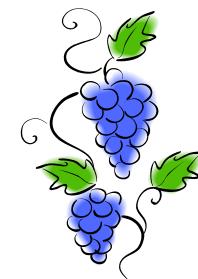




être française
et apprendre
à faire du vin
aux USA !



Trahison !

Dr Delphine Vincent
27 Mars 2023

PLACE DE LA VIGNE DANS LE MONDE

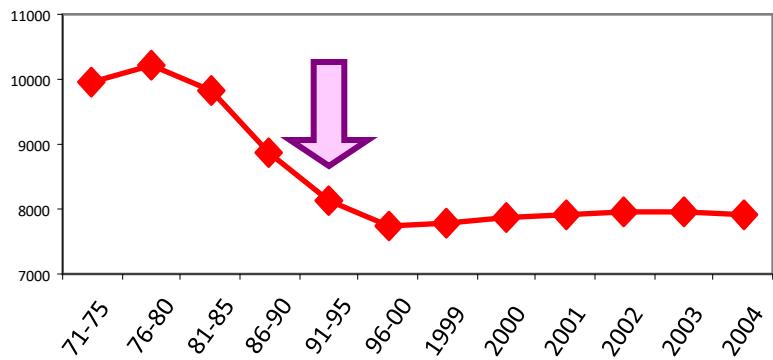
Production mondiale des principaux fruits



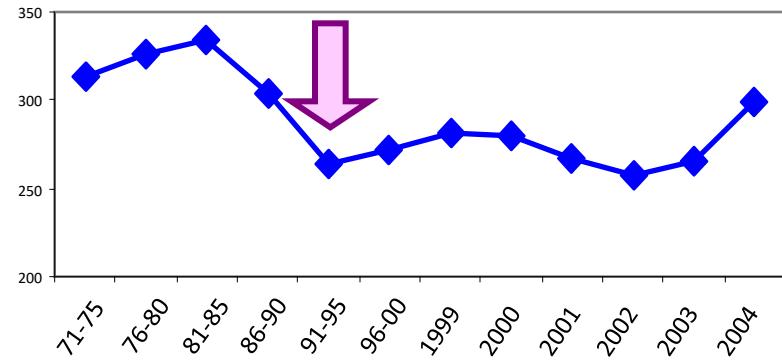
- agrumes
- banane
- raisin
- pomme
- mange
- poire
- ananas
- pêche
- prune
- papaye
- date
- fraise
- abricot
- avocat

QUELQUES CHIFFRES...

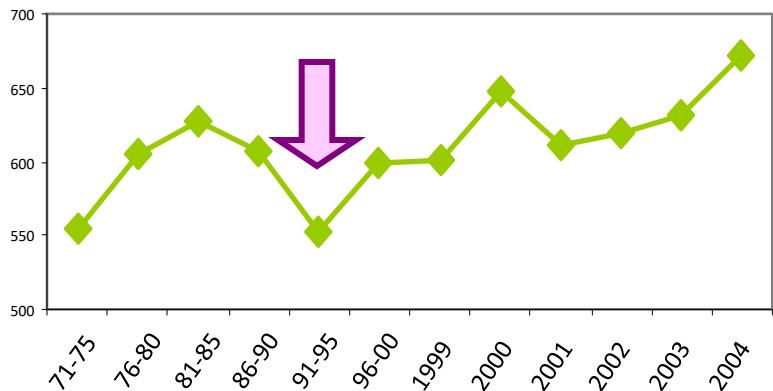
Superficie mondiale



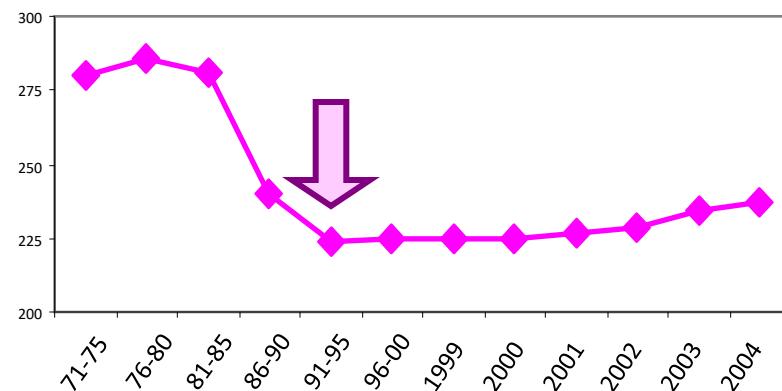
Production mondiale de vins



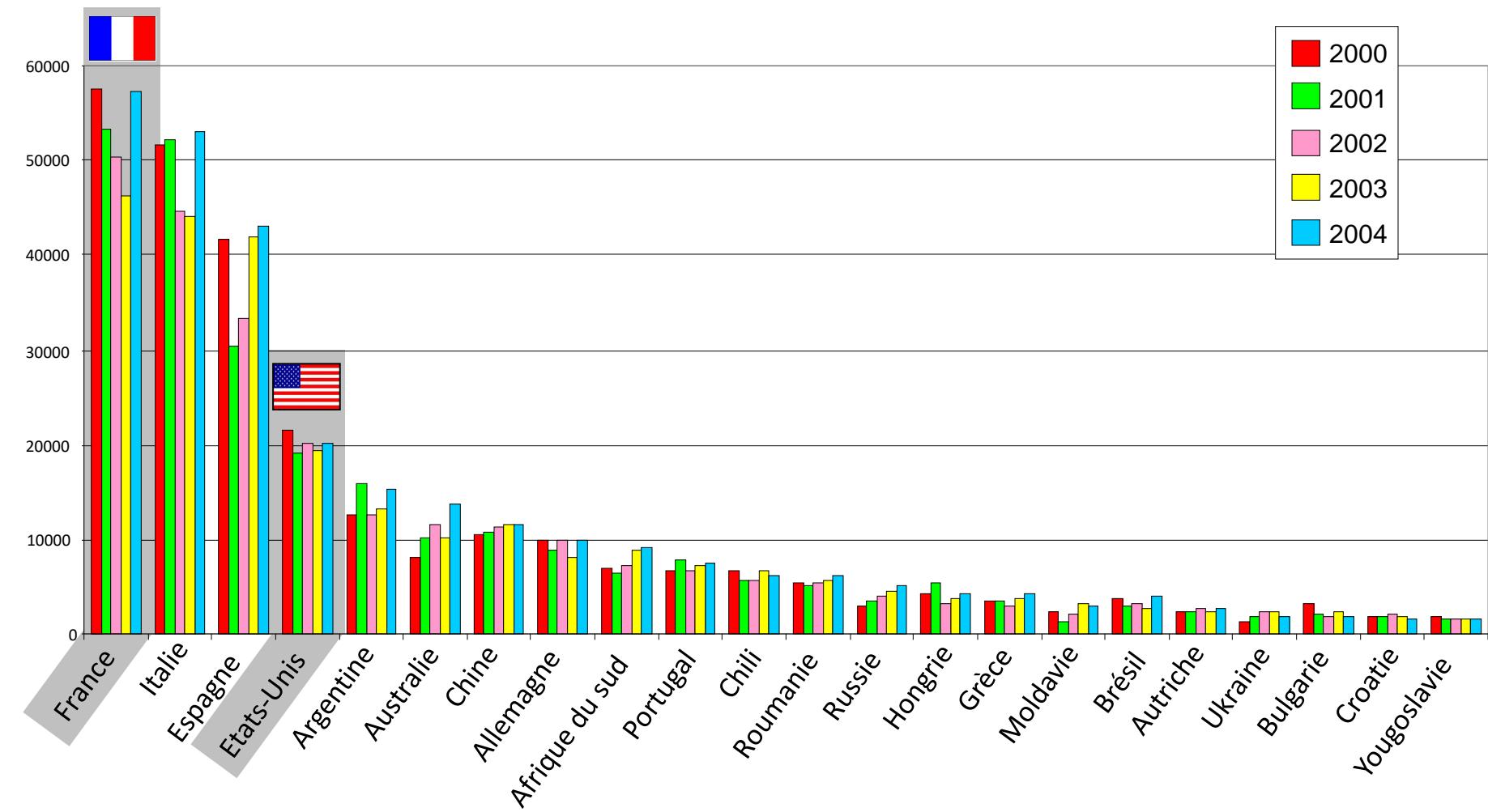
Production mondiale de raisins



Consommation mondiale de vins

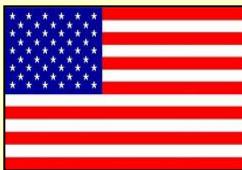


LES PRINCIPAUX PRODUCTEURS MONDIAUX

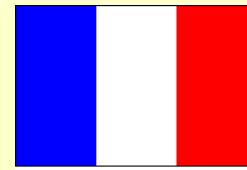


FRENCH PARADOX

une autre raison d'être jaloux !



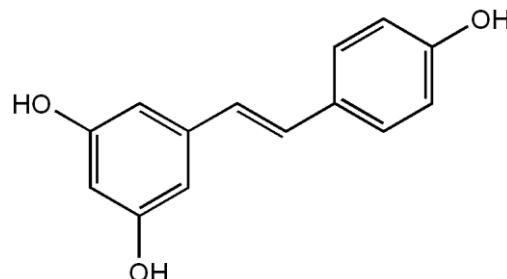
Gras 72 g/j
Graisses saturées +
Cholesterol +
Attaque cardiaque: 230/100000



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 !

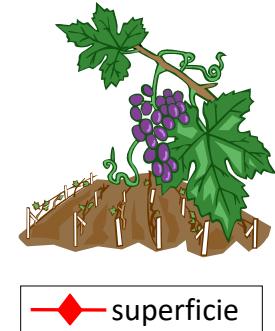
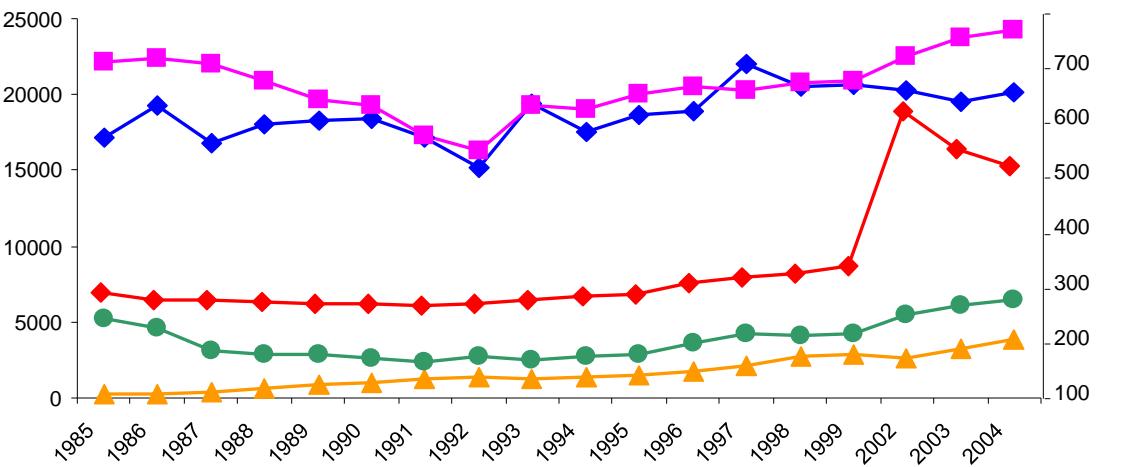
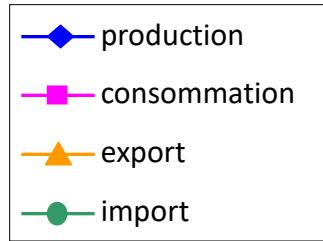


Resveratrol (phytoalexin)
antioxidant

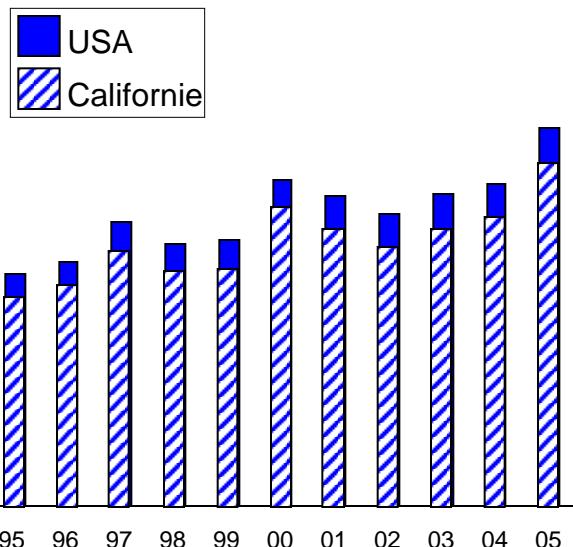
Présent dans divers fruits (myrtilles, fraises, cacahuètes, pignons !)
particulièrement concentré dans la peau des raisins rouges (donc dans les vins rouges)



