pas-à-pas (16)

$\Rightarrow$ Spots les plus abondants (186)

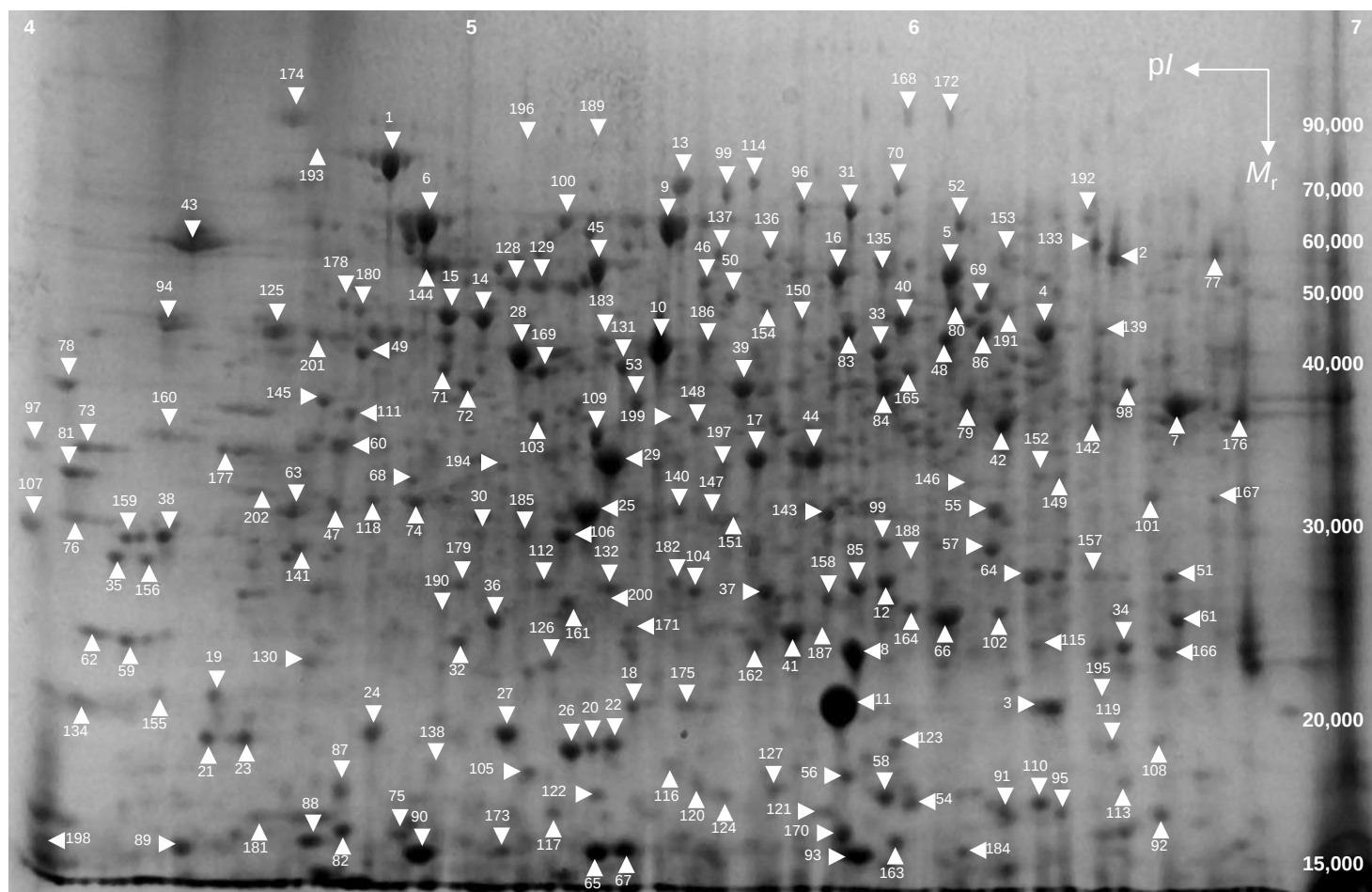


Classification fonctionnelle

Profils des protéines d'intérêt

CARTE PROTEIQUE

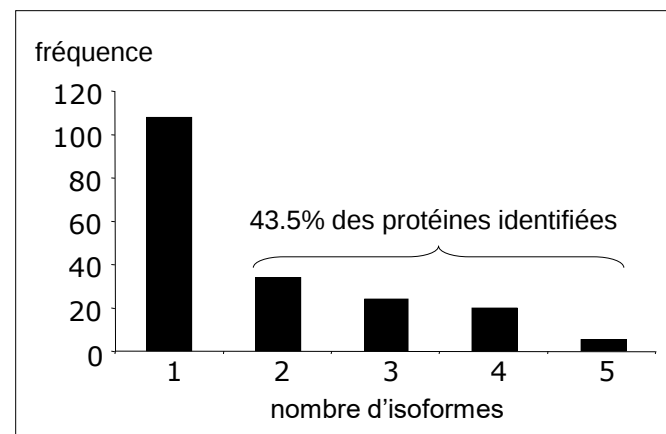
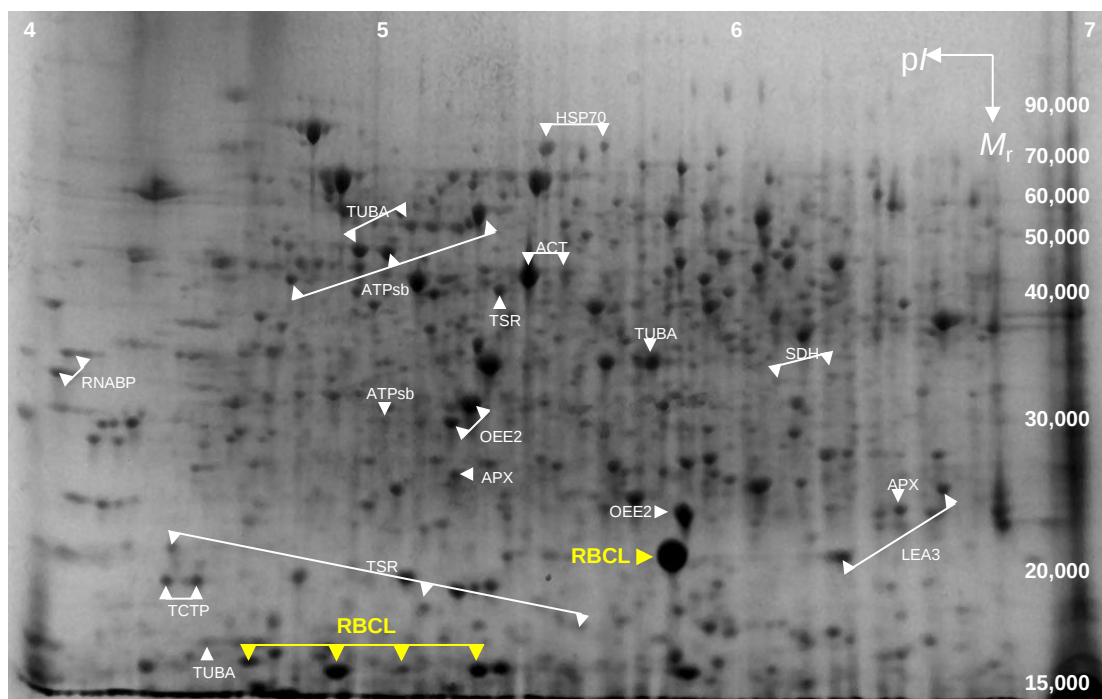
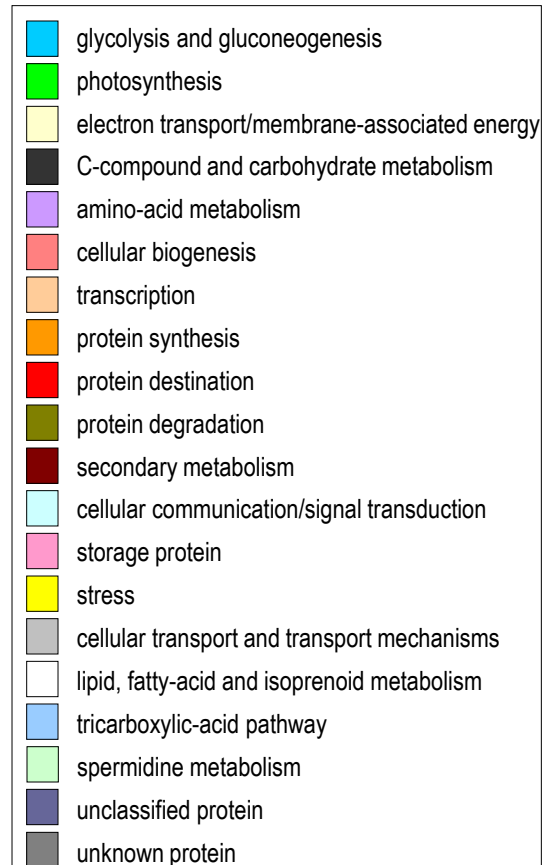
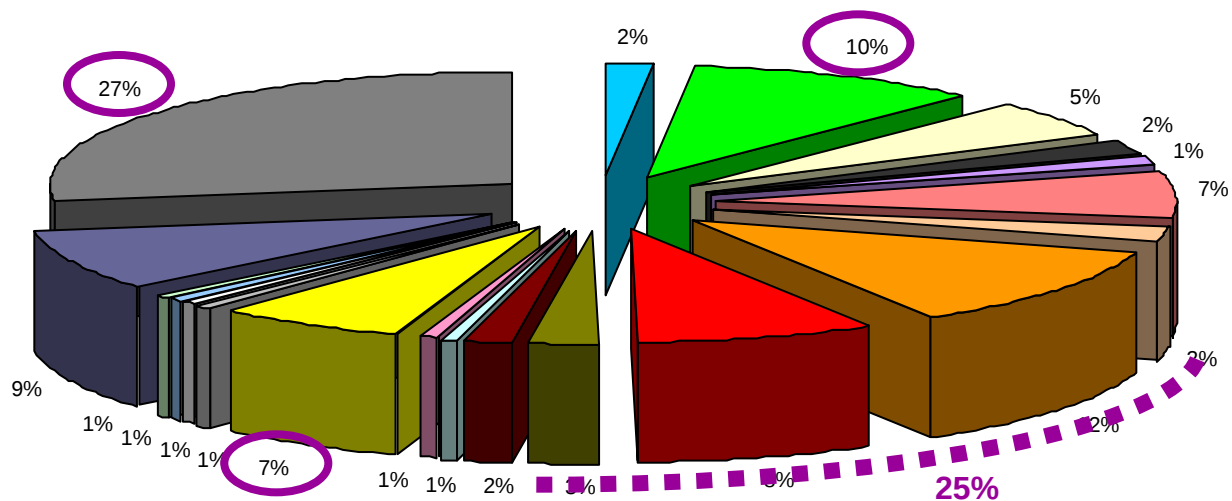
202 spots (186 abondants + 16 « régression ») analysés en MALDI-TOF/TOF



