



Grapevine genomics and ripening : on-going worldwide research



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TOPICS

I Grape berry development

II Grape Genome and ESTs

III What is transcriptomic?

IV Transcriptomic approaches

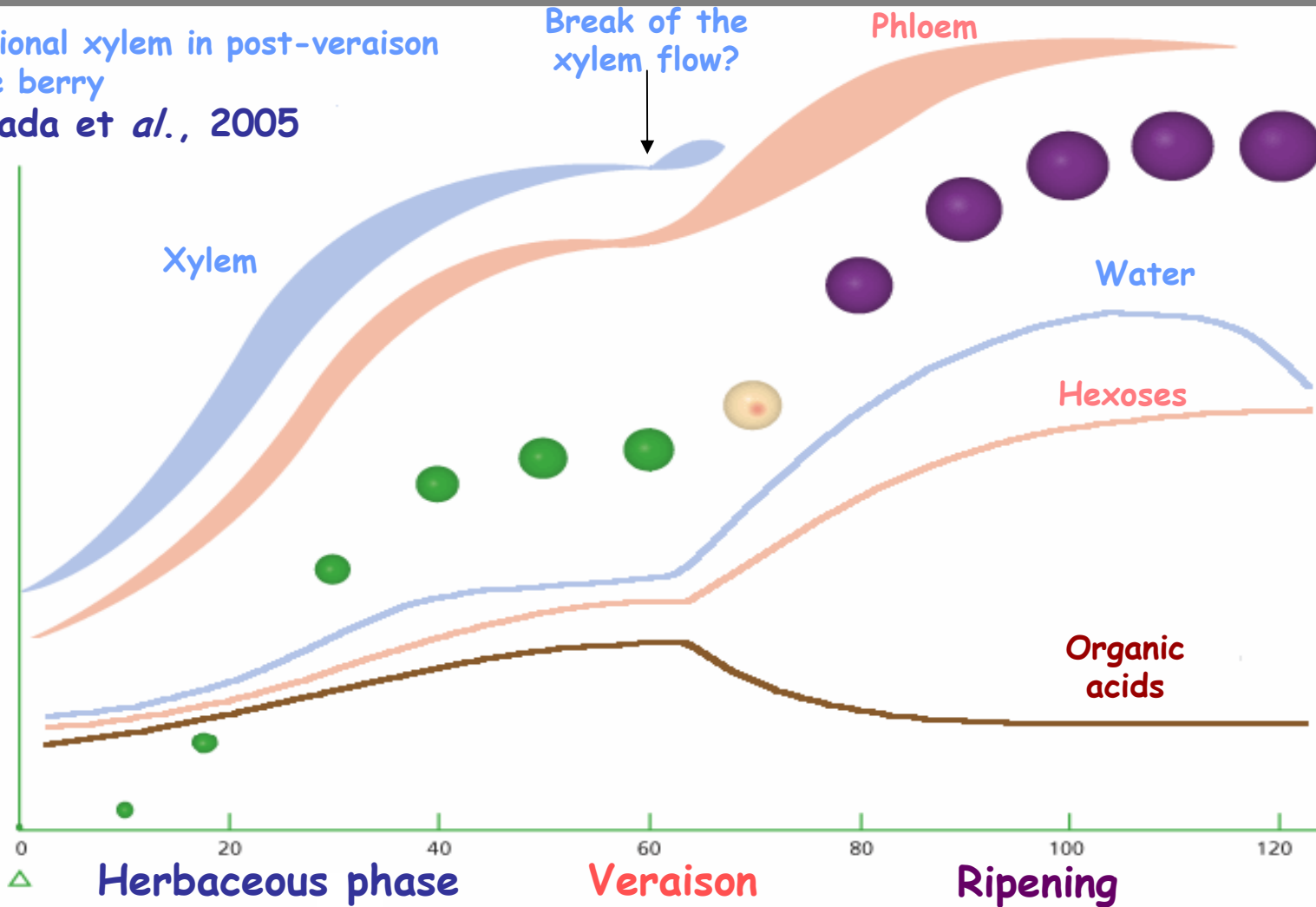
V Grape transcriptomic

- Spotted microarrays
- Vitis Genechip

VI Importance of transcriptomic studies

Grape berry development

Functional xylem in post-veraison
Grape berry
Bondada et al., 2005



Grape Genome 475 Mb ~ 25000 genes

International research program "*Mieux comprendre la Vigne*", based on grape sequencing genome program and exploitation of corresponding data.