LA VIGNE AUX USA



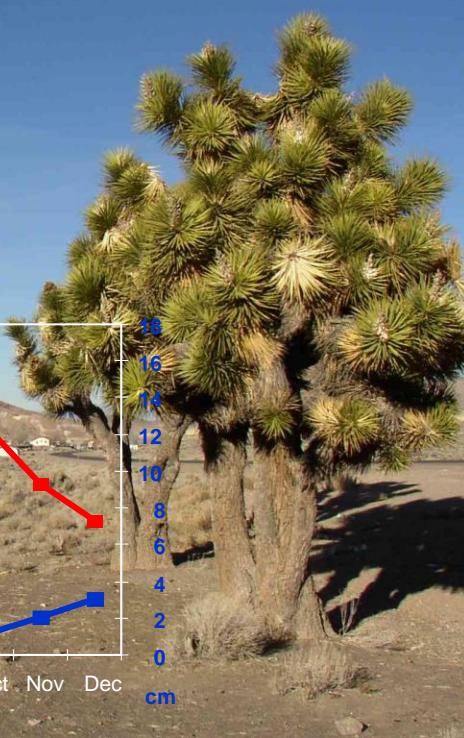
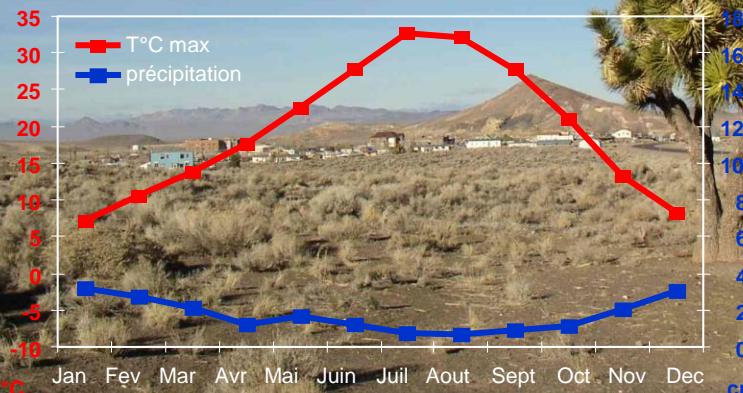
Production de vin aux USA



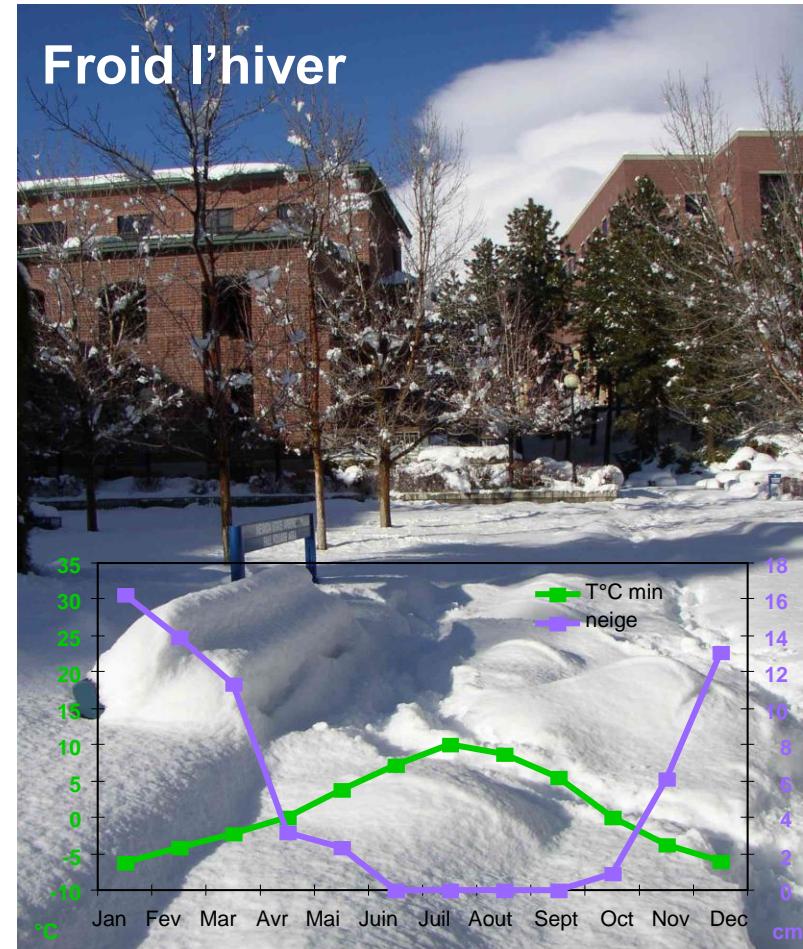
LA VIGNE DANS LE NEVADA

A priori, c'est pas gagné !

Chaud l'été
Sec toute l'année



Froid l'hiver



LA VIGNE DANS LE NEVADA

Jamais 2 sans 3...

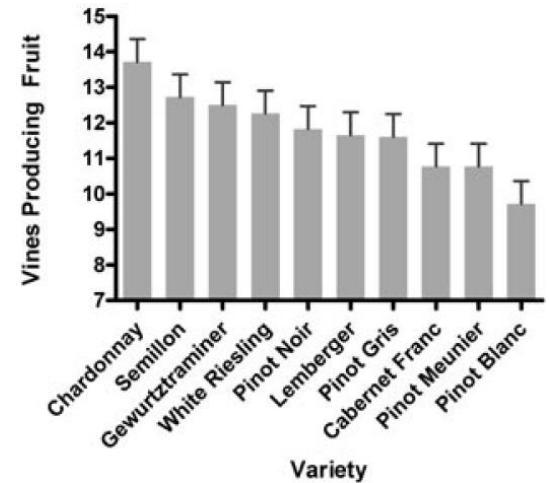


Positivons !

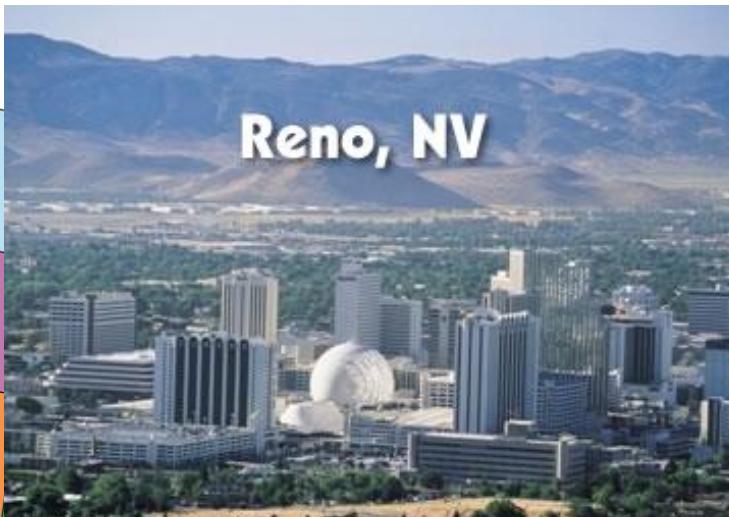
Pression biotique faible voire inexisteante!

LA VIGNE DANS LE NEVADA

en effet...



PROJET VIGNE A RENO, NEVADA



CONN: CONNECTICUT
MASS: MASSACHUSETTS
NH: NEW HAMPSHIRE
RI: RHODE ISLAND
VT: VERMONT

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

STRATEGIE: SYSTEMS BIOLOGY

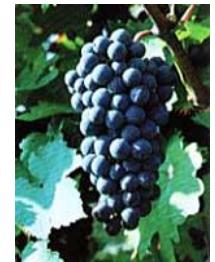
espèce

Vitis vinifera



variétés

Chardonnay



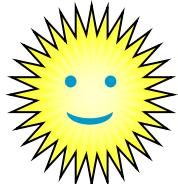
Cabernet Sauvignon

stress abiotiques

sécheresse

salinité

froid



conditions de culture

champ



serre



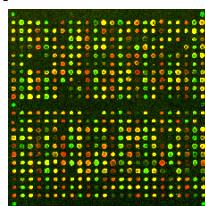
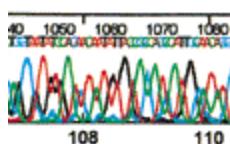
chambre de culture



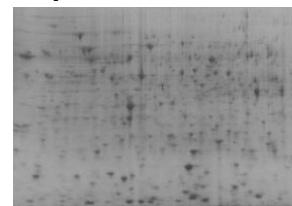
niveaux
d'expression
de gènes

transcriptome

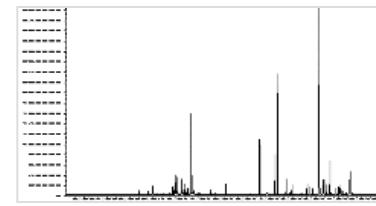
ESTs



protéome



métabolome

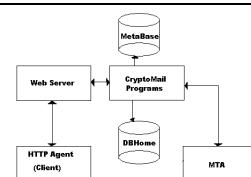


informatique

statistical analyses



database
integration



STRATEGIE: SYSTEMS BIOLOGY

espèce

Vitis vinifera



variétés

Chardonnay

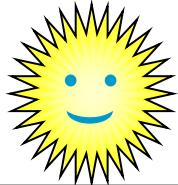


Cabernet Sauvignon

stress abiotiques

sécheresse

salinité



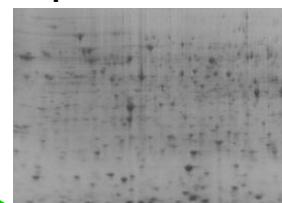
conditions de culture

serre



niveaux
d'expression
de gènes

protéome

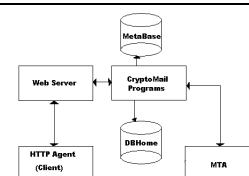


informatique

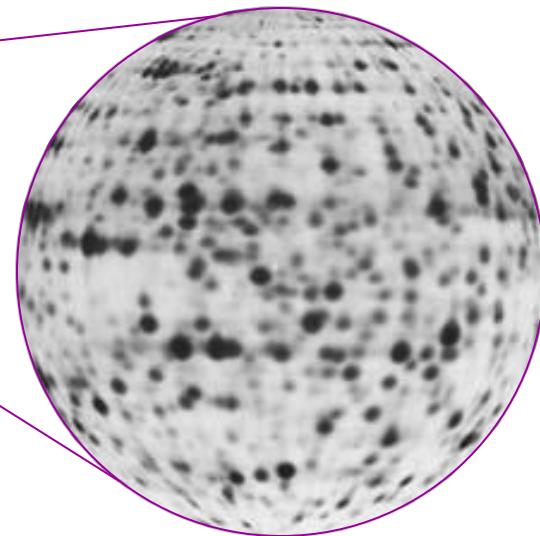
statistical analyses



database
integration



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



MATERIAL VEGETAL ET MESURES

salinité	 10 mM [NaCl]	length Ψ_w	length Ψ_w	length Ψ_w	length Ψ_w
sécheresse		length Ψ_w	length Ψ_w	length Ψ_w	length Ψ_w
témoin	Ψ_w	length Ψ_w	length Ψ_w	length Ψ_w	length Ψ_w

d-4 d0 d4 d8 d12 d16 temps

2 variétés
 (CH & CS)

3 répl. biol.
 (3 x 2 shoots)

42

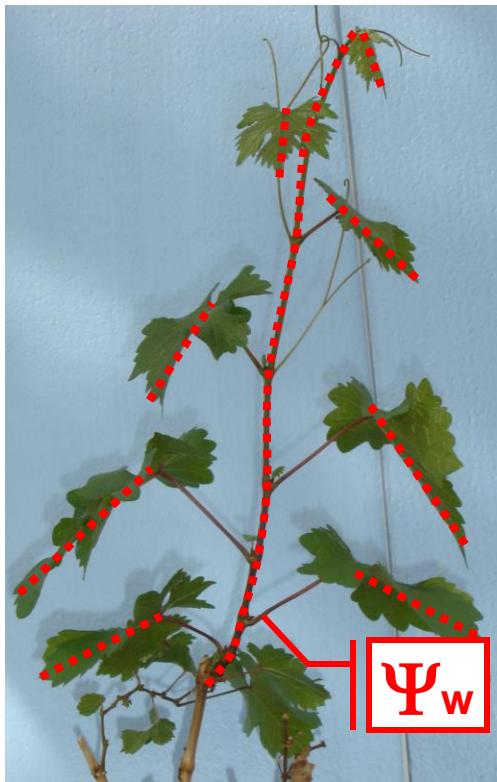
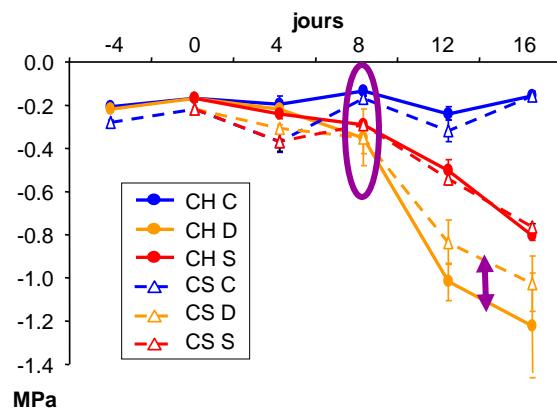