co-migration: témoin d0 CS+CH

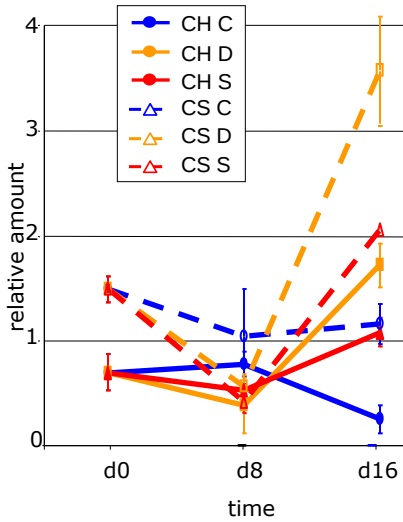
11 x 8 cm² 12,5% acrylamide Bleu de Coomassie Colloïdal

CARTE PROTEIQUE

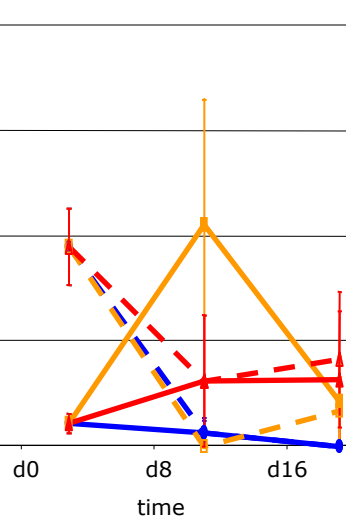


EXEMPLE D'ISOFORMES: LA RUBISCO (RBCL)

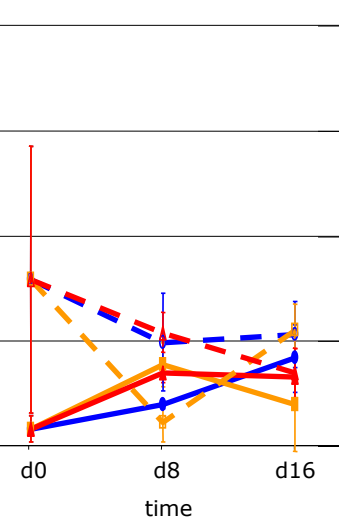
A Spot#88



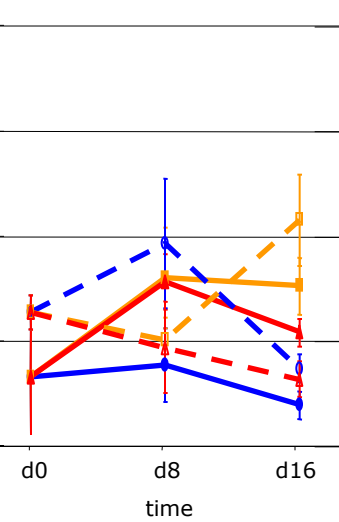
B Spot#90



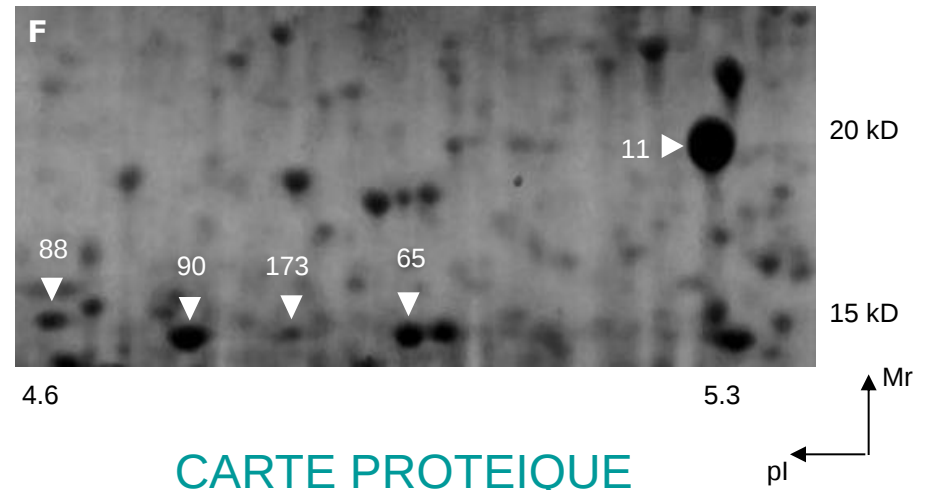
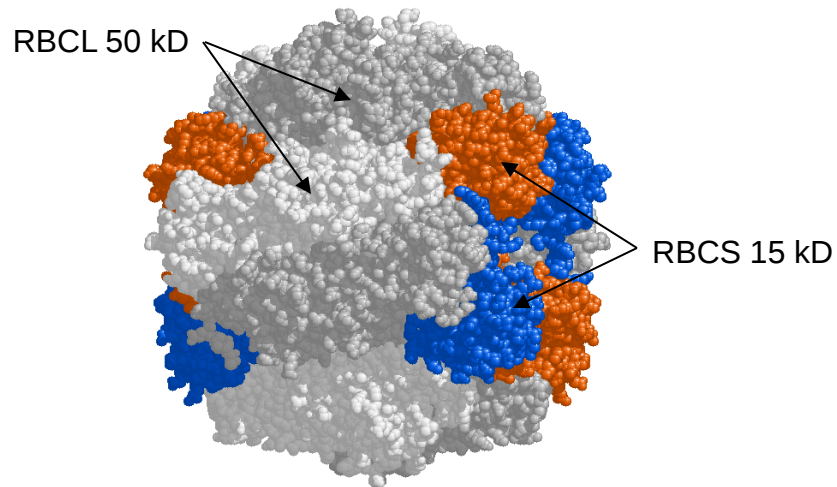
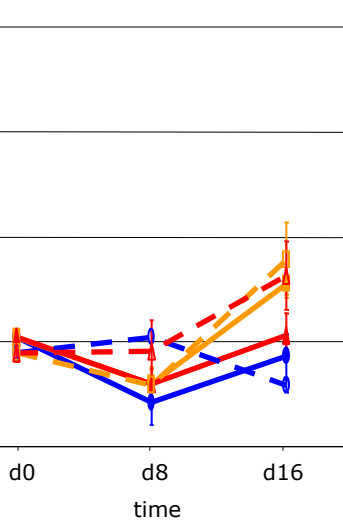
C Spot#173



D Spot#65



E Spot#11



RESULTATS STATISTIQUES

758 spots reproductibles (2/3 rép. biol.)

Régressions pas-à-pas

Potentiel hydrique

pas	R ²	Protéine
1	0.8175	NtPRp27
2	0.9438	Triosephosphate isomerase, chloroplastic
3	0.9754	Ribosomal protein S7
4	0.9910	Xyloglucan endotransglycosylase
5	0.9973	AT5g28840/F7P1_20
6	0.9992	Ribosomal protein L36, putative
7	0.9998	P0431H09.23
8	1.0000	Spermidine synthase

Elongation de la tige

pas	R ²	Protéine
1	0.7705	RuBisCo
2	0.9310	Heat shock protein 70 kD
3	0.9777	Ascorbate peroxidase, thylakoid-bound
4	0.9922	60S acidic ribosomal protein P1
5	0.9988	60S acidic ribosomal protein P0
6	0.9995	Unknown protein
7	0.9998	Heat shock protein 70 kD
8	0.9998	Chaperonin-60 LS2

Sur-exprimée par sécheresse (++) et salinité (+)
 Marqueur de stress biotique
 Induite par stress hydrique et ABA

ANOVA

$$y_{ijk} = V_i + D_j + T_k + (VD)_{ij} + (VT)_{ik} + (DT)_{kj} + (VDT)_{ijk} + \varepsilon_{ijk}$$