Vitis vinifera ESTs

Cultivars	Total ESTs (%)	Plant Organ	Total ESTs (%)	Developmental Stage	Total ESTs (%)	Stress Treatment	Total ESTs (%)
Canernet Sauvignon	63,799 (47)	Berry	48,856 (25.6)	Preveraison	34,751 (40)	Abiotic	51,492
Chardonnay	56,337 (41.3)	Leaf	42,953 (31.7)	Postveraison	17,872 (13.2)	Biotic	3,650 (3.0)
Pinot noir	5,681 (4.2)	Flower	11,326 (8.4)	Veraison	10,689 (8.0)	ND	74,399 (57.0)
Shiraz	7,393 (5.5)	Roots	7,271 (5.4)	Pre-anthesis	6,117 (4.5)		
Regent	1,755 (1.3)	Compound bud	6,112 (4.5)	Anthesis	4,426 (3.3)		
Ugni Blanc	675 (0.5)	Berry without seeds	5,576 (4.1)	Postmeiosis	3,748 (2.8)		
Chasselas	261 (0.2)	Stem	4,760 (3.5)	Bud swelling	1,750 (1.3)		
Total	135601	Petiole	4,491 (3.3)	ND*	56,046 (41.3)		
		Berry pedicle	2,722 (2.0)				
		Shoot tip	557 (0.4)				
		Seeds	478 (0.4)				
		Berry skin	309 (0.2)				

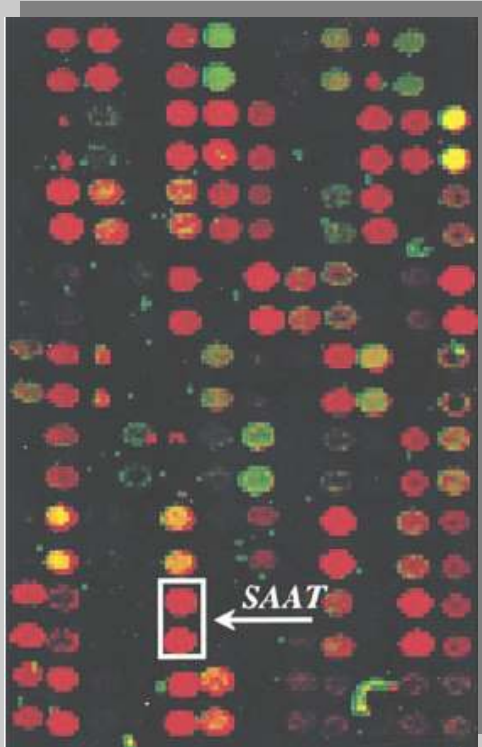
Genbank data
217279 ESTs
15181 Unigenes

From Goes da Silva et al., 2005

Transcriptomic

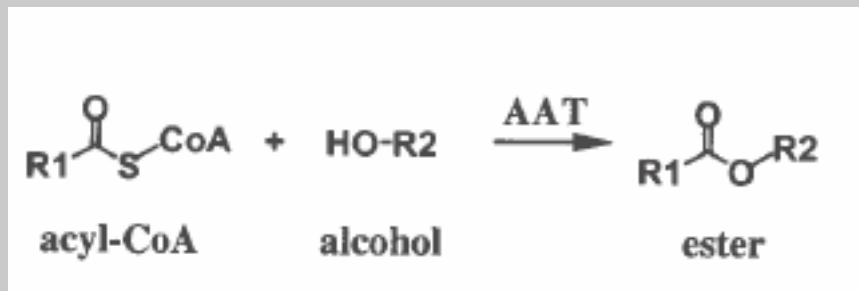
- Study of the transcriptome, ie the complete set of messenger RNA transcripts that can be translated into proteins and enzymes
- Very powerful approach towards understanding gene function
- Can lead to important strategies for plant improvement