1 shoot =
1 bourgeon
4 feuilles
tige
tendrons

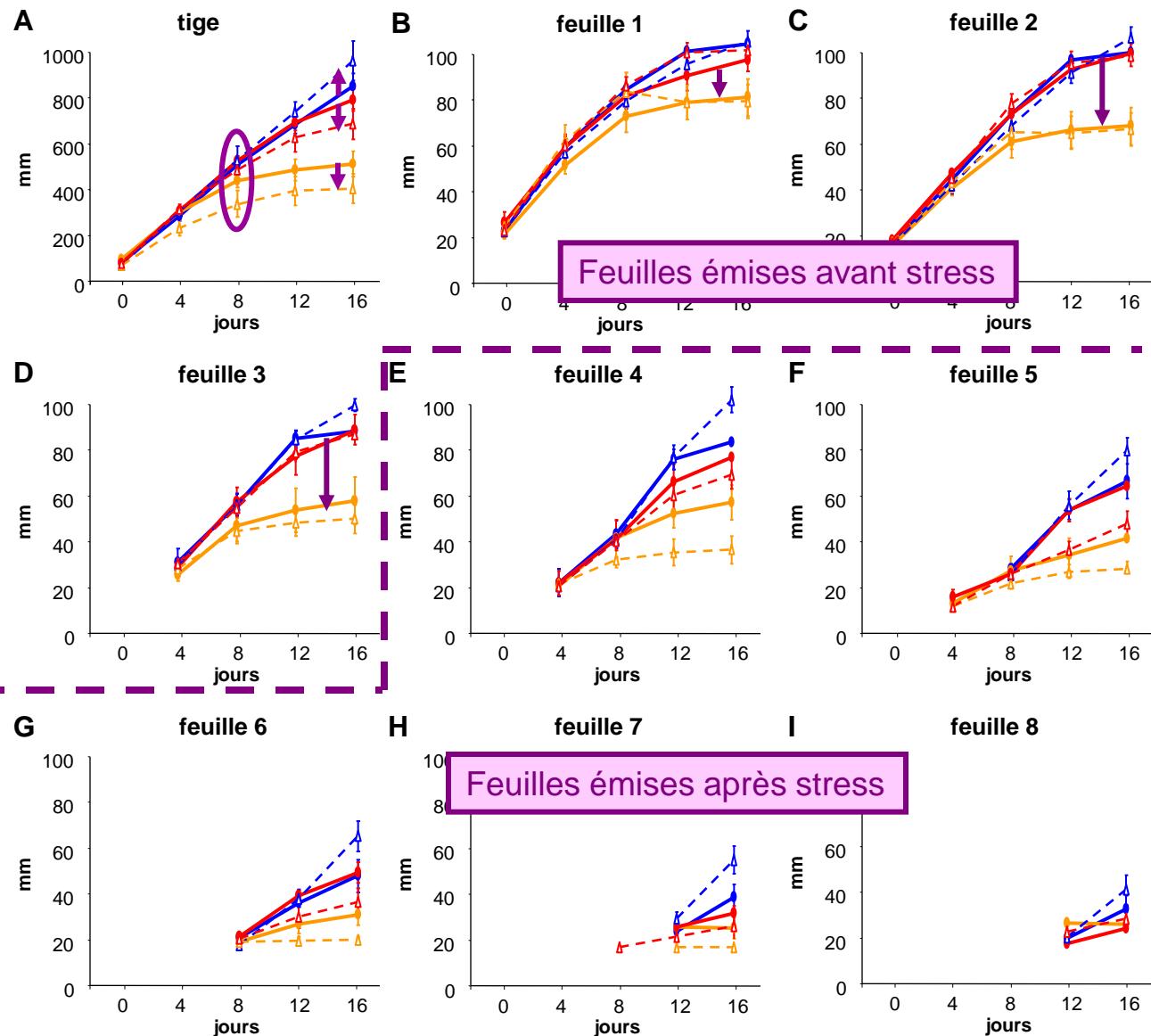


DONNEES PHYSIOLOGIQUES

Potentiel hydrique



Cinétique de croissance des tiges/feuilles



DONNEES PHYSIOLOGIQUES

Au niveau physiologique...

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

Feuilles présentes avant stress moins affectées

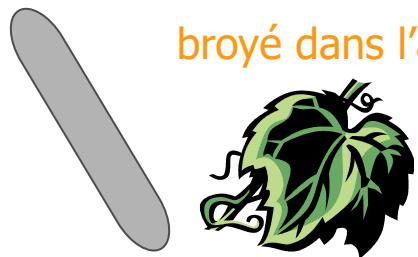
Quant est-il au niveau moléculaire ?

Réponses avec les protéines...

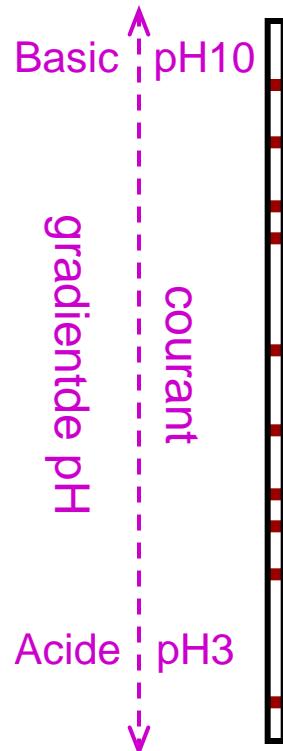
ELECTROPHORESE BIDIMENSIONNELLE

Matériel végétal

broyé dans l'azote

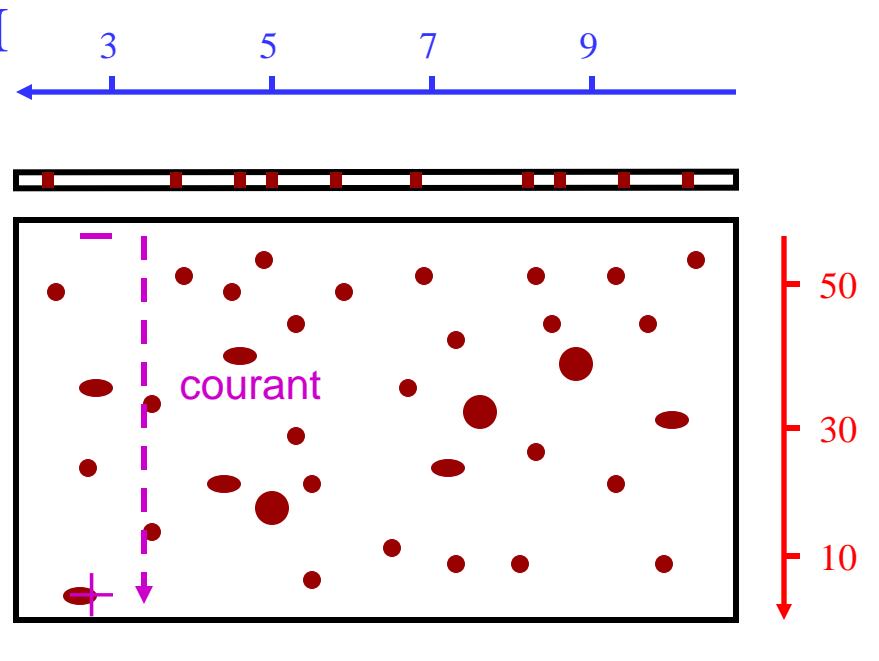


extraction de
protéines



1ère dimension
selon le pI

2ème dimension
selon le PM



Coloration
nitrate d'argent, BBC

ANALYSES STATISTIQUES

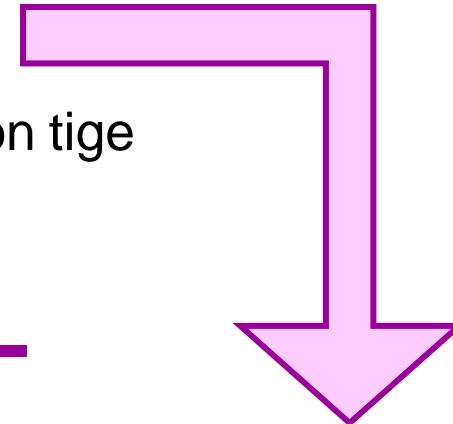
~1500 spots détectés \Rightarrow 758 spots reproductibles (2/3)

\Rightarrow Régressions pas-à-pas

2 modèles: Ψ_w + élongation tige

\Rightarrow ANOVA

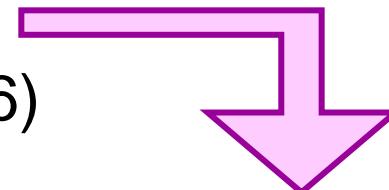
\Rightarrow corrélations, ACP, ACH



IDENTIFICATION DE PROTEINES

\Rightarrow Régressions pas-à-pas (16)

\Rightarrow Spots les plus abondants (186)