Traduction

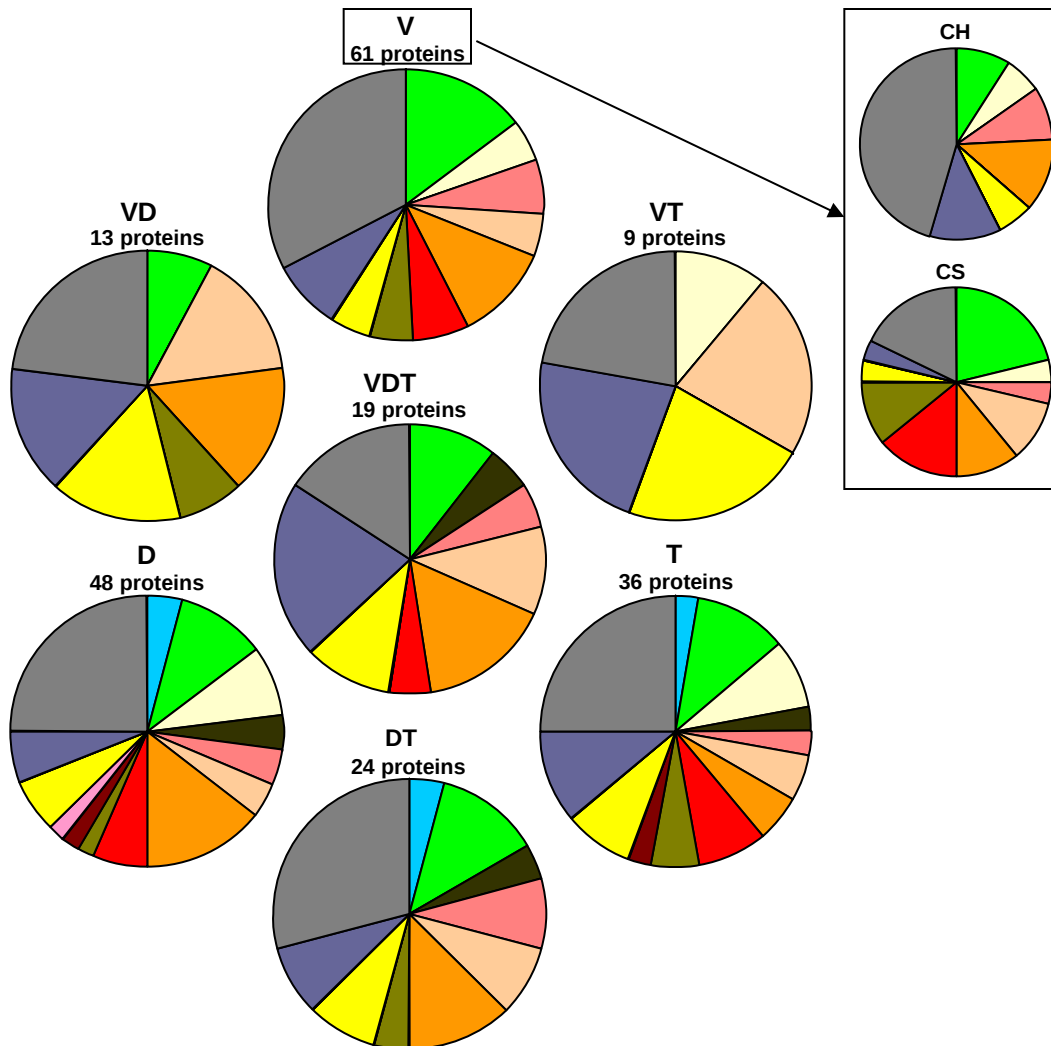
Biogénèse des parois

facteurs	p < 0.05
V	305 (40%)
D	199 (26%)
T	115 (15%)
V*D	89 (12%)
V*T	79 (10%)
D*T	120 (16%)
V*D*T	111 (15%)

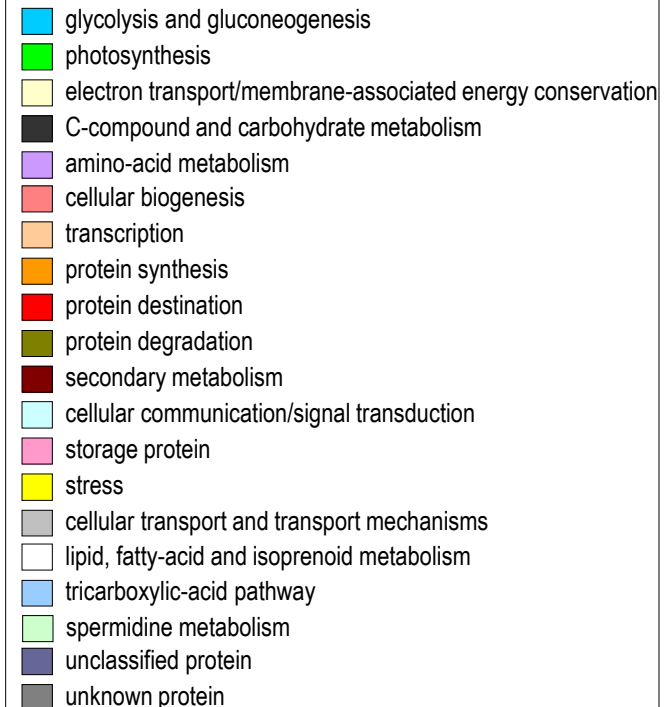
Métabolisme protéique
 (synthèse + maturation)

REPARTITION DES PROTEINES SIGNIFICATIVES PARMI LES 202 PROTEINES IDENTIFIEES

ANOVA: $y_{ijk} = V_i + D_j + T_k + (VD)_{ij} + (VT)_{ik} + (DT)_{kj} + (VDT)_{ijk} + \varepsilon_{ijk}$



Classification fonctionnelle (GO)

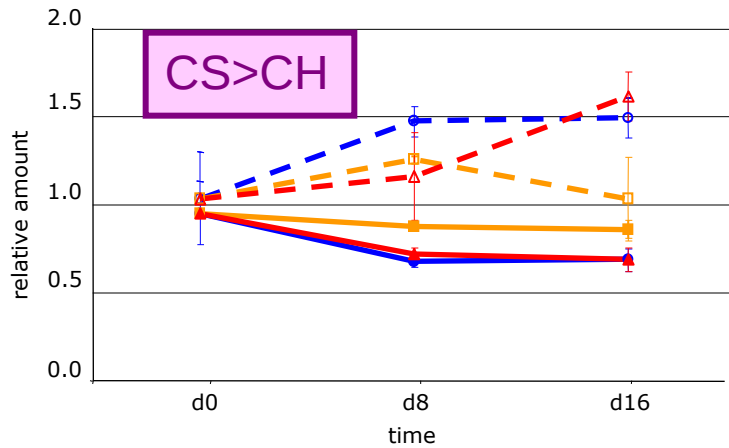


PROTEINES VARIETES-DEPENDANTES

A Spot#22 PPIase

Peptidyl-prolyl cis-trans isomerase, chloroplastic

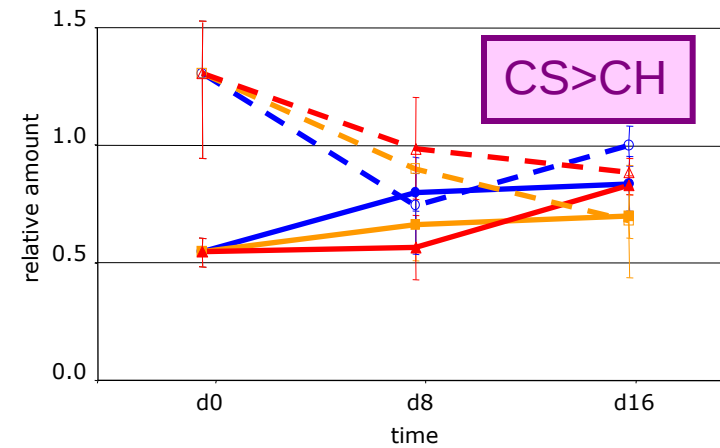
Repliement de protéines



B Spot#51 PSA4

Proteasome subunit alpha type 4

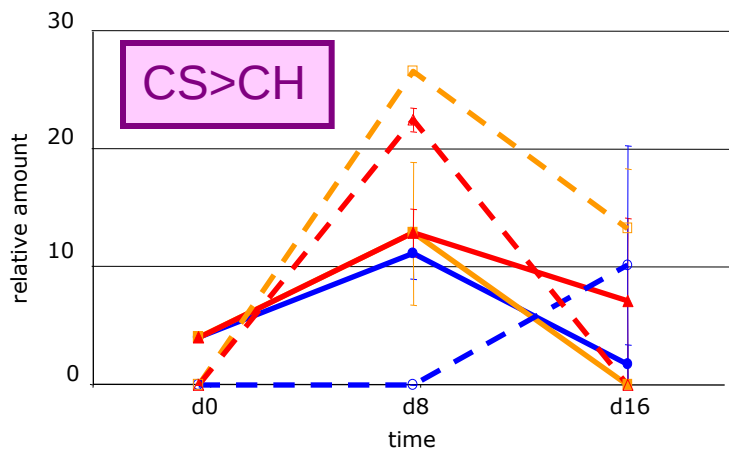
Dégradation de protéines



C Spot#92 CE

Ubiquitin-conjugating enzyme

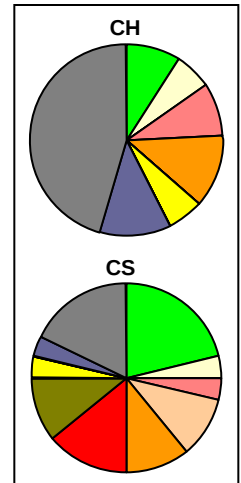
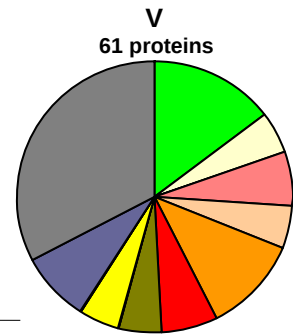
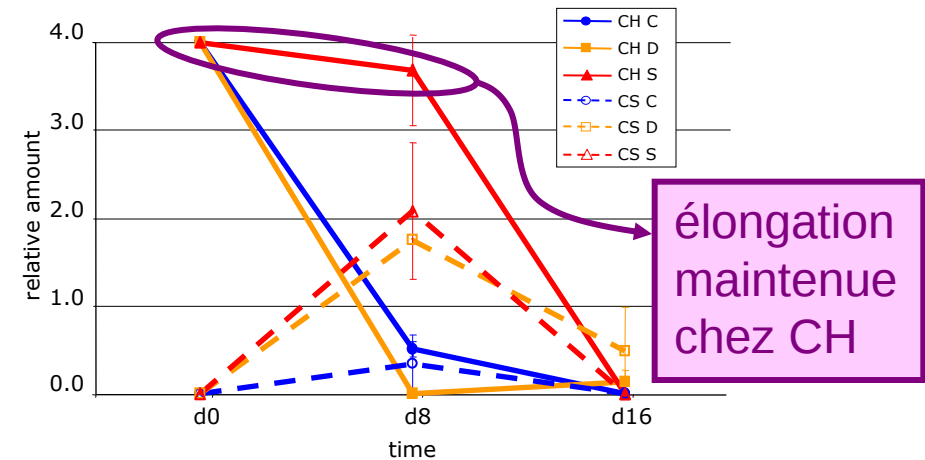
Dégradation de protéines