Strawberry transcriptomic approach 1



2181 unigenes

SAAT : Strawberry alcohol acyltransferase



→ Identification of a gene involved in flavour components biosynthesis

Strawberry transcriptomic approach 2

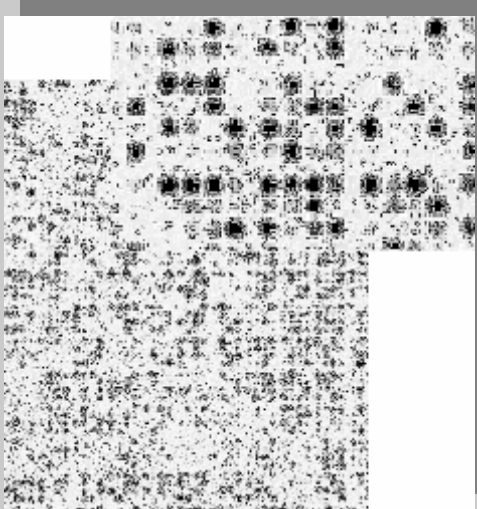
Substrate specificity of the recombinant enzyme

Alcohol	Carbon No.	Ester Formed	Activity ^a	Reported in Strawberry
Methanol	C1:0	Methyl acetate	1.11 ± 0.28	Yes
Ethanol	C2:0	Ethyl acetate	0.87 ± 0.10	Yes
1-Propanol	C3:0	1-Propyl acetate	2.60 ± 0.76	Yes
2-Propanol	C3:0	2-Propyl acetate	1.30 ± 0.15	Yes
1-Butanol	C4:0	1-Butyl acetate	2.29 ± 0.25	Yes
2-Butanol	C4:0	2-Butyl acetate	3.11 ± 0.02	Yes
3-Methyl-1-butanol (isomylalcohol)	C5:0	3-Methyl-1-butyl acetate (isomyl acetate)	3.68 ± 0.25	Yes
1-Hexanol	C6:0	1-Hexyl acetate	2.44 ± 0.37	Yes
cis-2-Hexanol-1-ol	C6:1	cis-2-Hexenyl acetate	0.55 ± 0.55	No
cis-3-Hexanol-1-ol	C6:1	cis-3-Hexenyl acetate	4.55 ± 0.14	Yes
trans-2-Hexanol-1-ol	C6:1	trans-2-Hexenyl acetate	9.20 ± 0.65	Yes
trans-3-Hexanol-1-ol	C6:1	trans-3-Hexenyl acetate	1.25 ± 0.07	No
1-Heptanol	C7:0	Heptyl acetate	14.88 ± 4.12	No
1-Octanol	C8:0	1-Octyl acetate	12.36 ± 2.62	Yes
1-Nonanol	C9:0	1-Nonyl acetate	14.50 ± 0.11	No
1-Decanol	C10:0	1-Decyl acetate	7.78 ± 0.10	Yes
Furfurylalcohol	C5:2	Furfuryl acetate	0.72 ± 0.06	No
Benzylalcohol	C7:3	Benzyl acetate	0.68 ± 0.04	Yes
2-Phenylethylalcohol	C8:3	2-Phenylethyl acetate	1.58 ± 0.12	Yes
Linalcol	C10:2	Linalyl acetate	ND ^a	No

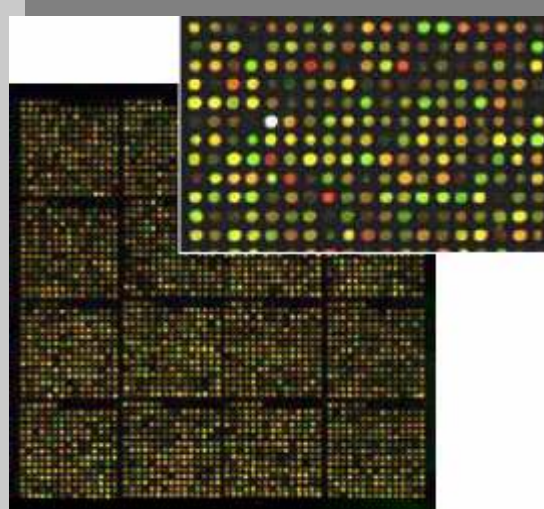
→ The SAAT enzyme shows maximum activity with alcohols whose corresponding esters are the major components of strawberry volatiles