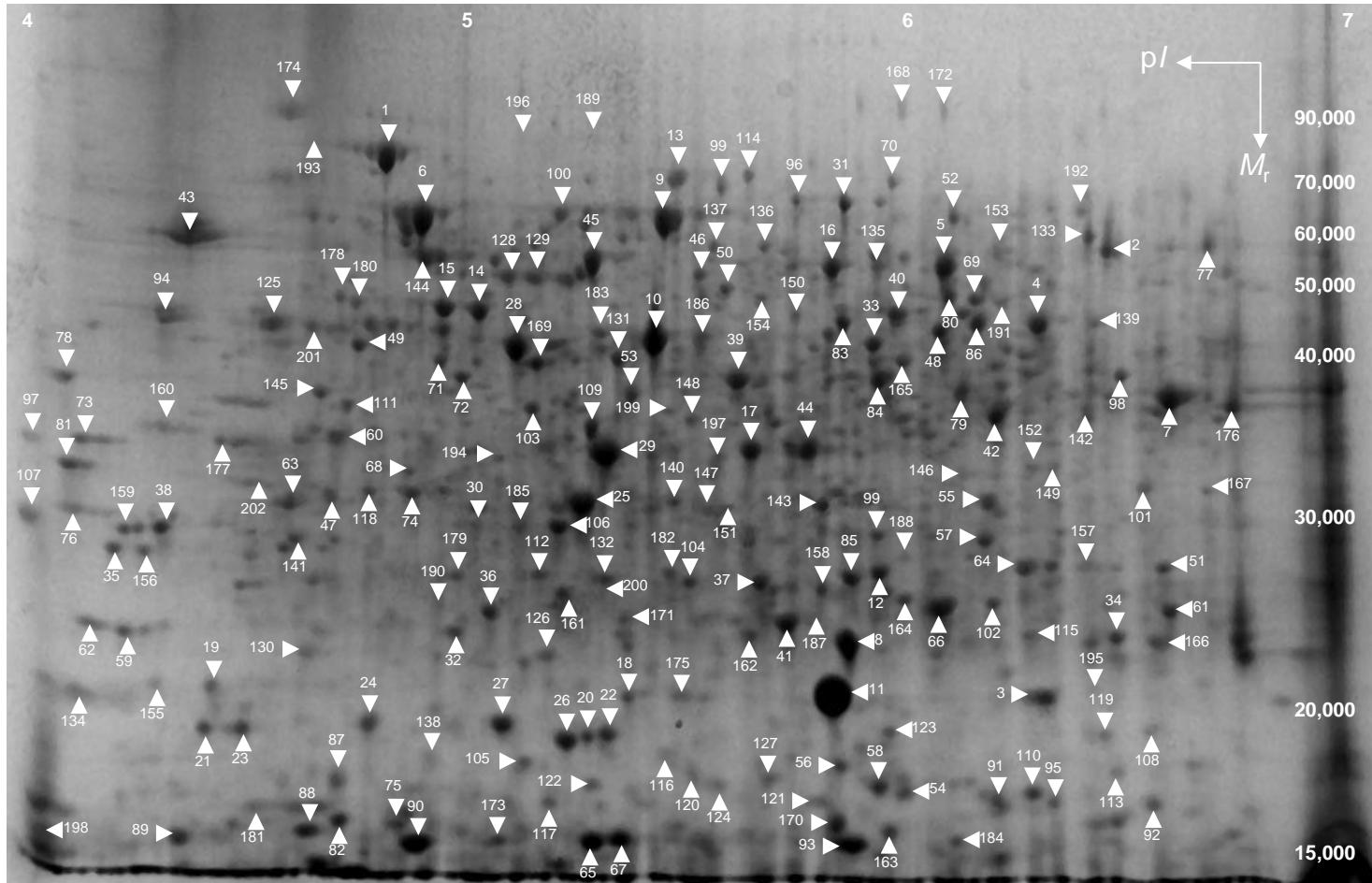


Classification fonctionnelle

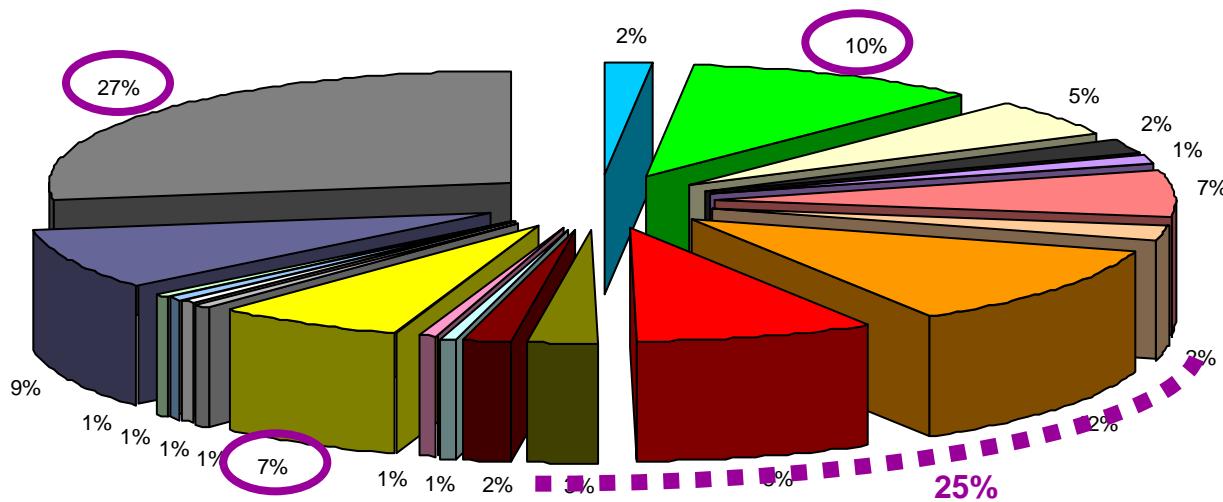
Profils des protéines d'intérêt

CARTE PROTEIQUE

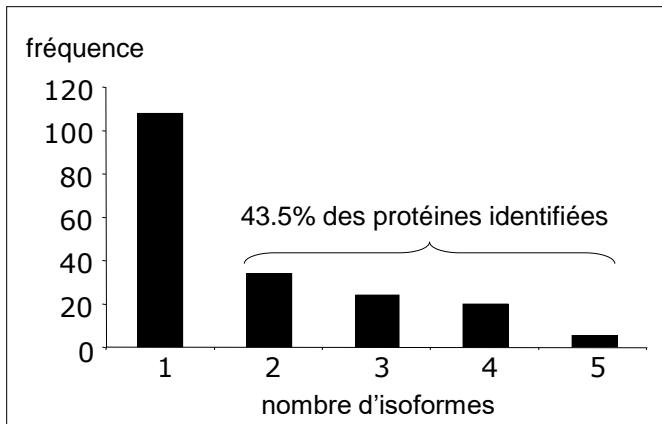
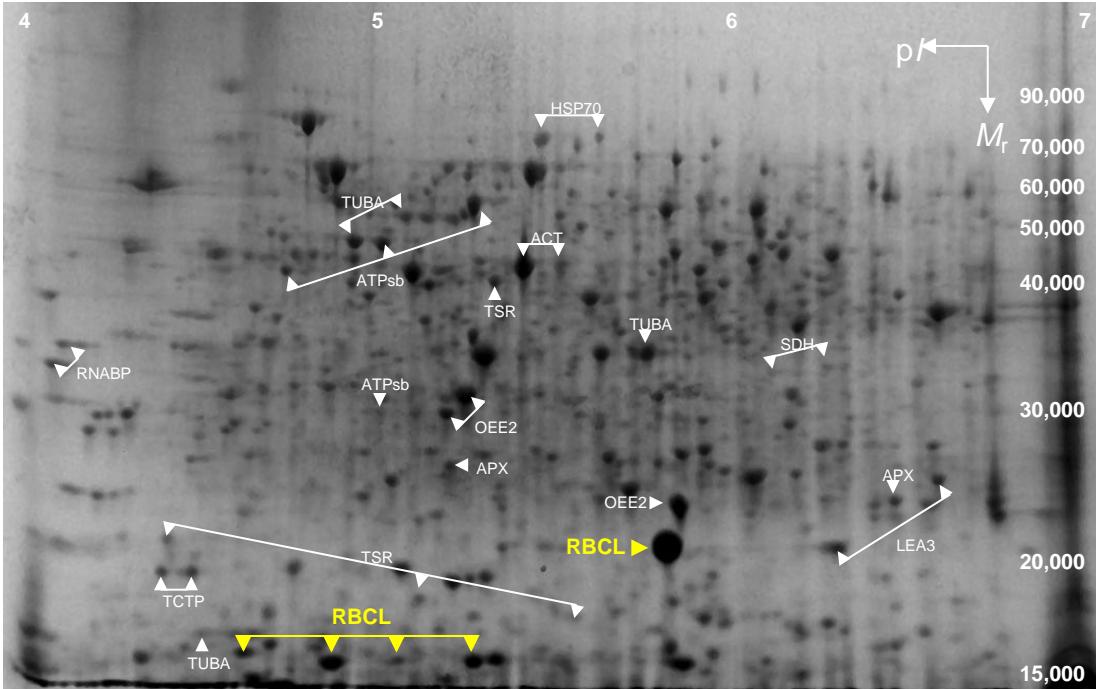
202 spots (186 abondants + 16 « régression ») analysés en MALDI-TOF/TOF



CARTE PROTEIQUE

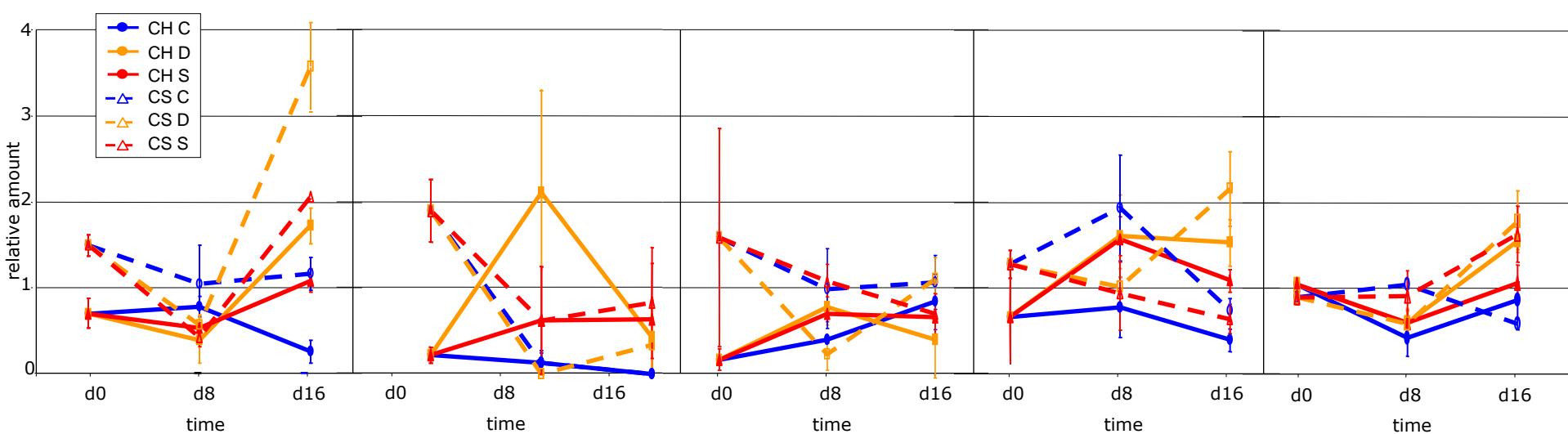


- █ glycolysis and gluconeogenesis
- █ photosynthesis
- █ electron transport/membrane-associated energy
- █ C-compound and carbohydrate metabolism
- █ amino-acid metabolism
- █ cellular biogenesis
- █ transcription
- █ protein synthesis
- █ protein destination
- █ protein degradation
- █ secondary metabolism
- █ cellular communication/signal transduction
- █ storage protein
- █ stress
- █ cellular transport and transport mechanisms
- █ lipid, fatty-acid and isoprenoid metabolism
- █ tricarboxylic-acid pathway
- █ spermidine metabolism
- █ unclassified protein
- █ unknown protein



EXEMPLE D'ISOFORMES: LA RUBISCO (RBCL)

A Spot#88

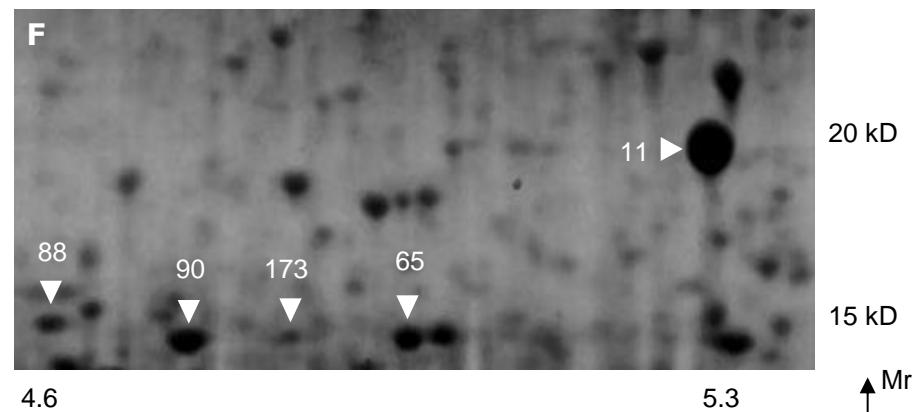
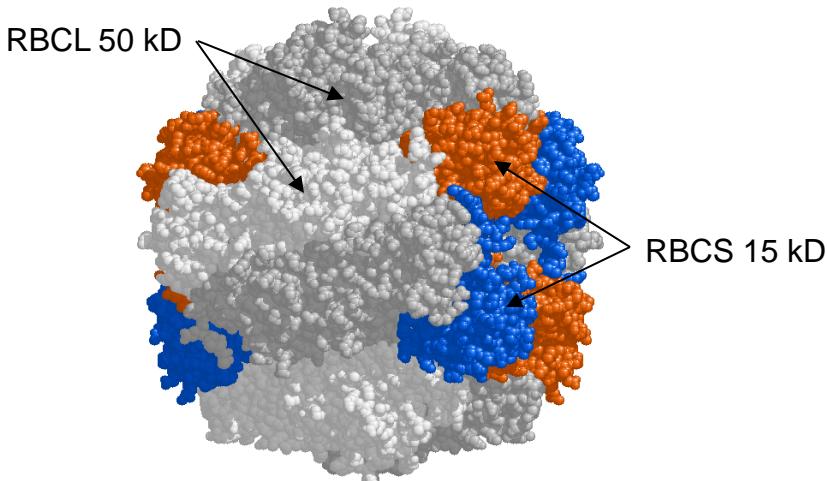


B Spot#90

C Spot#173

D Spot#65

E Spot#11



CARTE PROTEIQUE

Mr
pl

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

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

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

Traduction facteurs $p < 0.05$

Biogénèse des parois

V 305 (40%)

D 199 (26%)

T 115 (15%)

V*D 89 (12%)

V*T 79 (10%)

D*T 120 (16%)

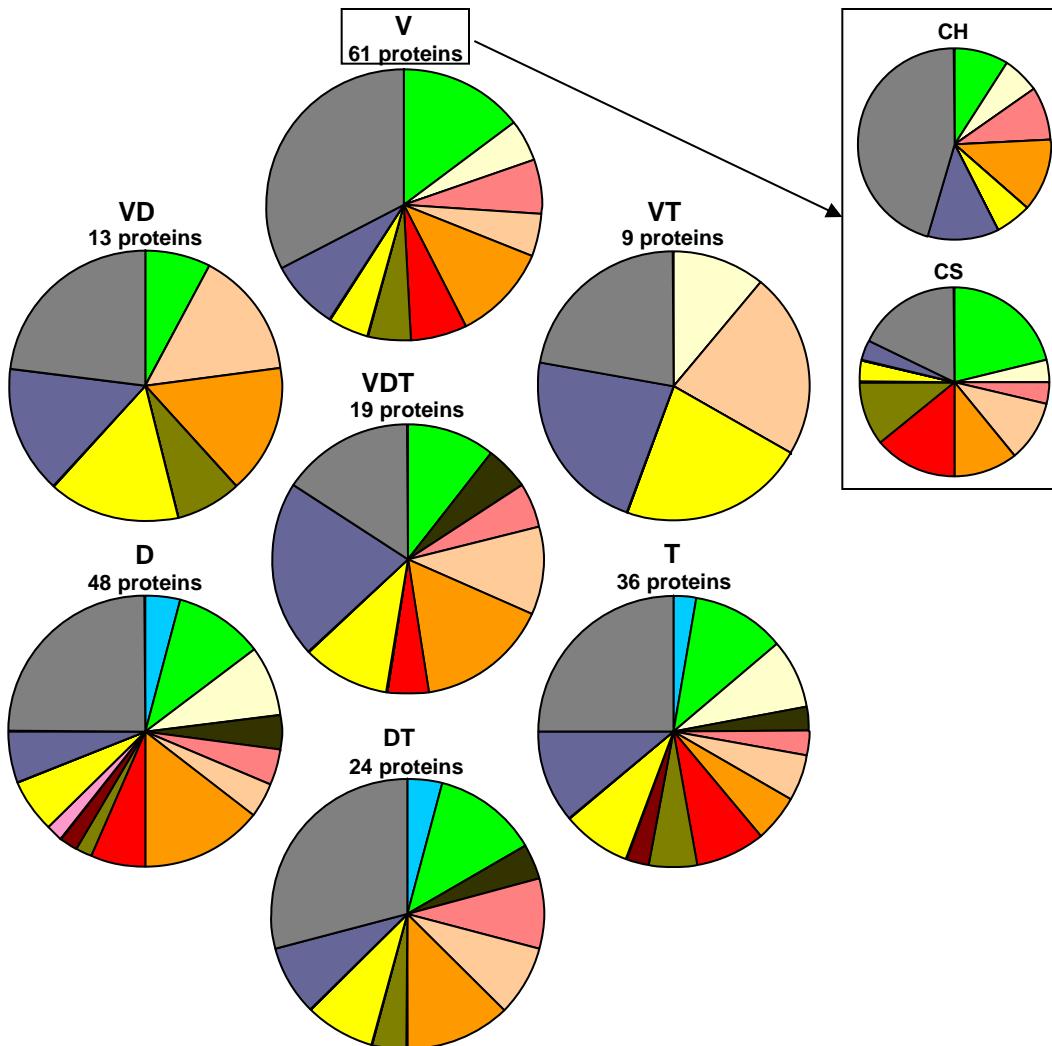
Elongation de la tige

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

Métabolisme protéique
(synthèse + maturation)

REPARTITION DES PROTEINES SIGNIFICATIVES PARMI LES 202 PROTEINES IDENTIFIEES

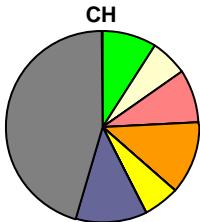
ANOVA: $y_{ijk} = V_i + D_j + T_k + (VD)_{ij} + (VT)_{ik} + (DT)_{kj} + (VDT)_{ijk} + \varepsilon_{ijk}$



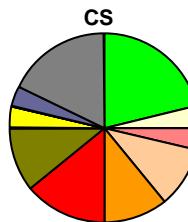
Classification fonctionnelle (GO)

- glycolysis and gluconeogenesis
- photosynthesis
- electron transport/membrane-associated energy conservation
- C-compound and carbohydrate metabolism
- amino-acid metabolism
- cellular biogenesis
- transcription
- protein synthesis
- protein destination
- protein degradation
- secondary metabolism
- cellular communication/signal transduction
- storage protein
- stress
- cellular transport and transport mechanisms
- lipid, fatty-acid and isoprenoid metabolism
- tricarboxylic-acid pathway
- spermidine metabolism
- unclassified protein
- unknown protein