D Spot#101 XET

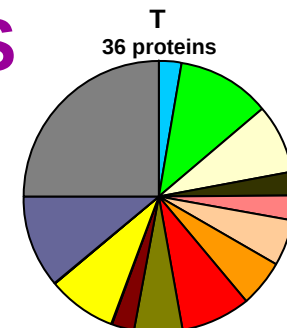
Xyloglucan endotransglycosylase

Biosynthèse des parois



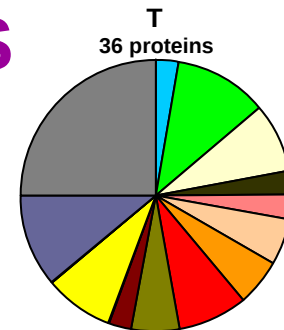
PROTEINES TRAITEMENT-DEPENDANTES

expression pattern	p-value for T factor	protein code	function annotation	MATdb Functional Classification
C=S>D	0.0034	ATPsf1	F1 ATPase subunit alpha	electron transport ^a
C=S>D	0.0046	RE	retroelement putative	unclassified protein
C=S>D	0.0060	DEL	GHDEL61	transcription
C=S>D	0.0108	na	Gene_id:T19N8.13	unknown protein
C=S>D	0.0145	HSP70	Heat shock 70 KD protein mitochondrial	protein destination
C=S>D	0.0727	HMMR	Hyaluronan mediated motility receptor-related protein	unclassified protein
C>D>S	0.0103	NAC	Alpha NAC-related protein	unknown protein
C>S=D	0.0055	ATPsb	ATP synthase beta chain	electron transport ^a
C>S=D	0.0089	na	Putative protein; protein id: At5g05210.1	unknown protein
C>S=D	0.0747	TCTP	Translationally controlled tumor protein homolog	unclassified protein
C>S=D	0.0747	na	Hypothetical protein; protein id: At2g31480.1	unknown protein
C>S>D	0.0006	na	Gene_id:T19N8.13	unknown protein
C>S>D	0.0124	HSP	Heat shock protein high molecular weight	protein destination
C>S>D	0.0205	na	OSJNBb0055I24.7	unknown protein
C>S>D	0.0382	PSII	Photosystem II	photosynthesis
C>S>D	0.0702	SRP	Serine/arginine rich protein putative	protein synthesis
C>S>D	0.0764	DRP	Desiccation-related protein	stress
C>S>D	0.0792	FH3	Naringenin,2-oxoglutarate 3-dioxygenase	secondary metabolism
D>S=C	0.0641	RBCL	Ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit	
D>S>C	0.0003	NMCP1	Nuclear matrix constituent protein 1 (NMCP1)-related	unclassified protein
D>S>C	0.0800	RBCL	Ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit	
D>S>C	0.0915	SBPase	Sedoheptulose-1,7-bisphosphatase, chloroplastic	
S=D>C	0.0337	SPK1	Guanine nucleotide exchange factor	
S>C=D	0.0199	ADK	Adenosine kinase	electron transport ^a
S>C=D	0.0702	LDL	Latex plastidic aldolase-like protein	glycolysis ^b
S>C>D	0.0050	RPL39	Ribosomal protein L39 putative	
S>C>D	0.0101	bHLH	bHLH protein	
S>C>D	0.0205	RPT3	Proteasome 26S AAA-ATPase subunit RPT3	
S>C>D	0.0354	na	Hypothetical protein At2g15000	unknown protein
S>C>D	0.0398	na	Hypothetical protein T32G9.36	unknown protein
S>C>D	0.0685	PSB1	Proteasome 20S beta 1 subunit	
S>C>D	0.0986	EPID	NAD-dependent epimerase/dehydratase family	C-compound ^c
S>D=C	0.0641	na	At1g79210/YUP8H12R_1	unknown protein
S>D>C	0.0008	PR10	Pathogenesis-related protein 10	stress
S>D>C	0.0558	H1	Histone H1-like protein	cellular biogenesis



115 spots

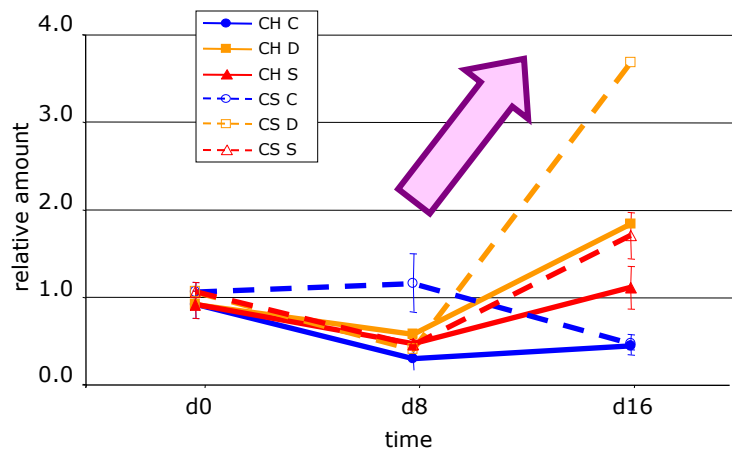
PROTEINES TRAITEMENT-DEPENDANTES



A Spot#82 NMCP1

Nuclear matrix constituent protein 1

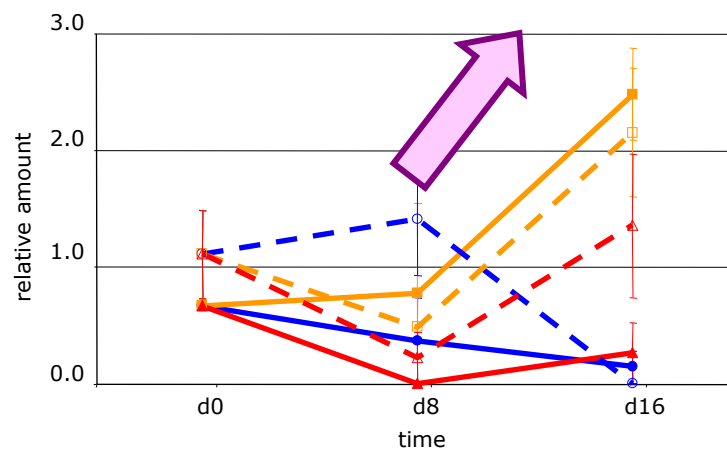
Restructuration de l'enveloppe nucléaire



B Spot#175 RPL39

Ribosomal protein L39

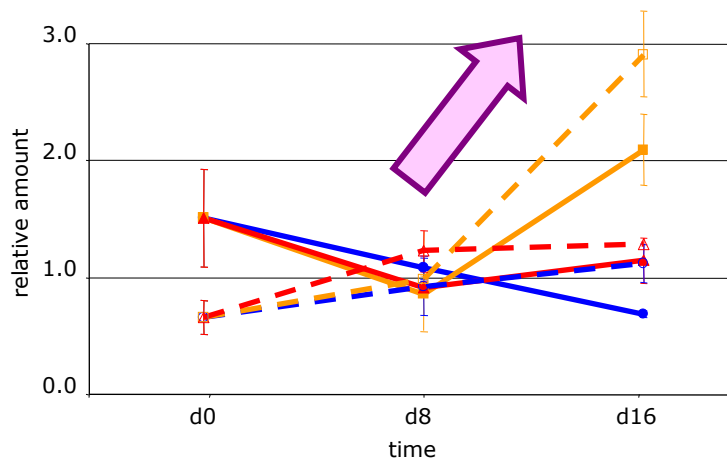
Traduction



C Spot#72 SBPase

Sedoheptulose-1,7-bisphosphatase, chloroplatic

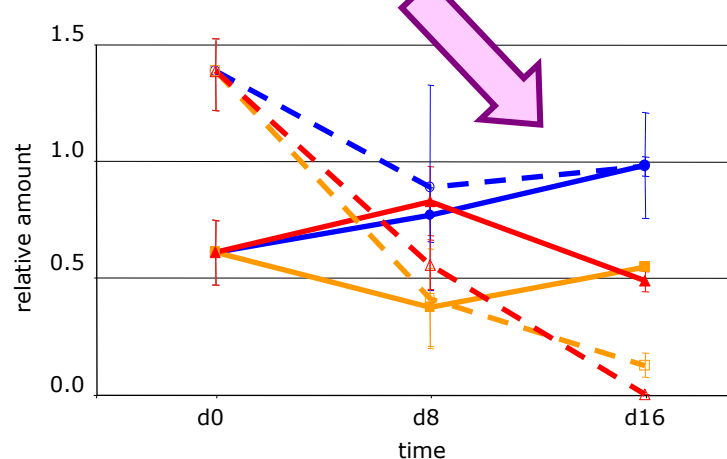
Photosynthèse



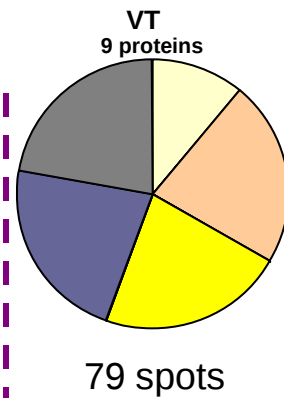
D Spot#145 PSII

Photosystem II

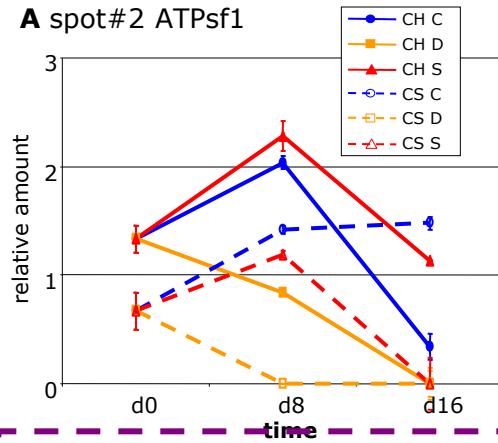
Photosynthèse



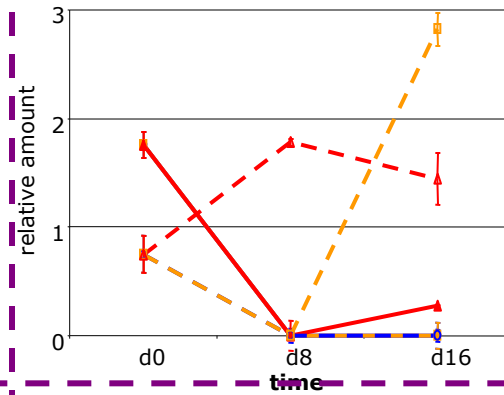
INTERACTION VARIETE-TRAITEMENT



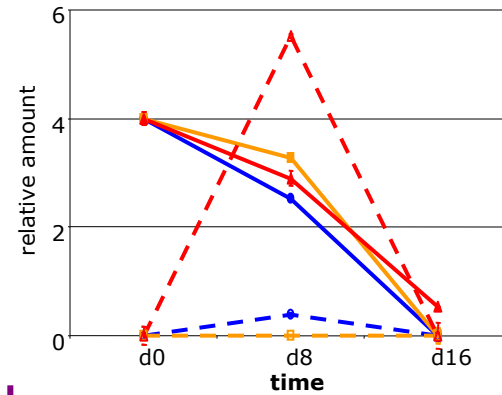
A spot#2 ATPsf1



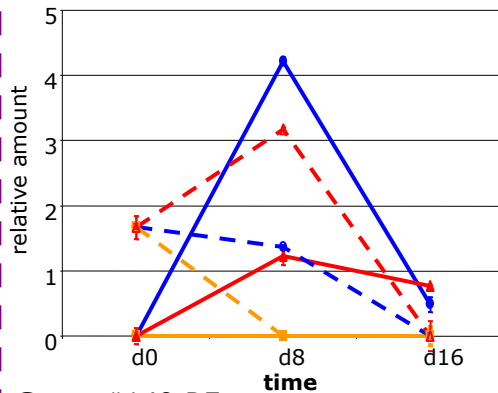
B spot#113 PR-10



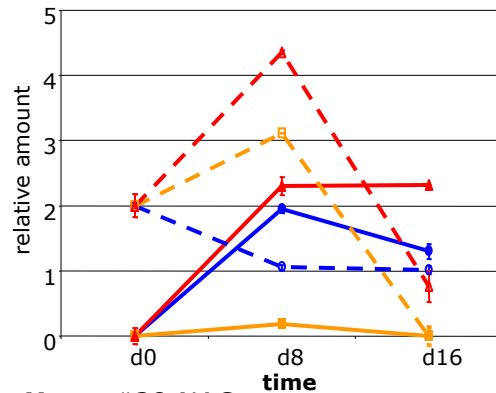
C spot#119 PRX



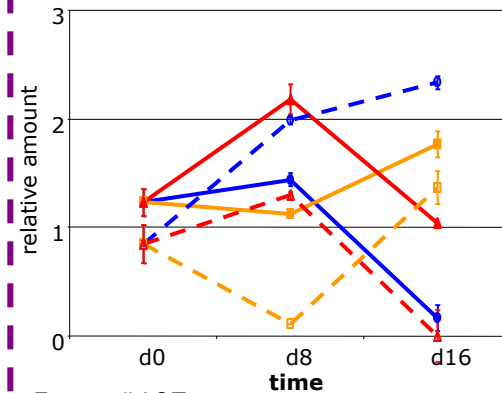
D spot#78 DEL



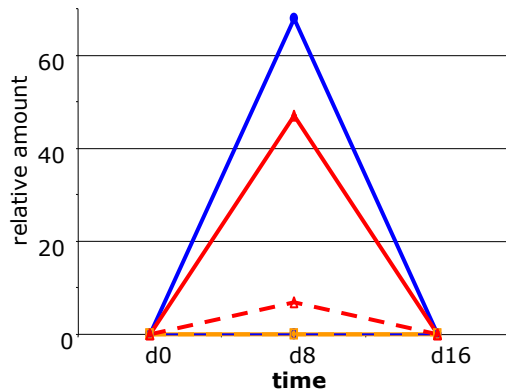
E spot#7 bHLH



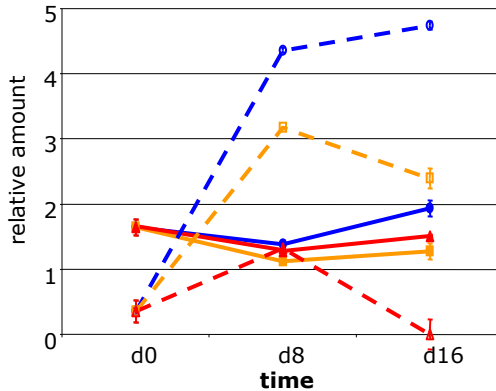
F spot#93 PPR



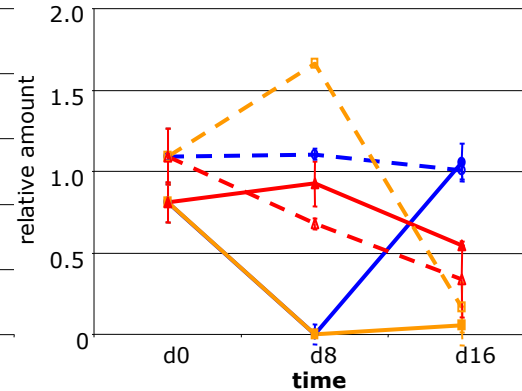
G spot#149 RE



H spot#38 NAC



I spot#107 na



CONCLUSIONS



Chardonnay plus tolérant aux stress hydrique et salin que Cabernet Sauvignon au niveau physiologique.



Peu de protéines répondent aux stress hydrique et salin.

Turn-over affecté (synthèse, maturation, dégradation).

Mise en évidence de protéines n'ayant jamais été associées au stress (NMCP1, RPL39, DEL, PPR).



La principale source de variation d'expression de protéines est d'origine génétique (variétés).



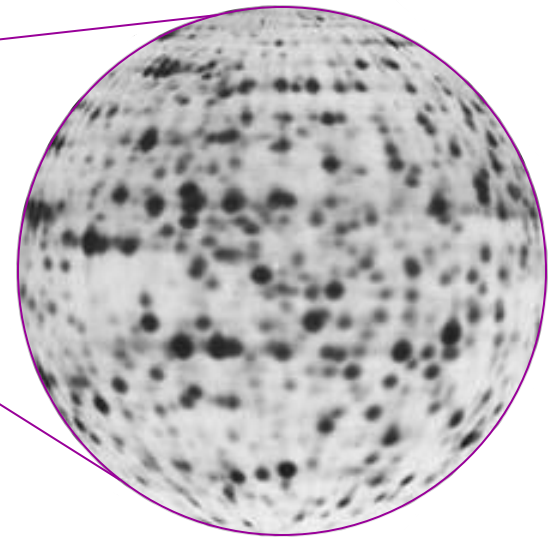
La majorité des protéines surexprimées chez CH en conditions de stress est de fonction inclassable ou inconnue tandis que les protéines surexprimées chez CS agissent dans le métabolisme protéique (transcription/traduction/maturation/dégradation) .



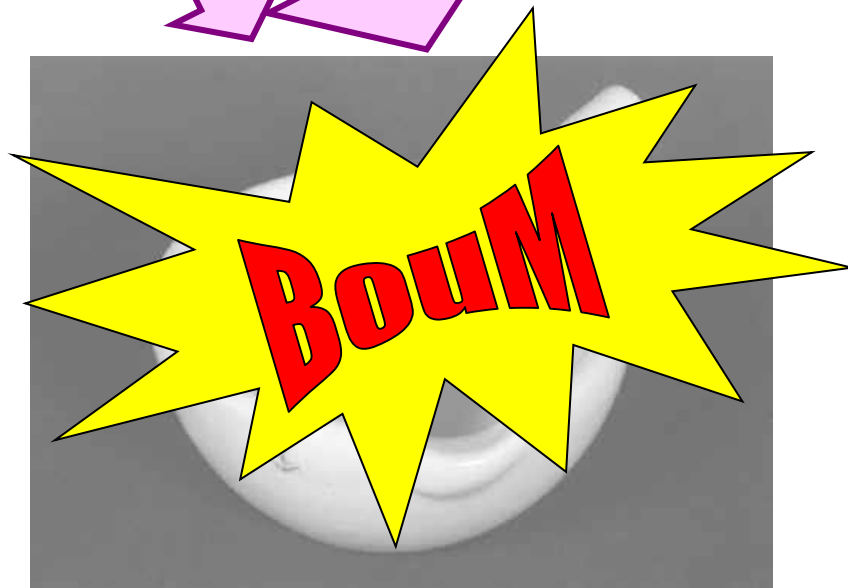
Les résultats transcriptomiques et métabolomiques confirment l'implication du métabolisme protéique dans la réponse à la salinité.