PROTEINES VARIETES-DEPENDANTES

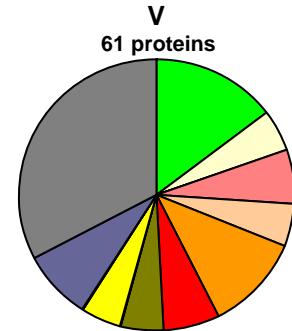


Chardonnay



Cabernet Sauvignon

variety in which the spot is more abundant	protein code	MATdb Functional Classification
Cabernet Sauvignon	PSII	photosynthesis
Cabernet Sauvignon	CPN60a	photosynthesis
Cabernet Sauvignon	RBCL	photosynthesis
Cabernet Sauvignon	RBCL	photosynthesis
Cabernet Sauvignon	RBCL	photosynthesis
Cabernet Sauvignon	PRK	photosynthesis
Cabernet Sauvignon	AK	electron transport ^a
Cabernet Sauvignon	TUBa3	cellular biogenesis
Cabernet Sauvignon	TFB3	transcription
Cabernet Sauvignon	DEL	transcription
Cabernet Sauvignon	bHLH	transcription
Cabernet Sauvignon	RPS14	protein synthesis
Cabernet Sauvignon	eEF1b	protein synthesis
Cabernet Sauvignon	TSR	protein synthesis
Cabernet Sauvignon	PPIase	protein destination
Cabernet Sauvignon	MAT	protein destination
Cabernet Sauvignon	CPN21	protein destination
Cabernet Sauvignon	PDI	protein destination
Cabernet Sauvignon	PSA4	protein degradation
Cabernet Sauvignon	CE	protein degradation
Cabernet Sauvignon	PBA1	protein degradation
Cabernet Sauvignon	PR10	stress
Cabernet Sauvignon	HSDI	unclassified protein
Cabernet Sauvignon	NAC	unknown protein
Cabernet Sauvignon	na	unknown protein
Cabernet Sauvignon	na	unknown protein
Cabernet Sauvignon	na	unknown protein
Cabernet Sauvignon	na	unknown protein



305 spots

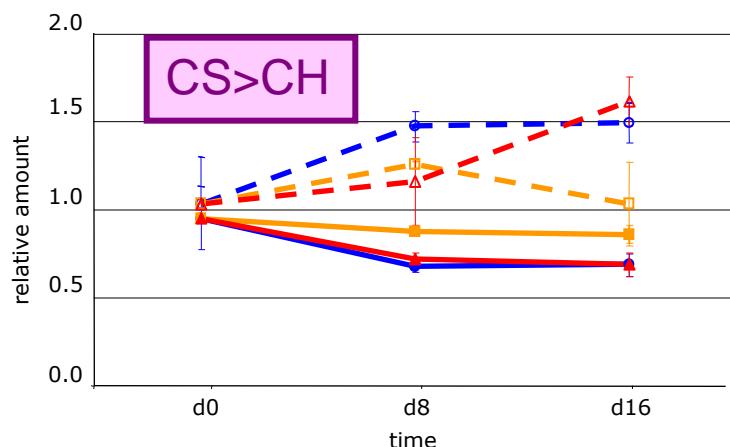
catégories absentes chez CH

PROTEINES VARIETES-DEPENDANTES

A Spot#22 PPIase

Peptidyl-prolyl cis-trans isomerase, chloroplastic

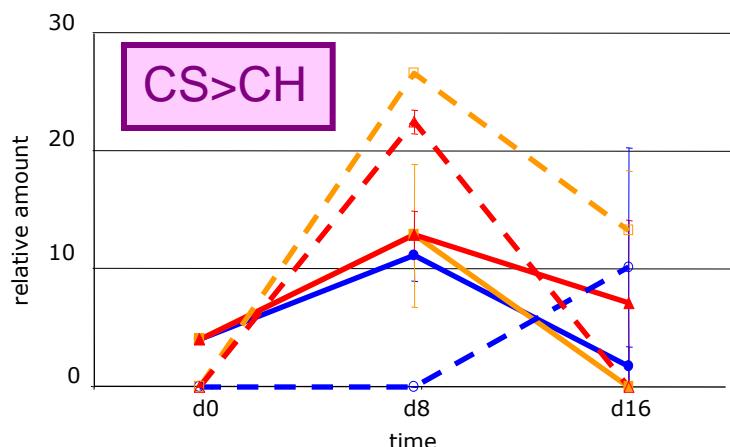
Reploiement de protéines



C Spot#92 CE

Ubiquitin-conjugating enzyme

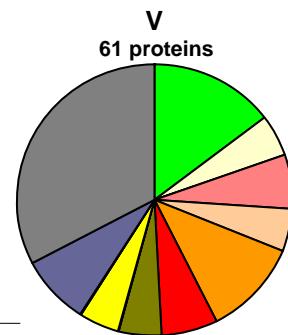
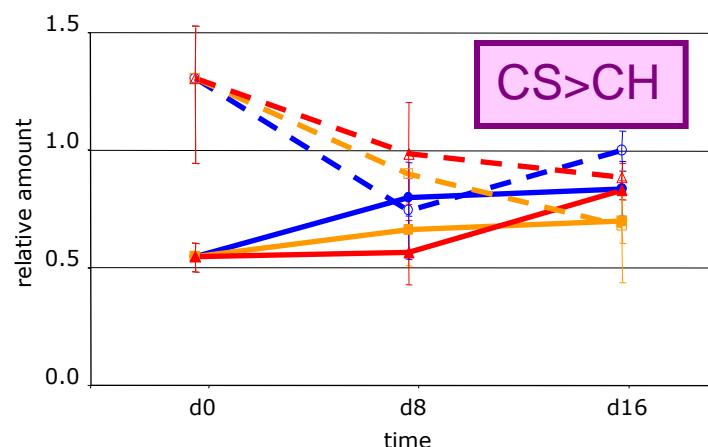
Dégradation de protéines



B Spot#51 PSA4

Proteasome subunit alpha type 4

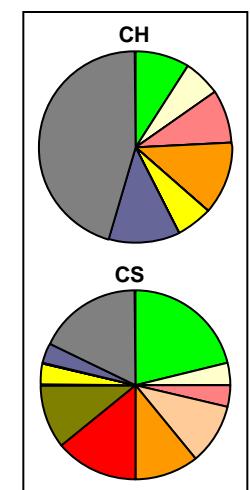
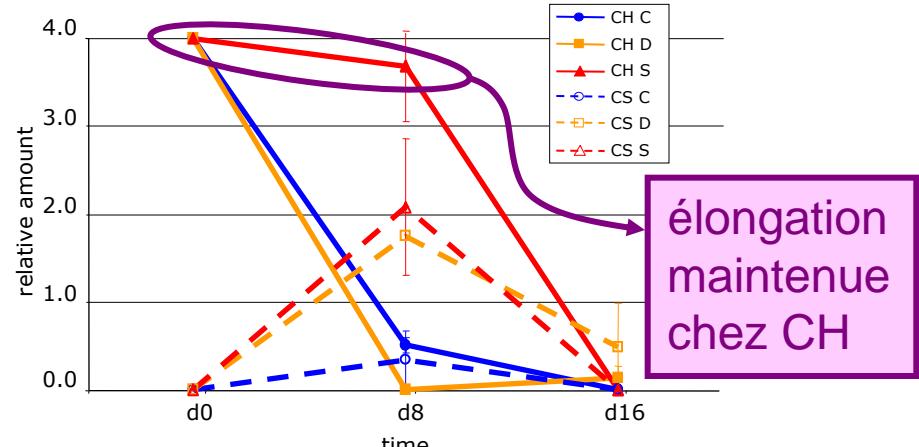
Dégradation de protéines



D Spot#101 XET

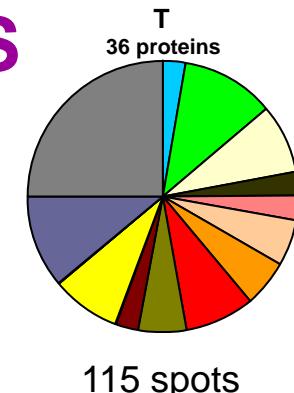
Xyloglucan endotransglycosylase

Biosynthèse des parois

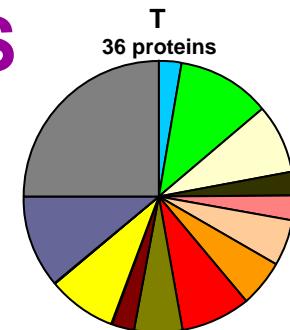


PROTEINES TRAITEMENT-DEPENDANTES

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



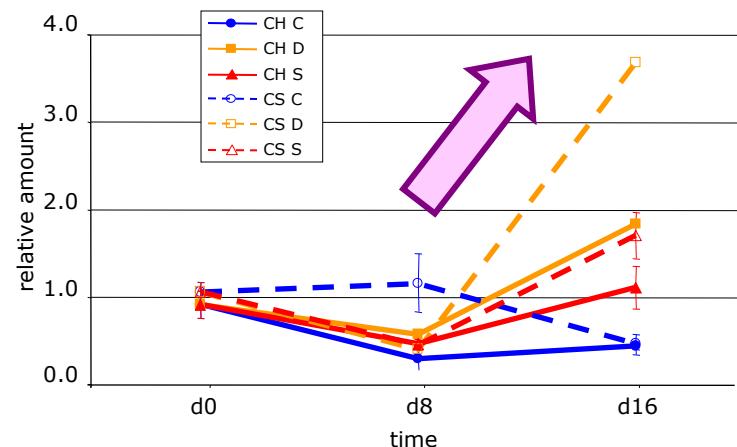
PROTEINES TRAITEMENT-DEPENDANTES



A Spot#82 NMCP1

Nuclear matrix constituent protein 1

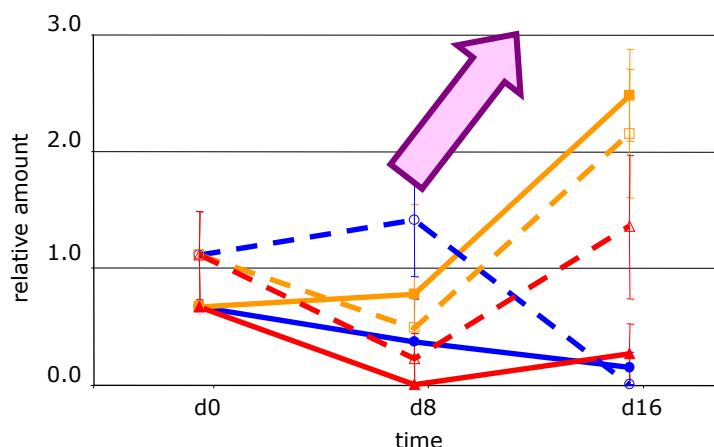
Restructuration de l'enveloppe nucléaire