Cramer et al. Functional & Integrative Genomics, 2007

Optimisation de l'extraction de protéines de grappes de raisin



BROYAGE DE GRAPPES DE RAISINS



EXTRACTION DE PROTEINES AU TCA/ACETONE



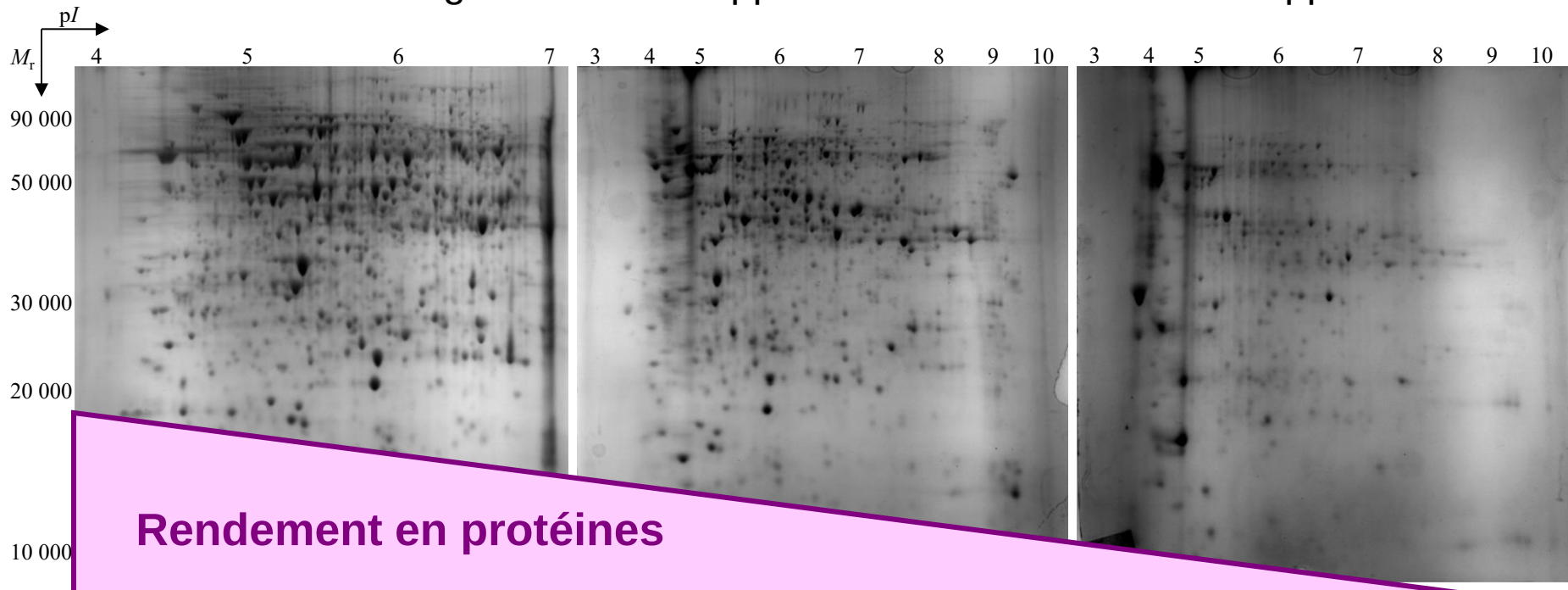
Extrémité de la tige



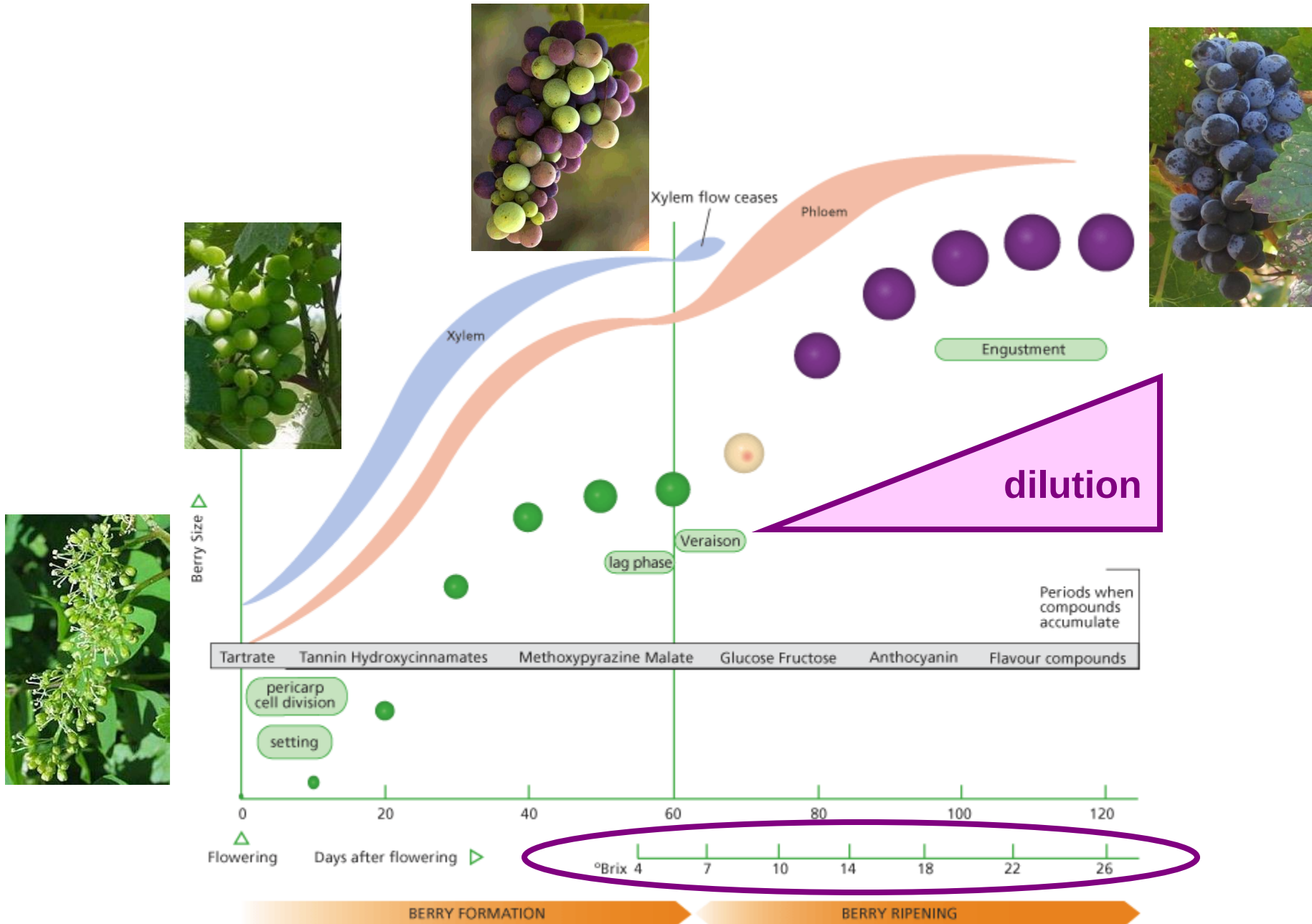
Grappe immature



Grappe mûre



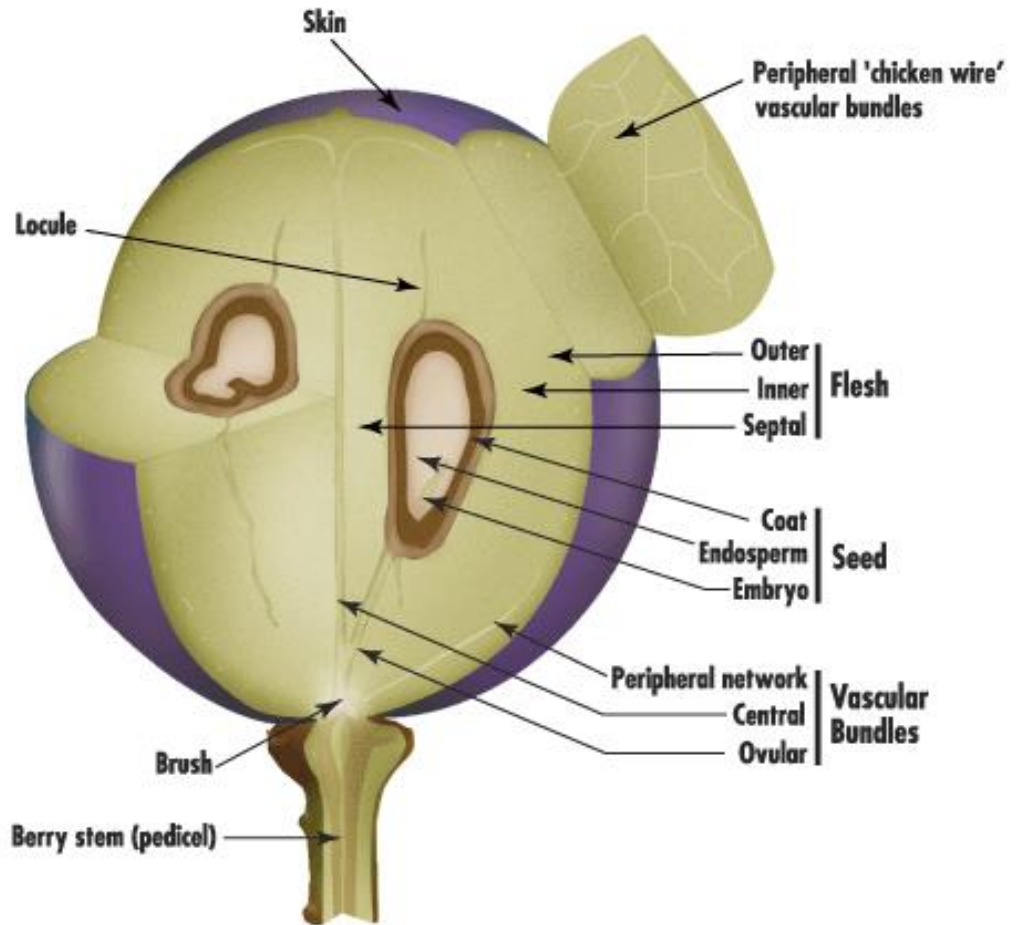
DEVELOPPEMENT DE LA BAIE DE RAISIN



DEVELOPPEMENT DE LA BAIE DE RAISIN

Baie de raisin = organe très spécialisé $\Rightarrow$ diversité de tissus:

- raffle/pédoncule **lignifié**
- épicarpe riche en **tannins** et composés **phénoliques**
- mésocarpe riche en **sucres**
- et au contenu en eau élevé
- pépins riches en **tannins**
- ...



Grappe de raisin = Organe composé de tissus récalcitrants, riches en composés interférant avec la 2-DE, jamais étudié au niveau protéomique
 $\Rightarrow$ Nécessité d'un protocole adapté

PROTOCOLES TESTES: TCA/ACETONE ET PHENOL

Finely ground frozen powder

Protocol 1

Precipitation

10% TCA
0.07% 2-ME
in acetone

Washing x3

0.07% 2-ME
in acetone

Drying

Resuspension (RB 1)

9 M urea
0.5% ASB-14
2% CHAPS
1.2% HED
0.5% CA pH 5-7
0.25% CA pH 3-10

Tous types de
tissus végétaux

Damerval et al. 1986

Protocol 2

Precipitation

12.5% TCA
28 mM 2-ME
in acetone

Washing x2

85% EtOH

Drying

Resuspension (RB 2)

7 M urea
2 M thiourea
4% CHAPS
0.5% Triton X-100
65 mM DTT
0.1% CA pH 5-7
0.2% CA pH 3-10

Mésocarpe de
baies de raisin

Sarry et al. 2003

Protocol 3

Precipitation/Washing x4

10% TCA
in acetone

Washing x2

10% TCA
in water

Washing x2

80% acetone

Drying

Resuspension (SB 3)

30% sucrose
2% SDS
0.1 M Tris-HCl (pH 7.5)
5% 2-ME
in phenol-Tris (pH 7.9)

Precipitation x3

0.1 M ammonium acetate
in MeOH

Washing x2

80% acetone

Drying

Resuspension (RB 3)

8 M urea
2 M thiourea
4% CHAPS
1% CA pH 5-7
1% CA pH 3-10

Limbes de
feuilles d'olivier

Wang et al. 2004

Protocol 4

Resuspension (SB 4)

0.7 M sucrose
50 mM EDTA
0.5 M Tris-HCl (pH 7.5)
0.1 M KCl
2% 2-ME
2 mM PMSF

Resuspension

in phenol-Tris (pH 7.9)

Precipitation x4

0.1 M ammonium acetate
in MeOH

Washing

100% acetone

Drying

Resuspension (RB 4)