B Spot#175 RPL39

Ribosomal protein L39

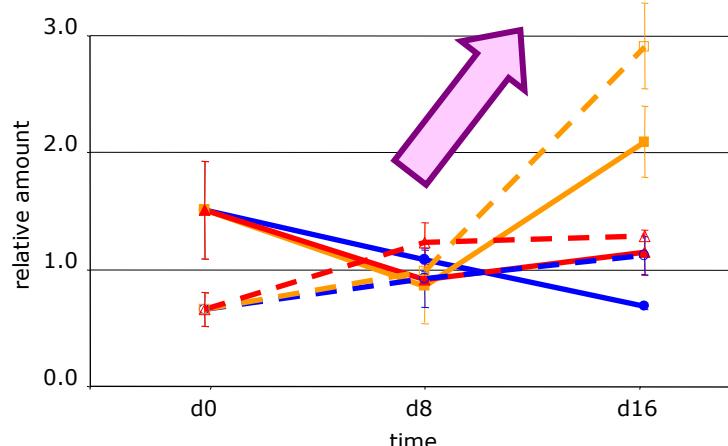
Traduction



C Spot#72 SBPase

Sedoheptulose-1,7-bisphosphatase, chloroplastic

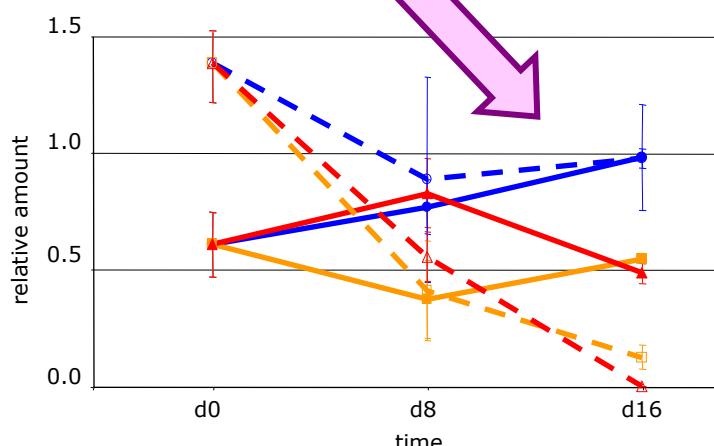
Photosynthèse



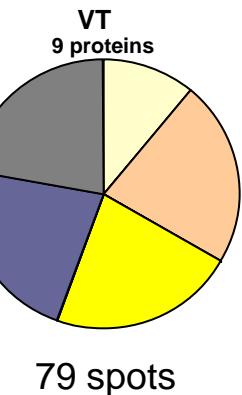
D Spot#145 PSII

Photosystem II

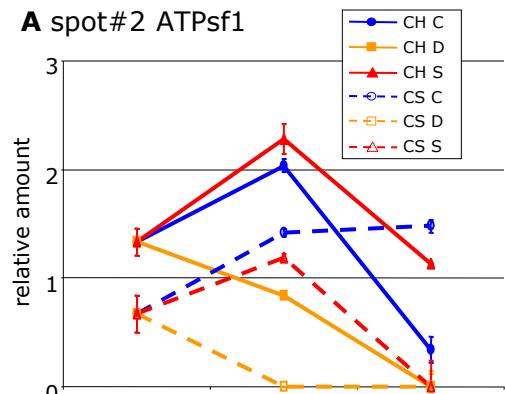
Photosynthèse



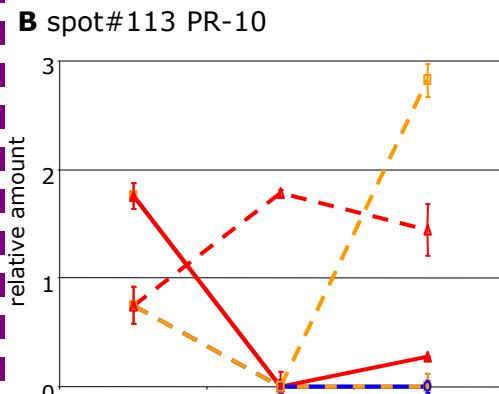
INTERACTION VARIETE-TRAITEMENT



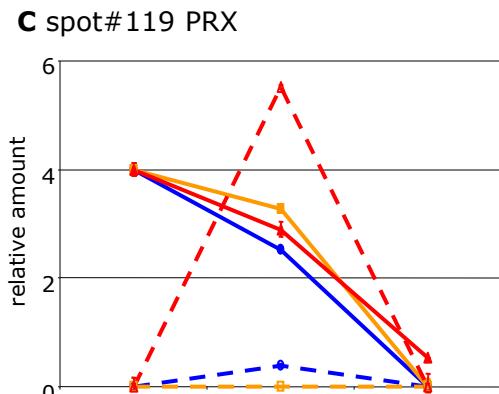
A spot#2 ATPsf1



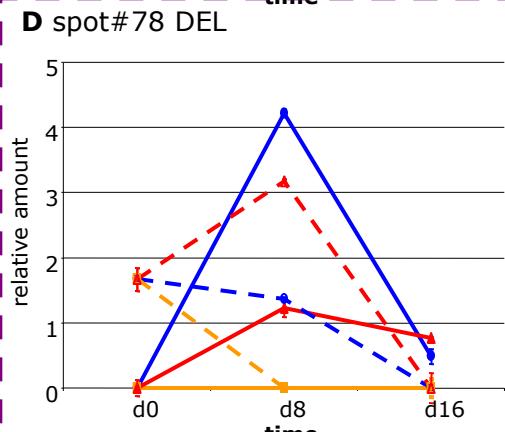
B spot#113 PR-10



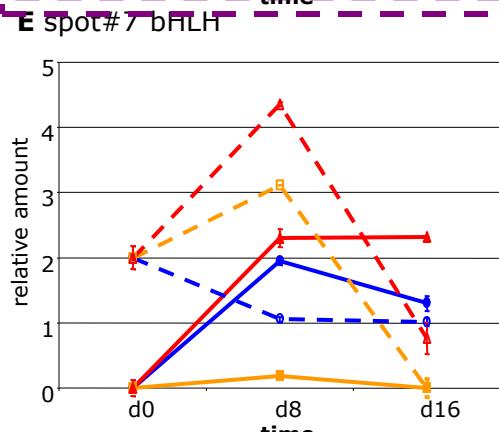
C spot#119 PRX



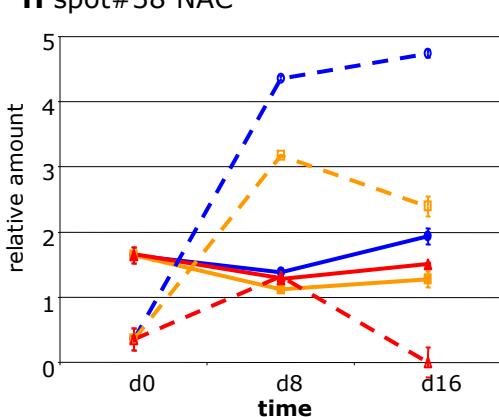
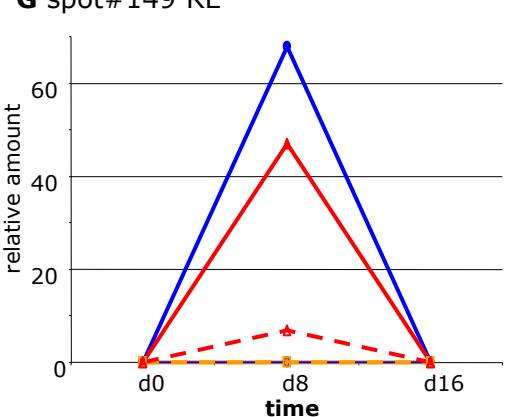
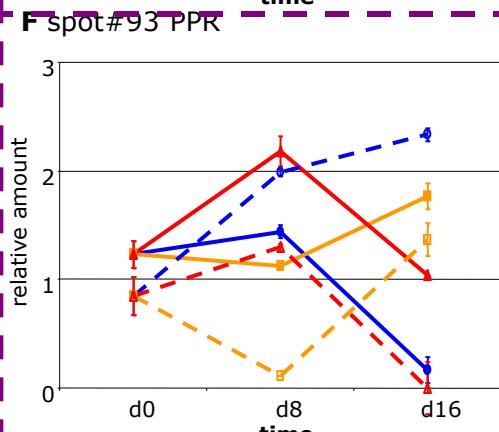
D spot#78 DEL



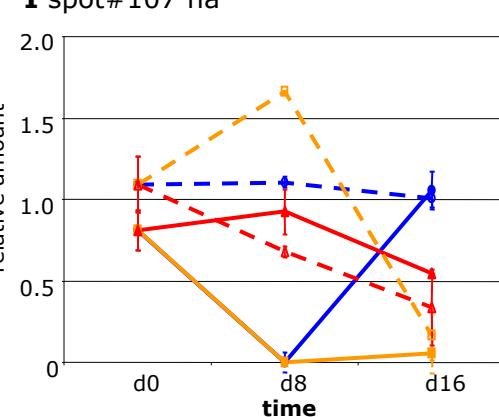
E spot#7 bHLH



F spot#93 PPR



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).



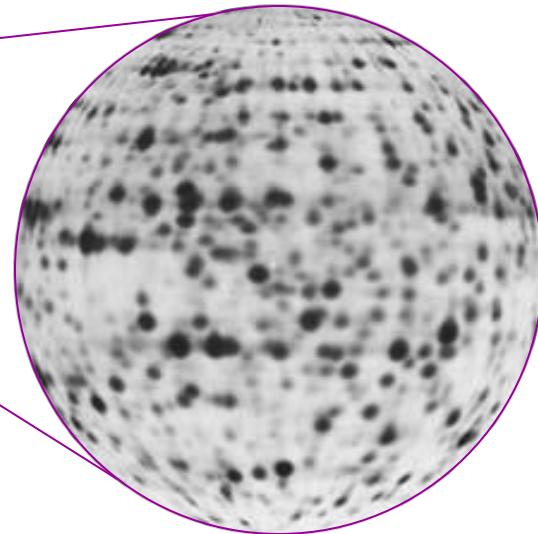
La majorité des protéines surexprimées chez CH en conditions de stress est de fonction inclassable ou inconnue tandis que les protéines surexprimées chez CS agissent dans le métabolisme protéique (transcription/traduction/maturation/degradation) .