7 M urea
2 M thiourea
10 mM DTT
4% CHAPS
1% CA pH 5-7
1% CA pH 3-10

Tomate, banane,
avocat, orange

Saravanan & Rose, 2002

SOLUBILISATION ET RESOLUTION AMELIOREES

TCA/acetone

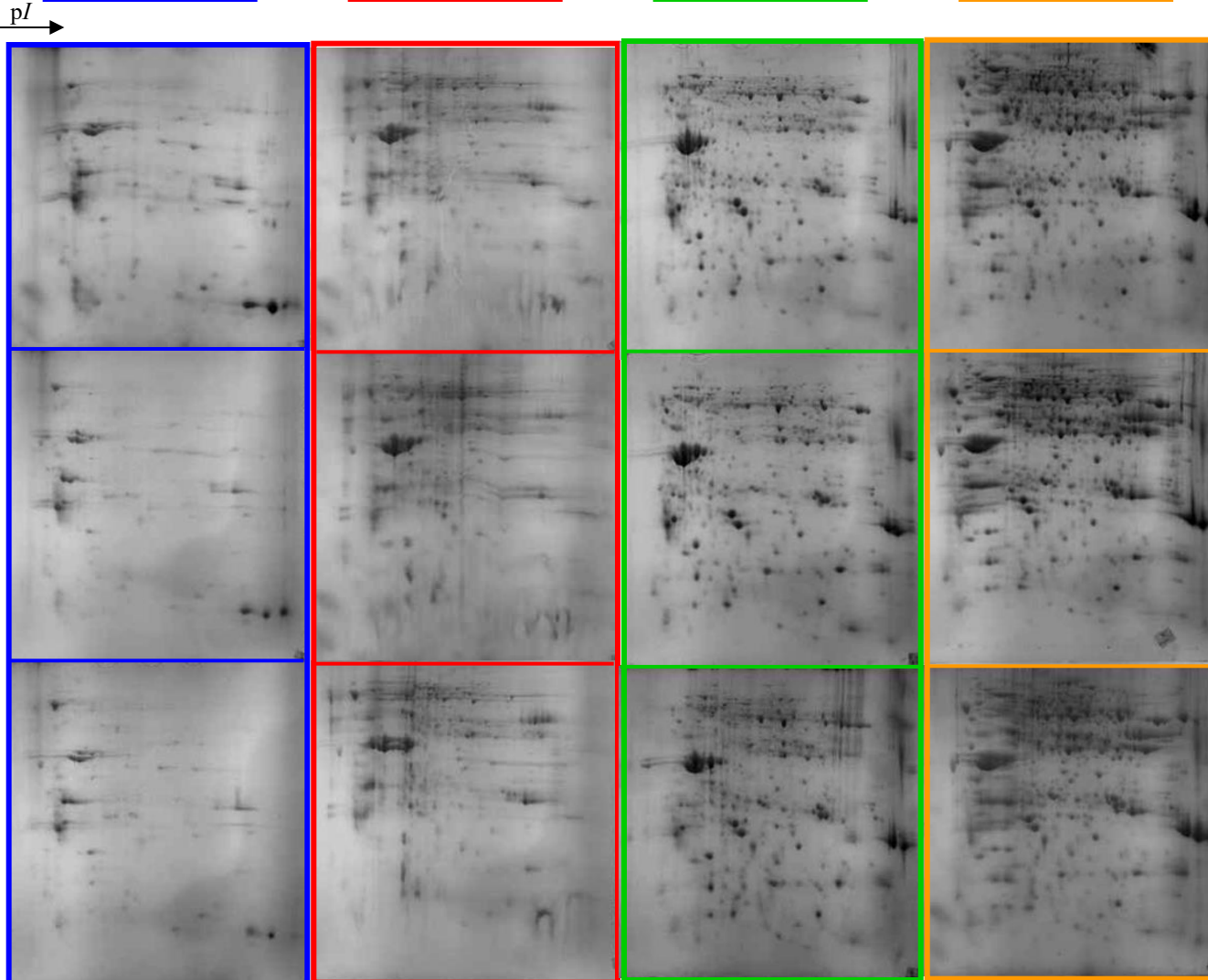
Phenol

Protocole 1

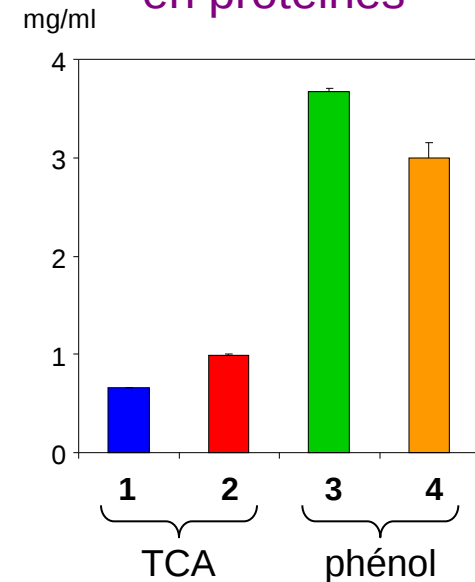
Protocole 2

Protocole 3

Protocole 4

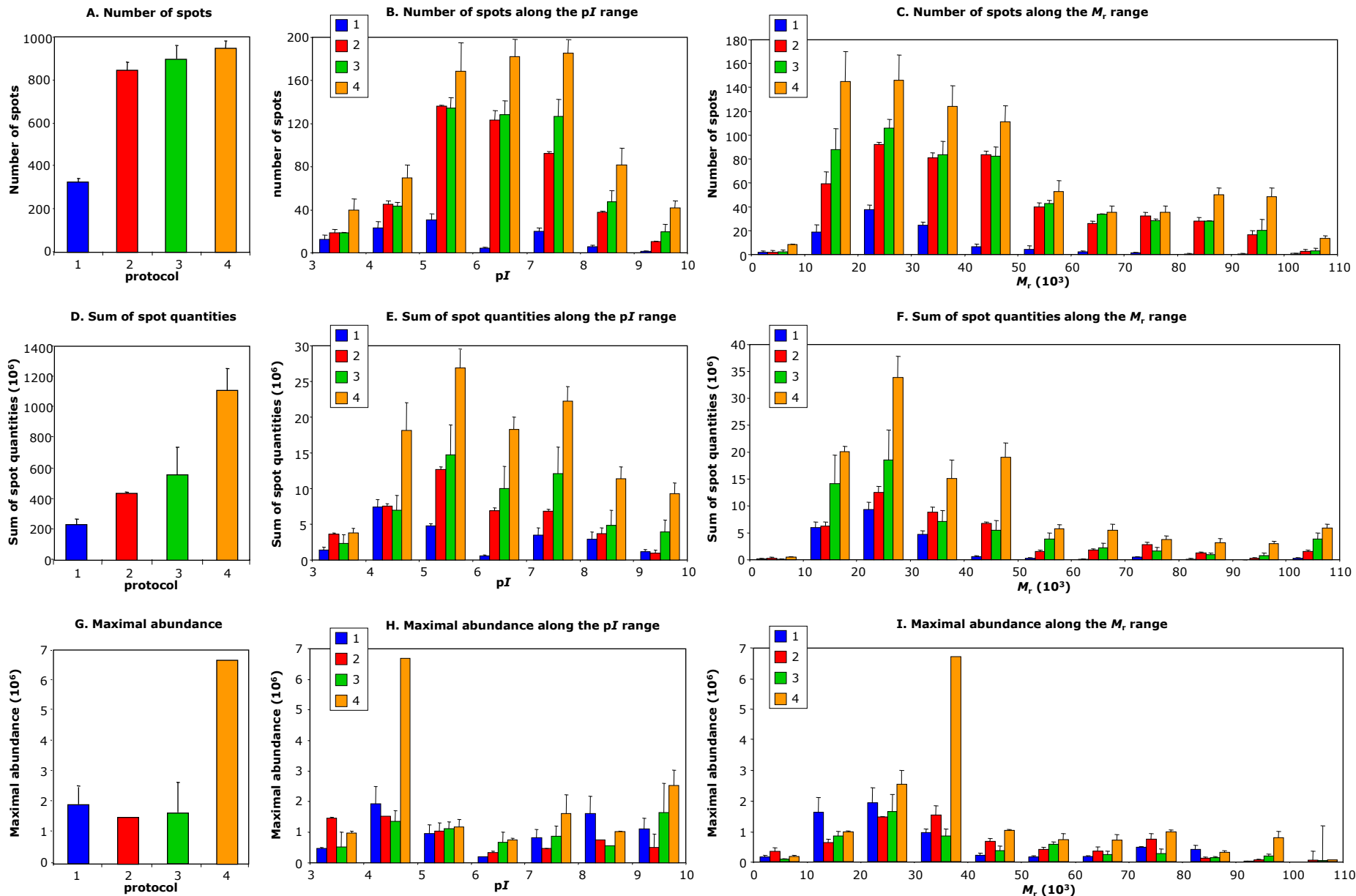


concentration
en protéines

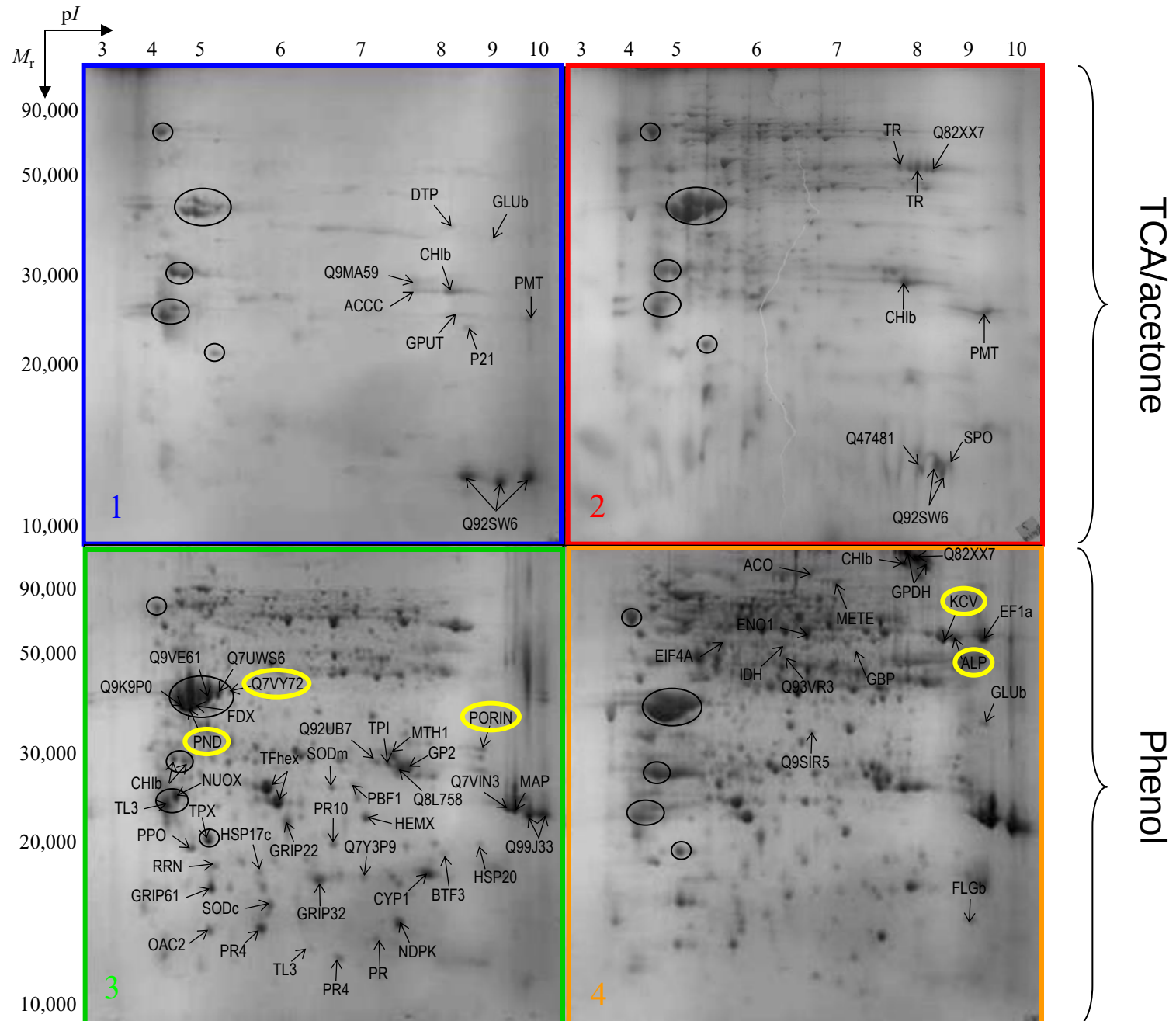


pH 3-10 NL
18 x 18 cm²
12% acrylamide
BCC

ESTIMATION DE L'EFFICACITE DES EXTRACTIONS



IDENTIFICATION DE PROTEINES



CONCLUSIONS



Mise en place d'une procédure facilitant le broyage de grappes de raisins (mixeur/carboglace + mortier/azote).



Test de 4 protocoles d'extraction ayant été utilisés sur divers tissus récalcitrants.



Extraction au phénol mieux adaptée au baies de raisin, riches en sucres et au contenu en protéines dilué. Enrichissement du taux de protéines précipitées $\Rightarrow$ limitation des pertes.



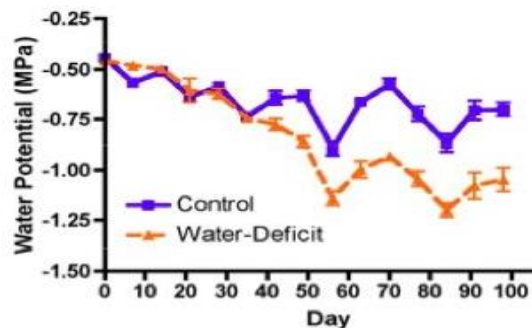
Effet du tampon de resolubilisation. Davantage de protéines précipitées dans le culot sont resolubilisées $\Rightarrow$ limitation des pertes.

L'AVENTURE CONTINUE...

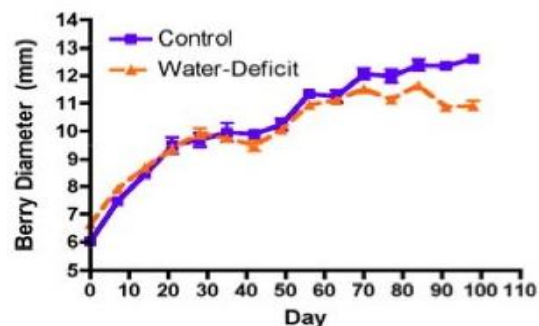
Effet de la sécheresse sur le développement des grappes de Cabernet Sauvignon.



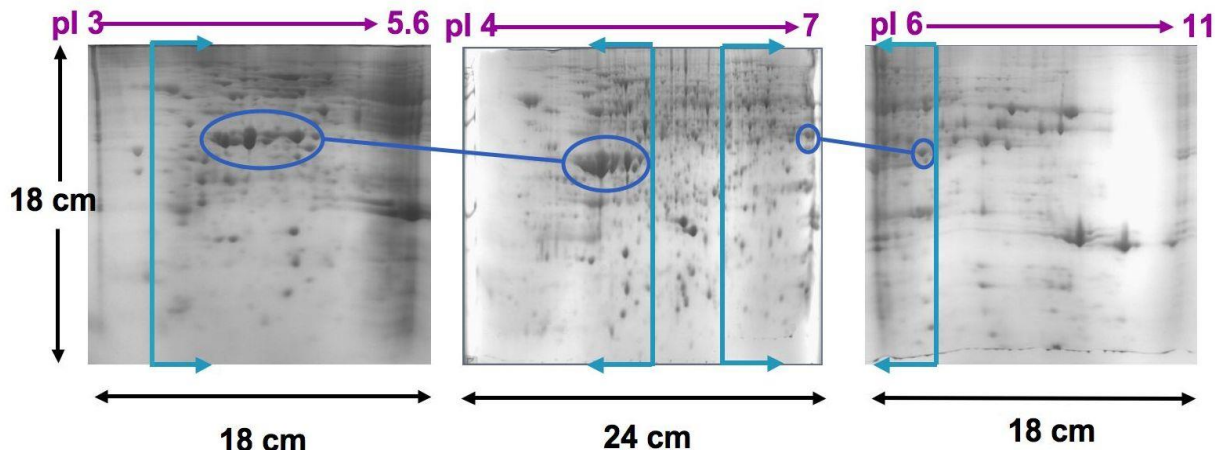
Average Stem Water Potential



Average Berry Diameter per Cluster

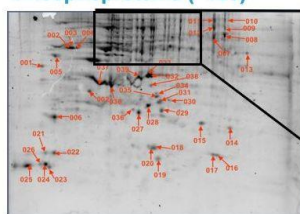


A comprehensive 2D map of the grape berry proteome was constructed using overlapping narrow-range 1st Dimension IPG strips. Approximately 1,000 unique spots have been picked and mass spectra obtained.

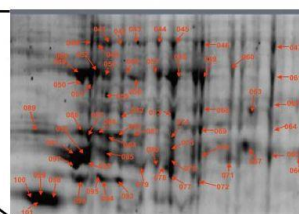


Phosphoproteins and glycoproteins tentatively identified for pI 4-7 using Molecular Probes ProQ Diamond and ProQ Emerald stains, followed by total protein staining with Sypro Ruby.

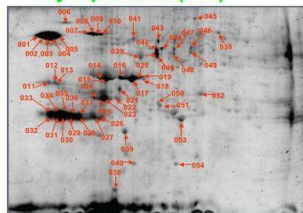
Phosphoproteins (~120)



ProQ® Diamond Phosphoprotein Stain

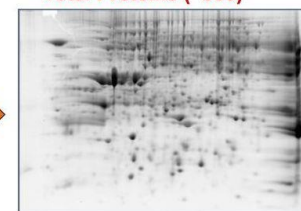


Glycoproteins (~60)



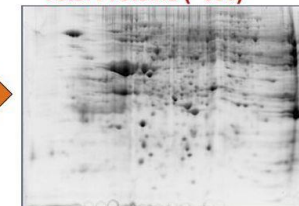
ProQ® Emerald300 Glycoprotein Stain

Total Proteins (~800)



Sypro Ruby® Stain for Total Proteins

Total Proteins (~800)



Sypro Ruby® Stain for Total Proteins

Following staining of gels, all spots were excised and mass spectra obtained using MALDI TOF-TOF MS/MS. This was followed by identity searching using BioRad IDQuest Software. Because mapping of the *Vitis vinifera* genome is incomplete, some identifications have yet to be obtained.

Un peu de tourisme...









Merci de votre attention !