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



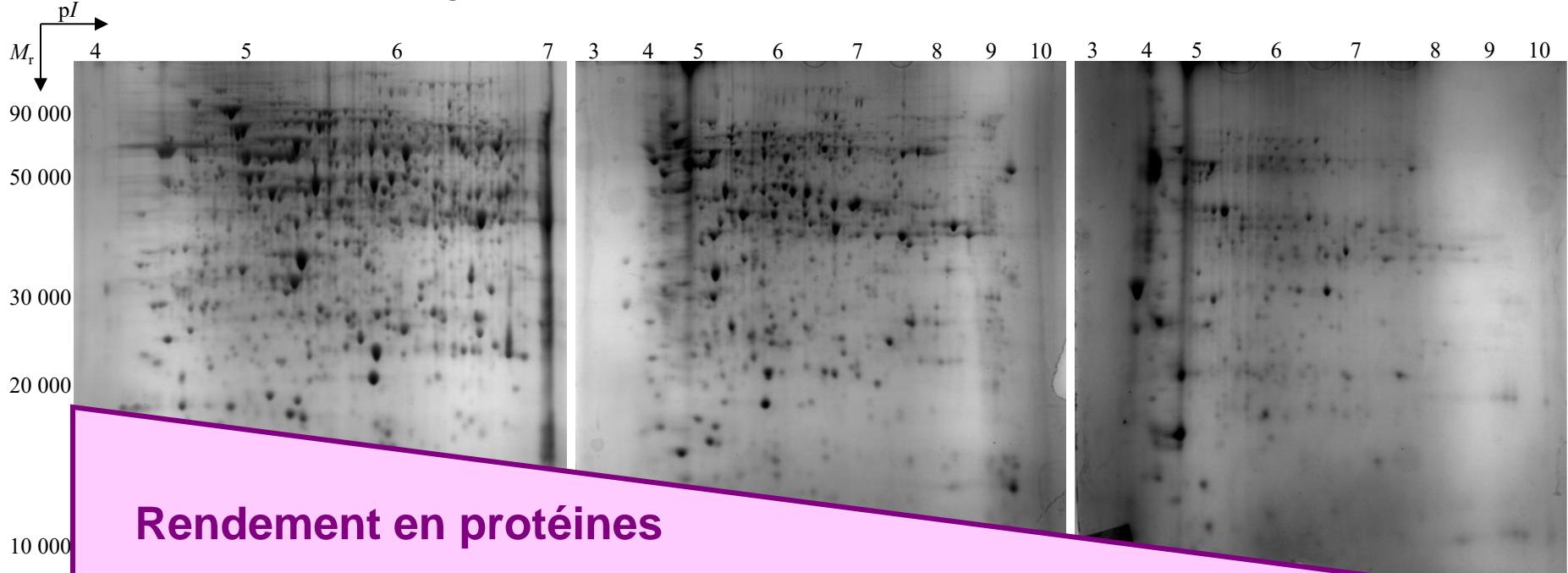
EXTRACTION DE PROTEINES AU TCA/ACETONE



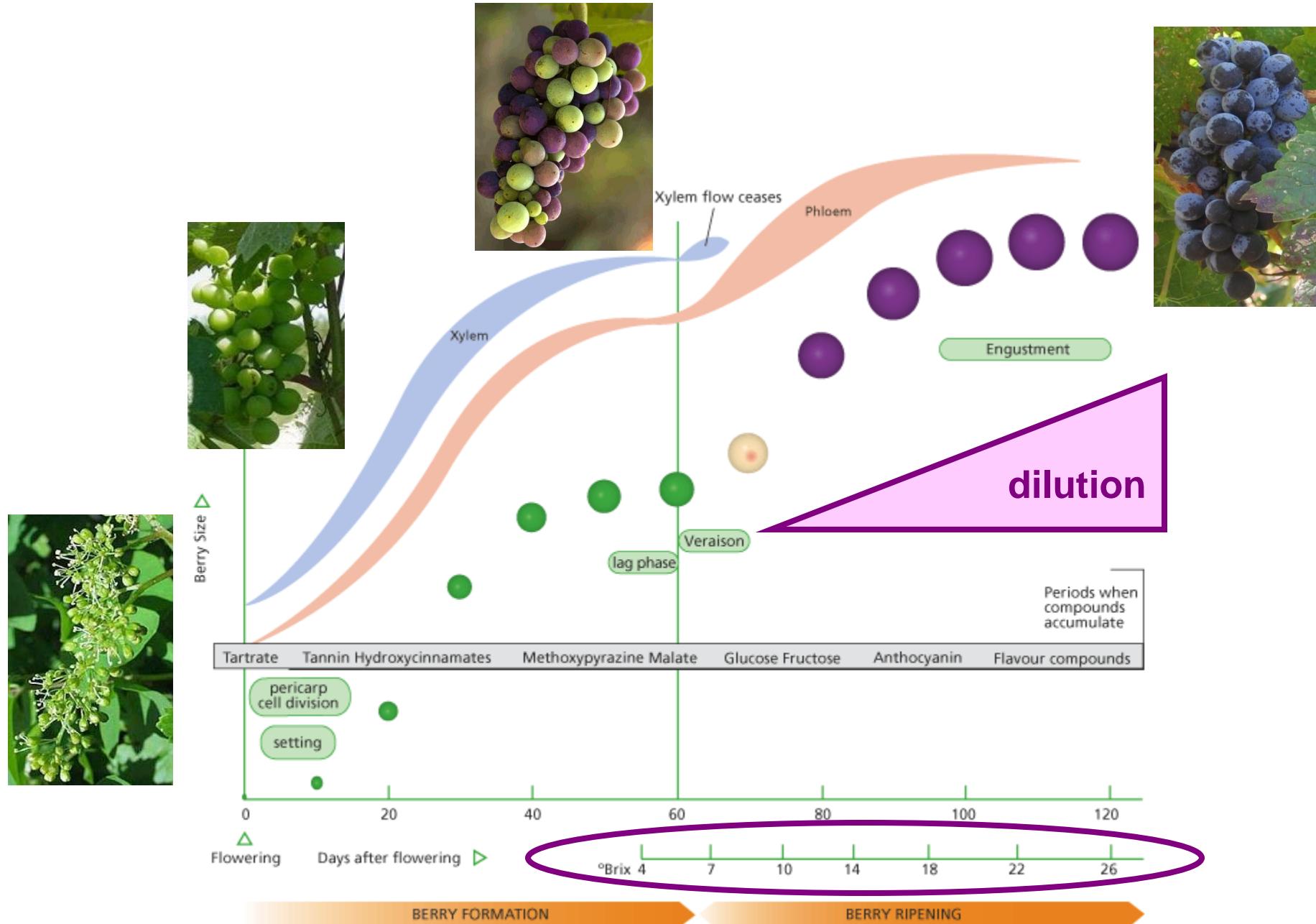
Extrémité de la tige

Grappe immature

Grappe mûre



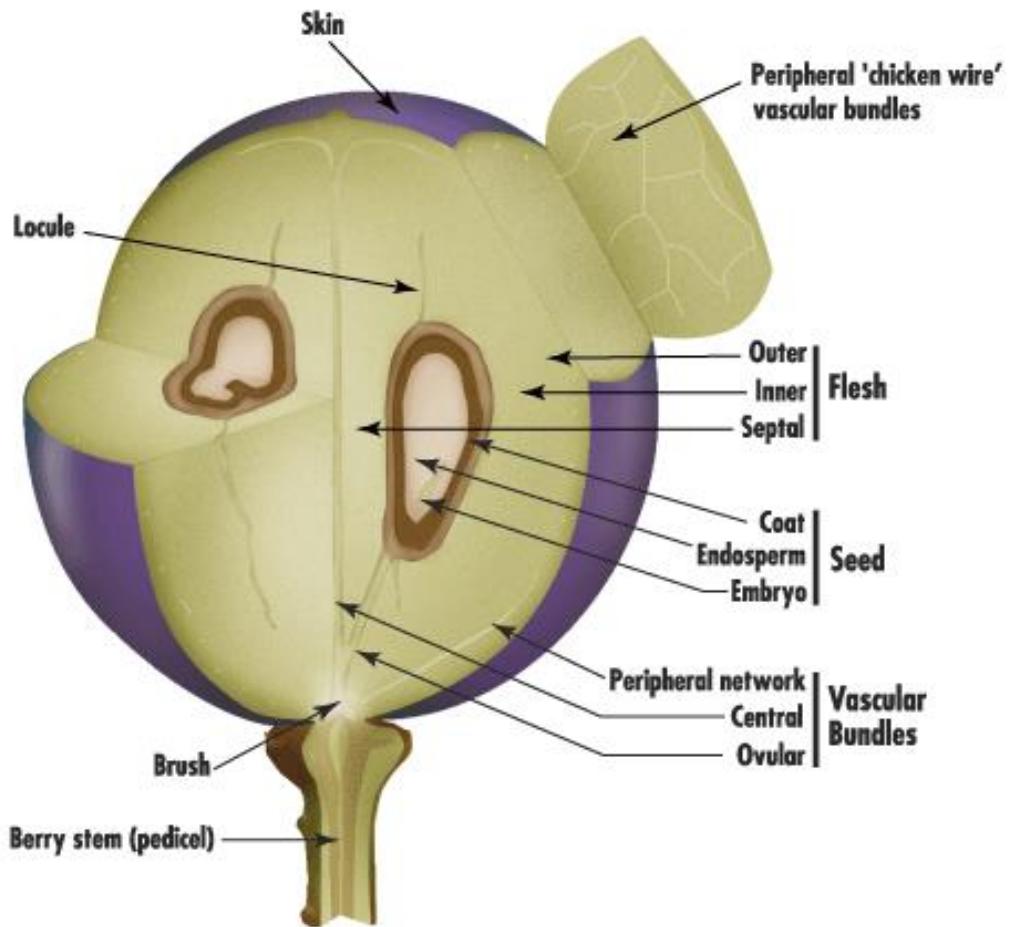
DEVELOPPEMENT DE LA BAIE DE RAISIN



DEVELOPPEMENT DE LA BAIE DE RAISIN

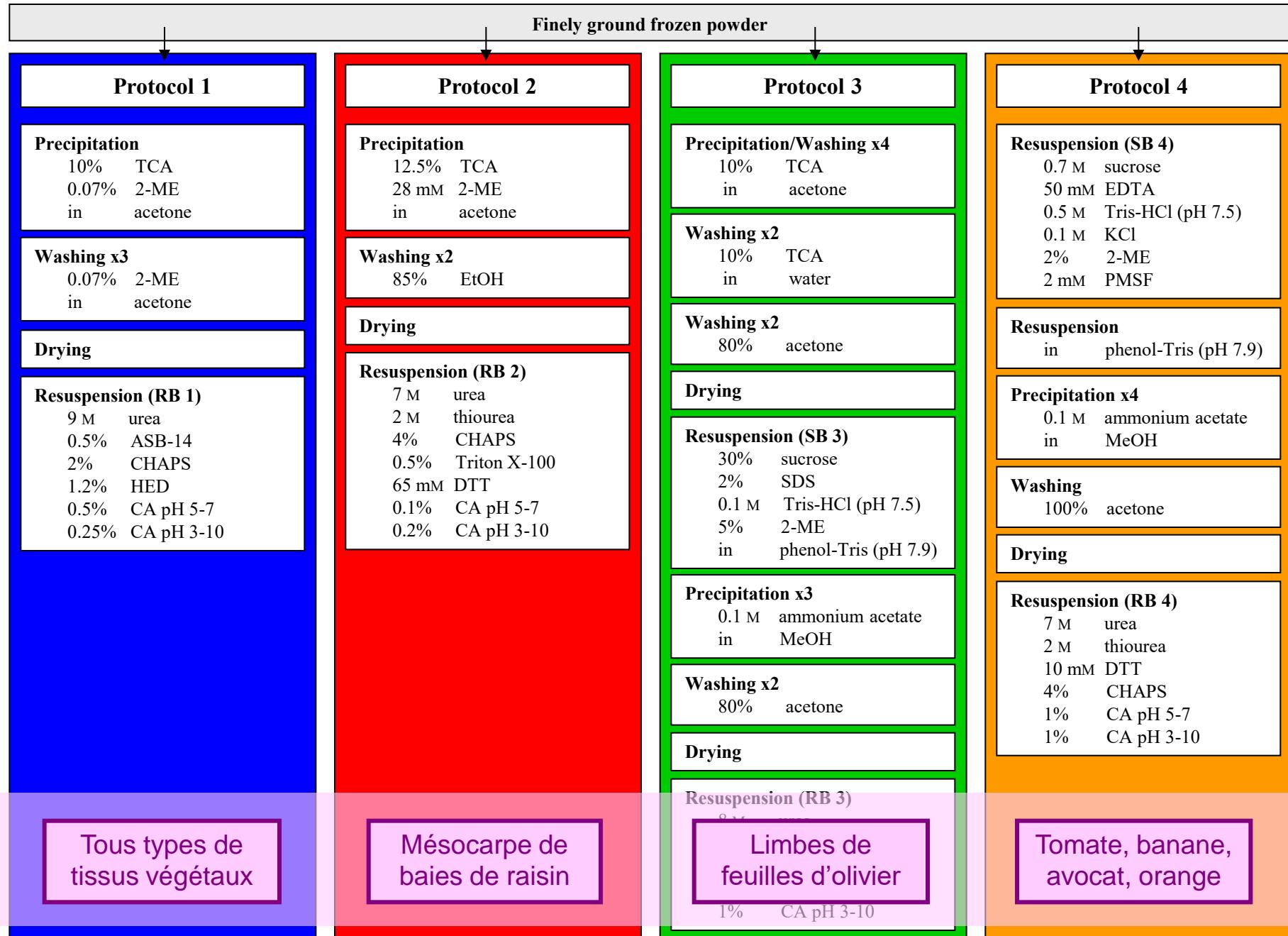
Baie de raisin = organe très spécialisé ⇒ diversité de tissus:

- 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
⇒ Nécessité d'un protocole adapté

PROTOCOLES TESTES: TCA/ACETONE ET PHENOL



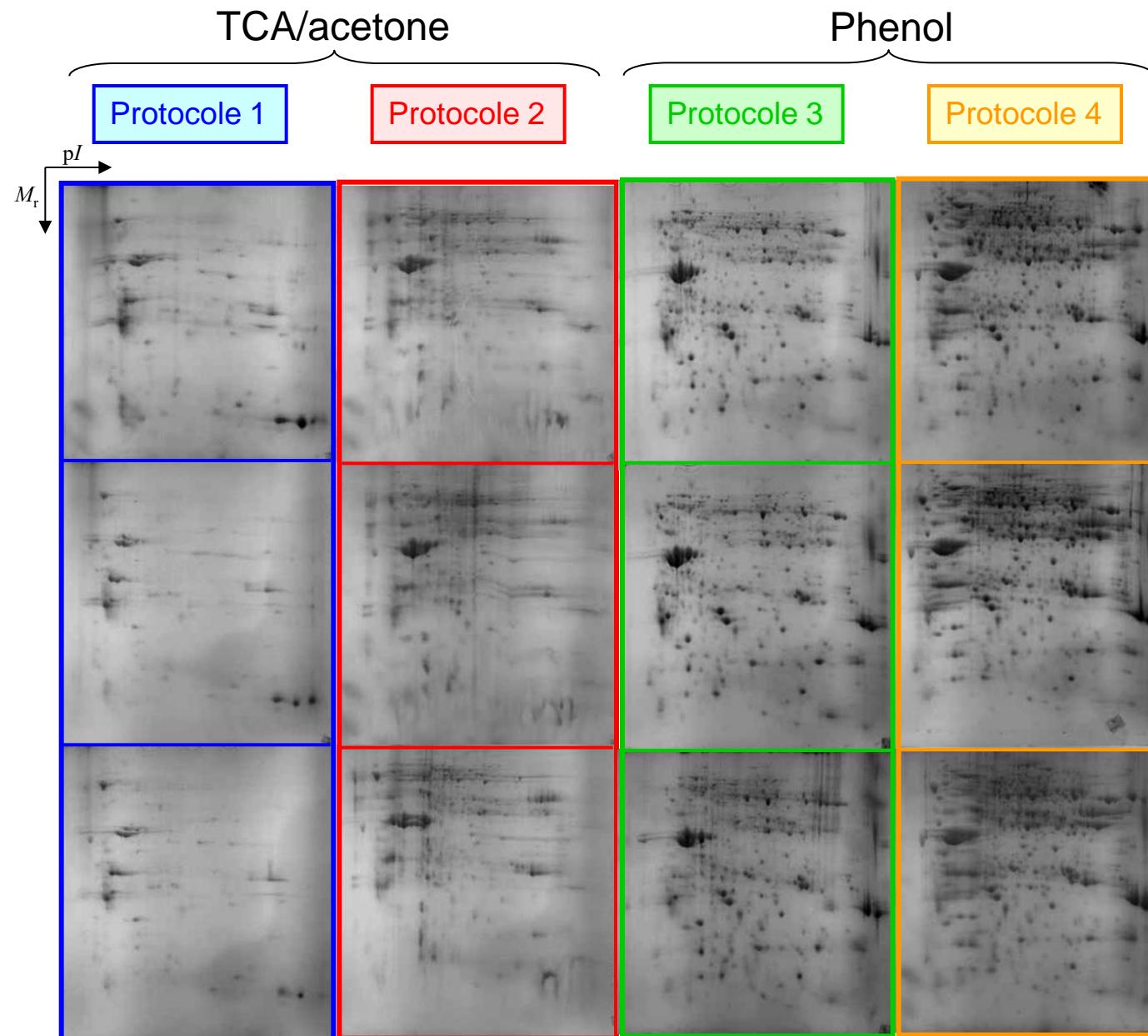
Damerval et al. 1986

Sarry et al. 2003

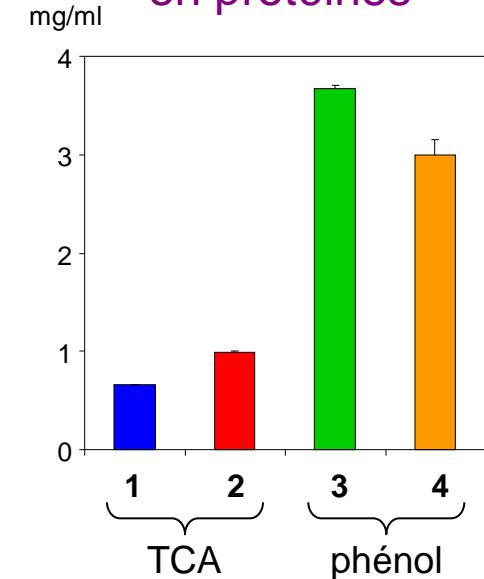
Wang et al. 2004

Saravanan & Rose, 2002

SOLUBILISATION ET RESOLUTION AMELIOREES

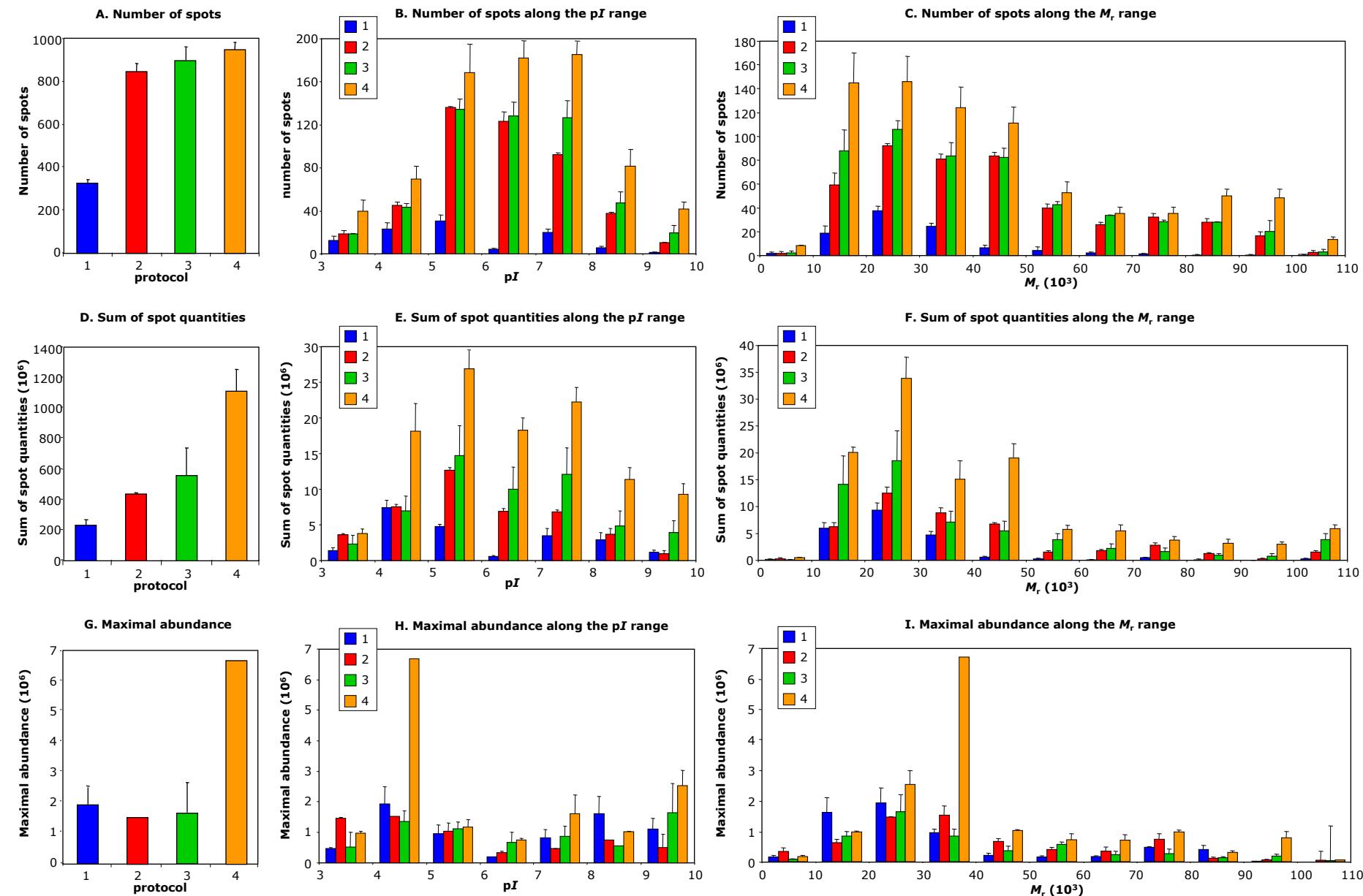


concentration
en protéines

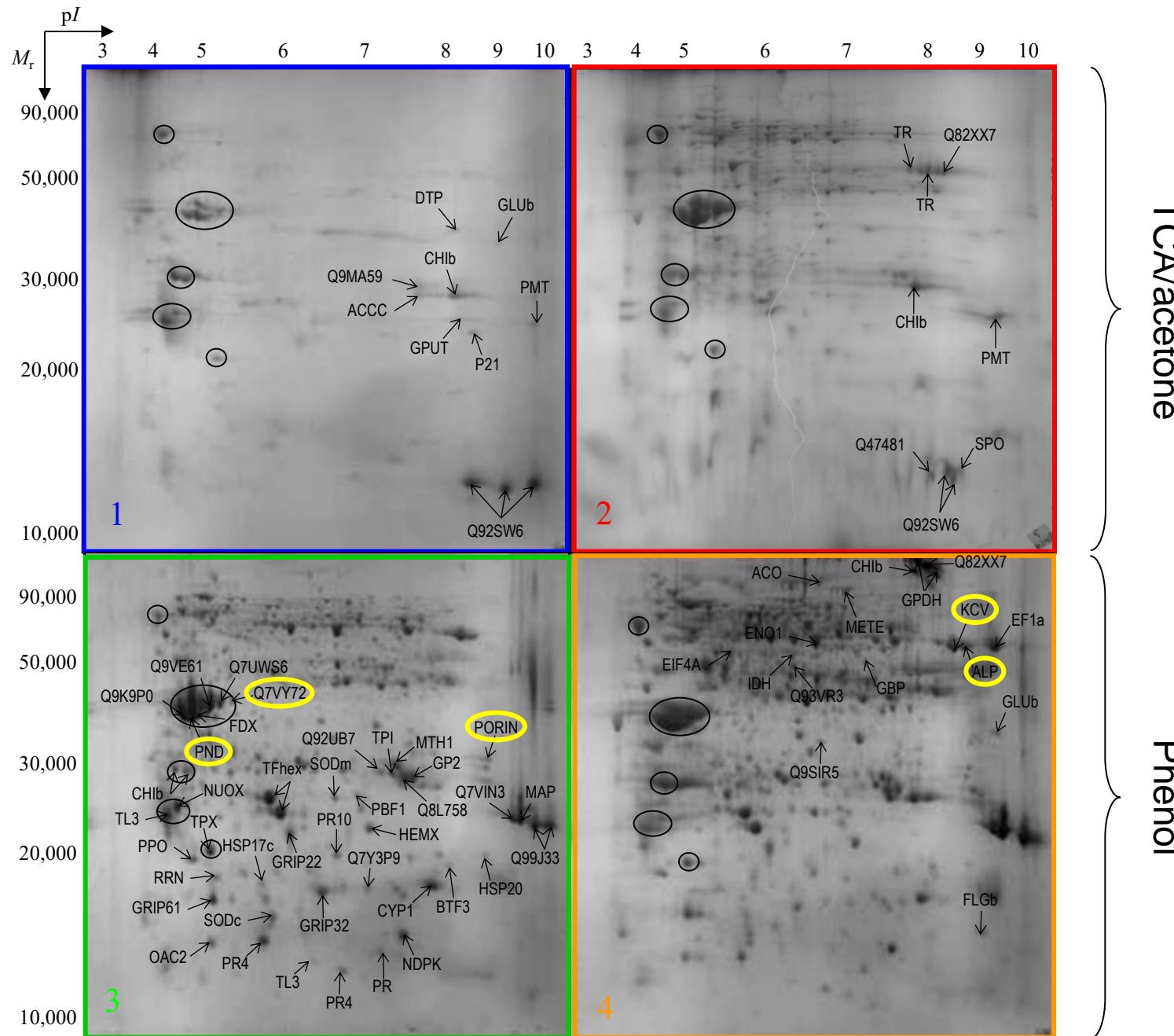


pH 3-10 NL
18 x 18 cm²
12% acrylamide
BCC

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.



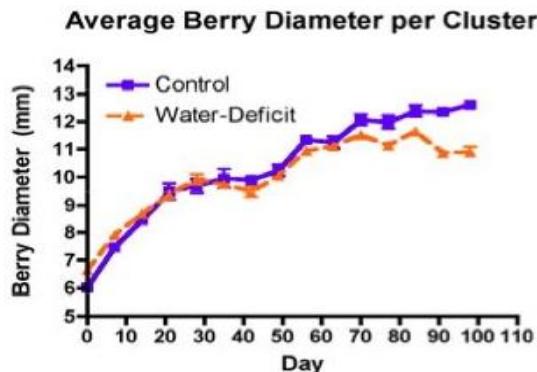
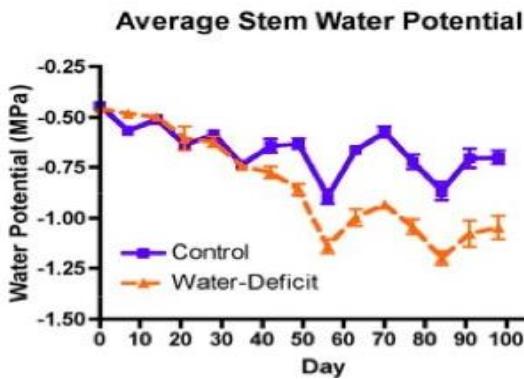
Extraction au phénol mieux adaptée au baies de raisin, riches en sucres et au contenu en protéines dilué. Enrichissement du taux de protéines précipitées \Rightarrow limitation des pertes.



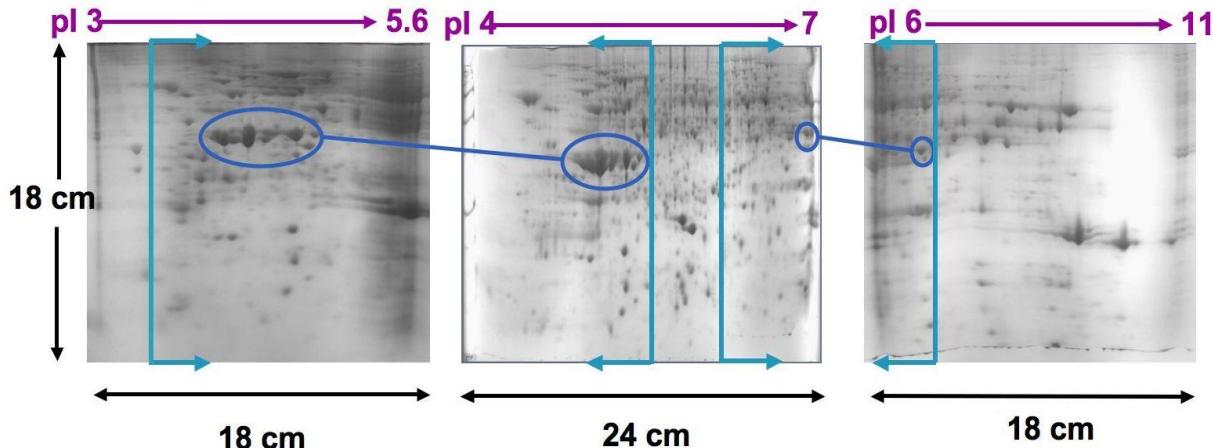
Effet du tampon de resolubilisation. Davantage de protéines précipitées dans le culot sont resolubilisées \Rightarrow limitation des pertes.

L'AVENTURE CONTINUE...

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

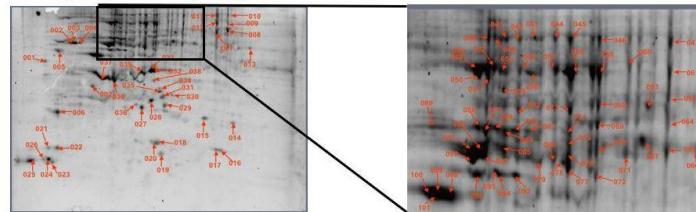


A comprehensive 2D map of the grape berry proteome was constructed using overlapping narrow-range 1st Dimension IPG strips. Approximately 1,000 unique spots have been picked and mass spectra obtained.

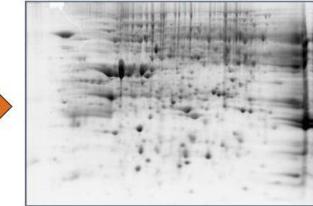


Phosphoproteins and glycoproteins tentatively identified for pl 4-7 using Molecular Probes ProQ Diamond and ProQ Emerald stains, followed by total protein staining with Sypro Ruby.

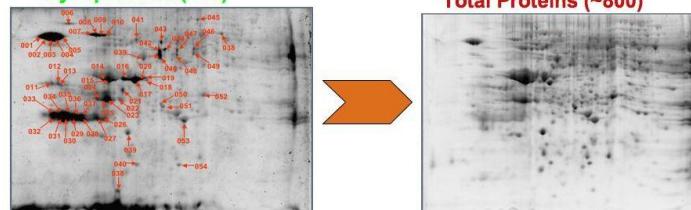
Phosphoproteins (~120)



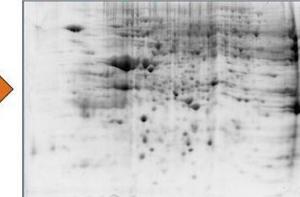
Total Proteins (~800)



Glycoproteins (~60)



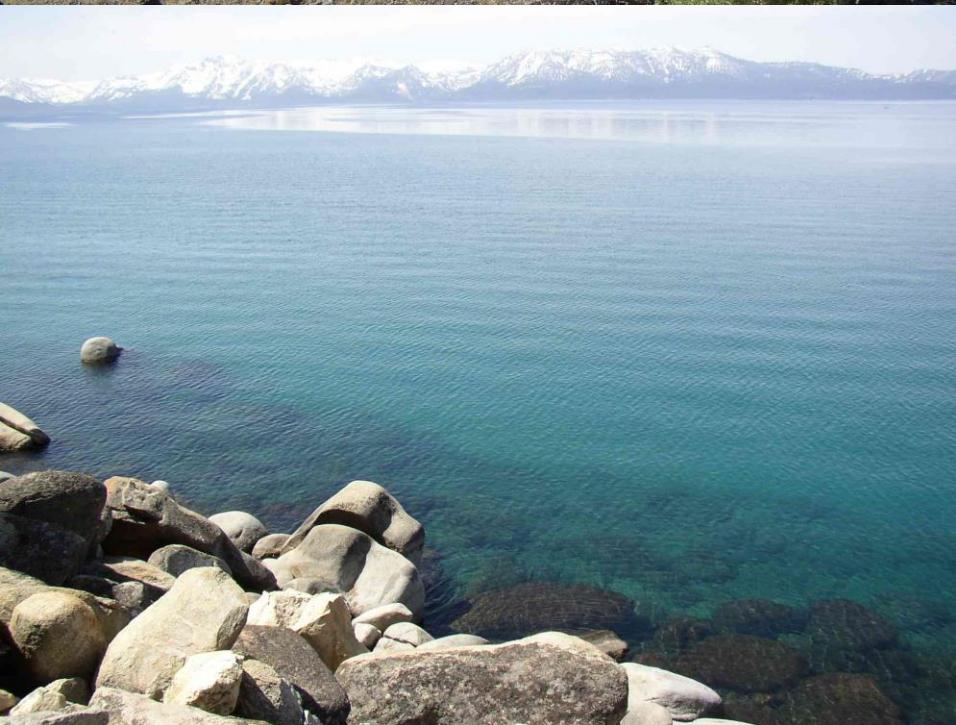
Total Proteins (~800)



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 !