Transcriptomic approaches

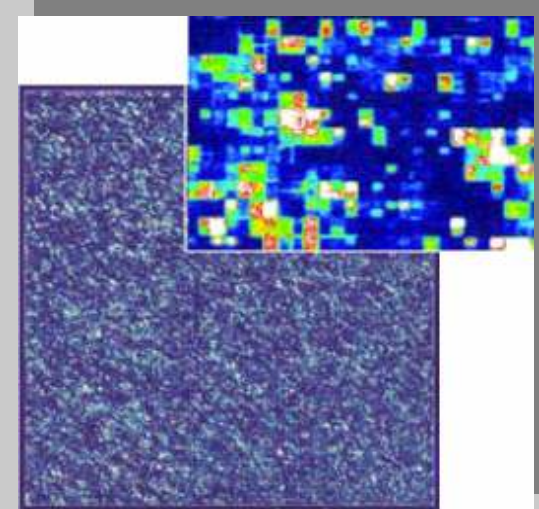
Macroarrays



Spotted microarrays



Gene Chips, Affymetrix



Support

Nylon membrane

Glass slide

Glass chip with chemical coating

Ø spots

0,5-1 mm

100 µm

20 µm

Density

100 to 300/cm²

100 to 1,000/cm²

Up to 25,000/cm²

Probes

PCR products

PCR products or oligonucleotides

Small oligonucleotides synthesised *in situ*

Targets

Radiolabeled cDNA

cDNA or PCR products labeled with fluorophore dyes (Cy3/Cy5)

ARNc or PCR products labeled with fluorophore dyes(Biotine/Streptavidine)

Overview of microarray chips

The GeneChip® Vitis Array
16,600 unigenes

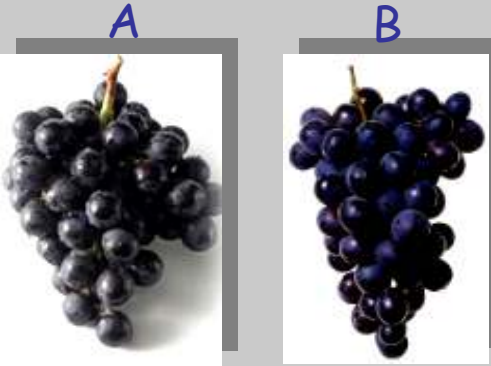


Spotted microarray

Glass slides with
14,562 unigenes



Using spotted microarrays



Specific oligo synthesis



Spotter



INRA de Montpellier

RNA extraction

Differential labeling
Cy3 Cy5

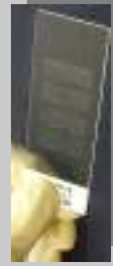
Co-hybridization



Hybridization station

Analysis

Spotted microarrays analysis



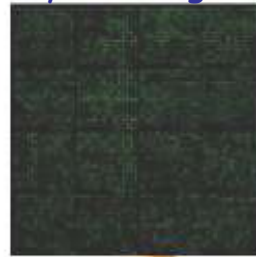
scanner



Cye 5 image



Cye 3 image



Data analysis

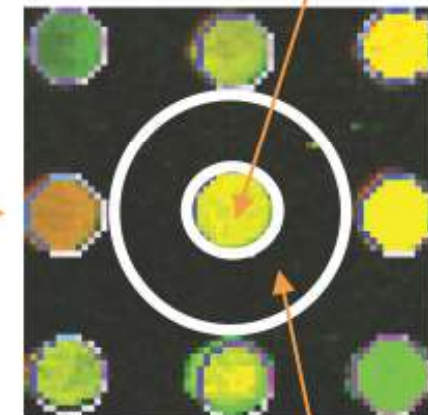


Cy5>Cy3

Cy5=Cy3

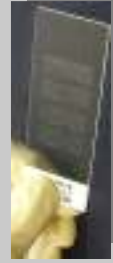
Cy5<Cy3

Area for signal intensity calculation



Area for local background calculation

Grape berry transcriptomic approaches 1



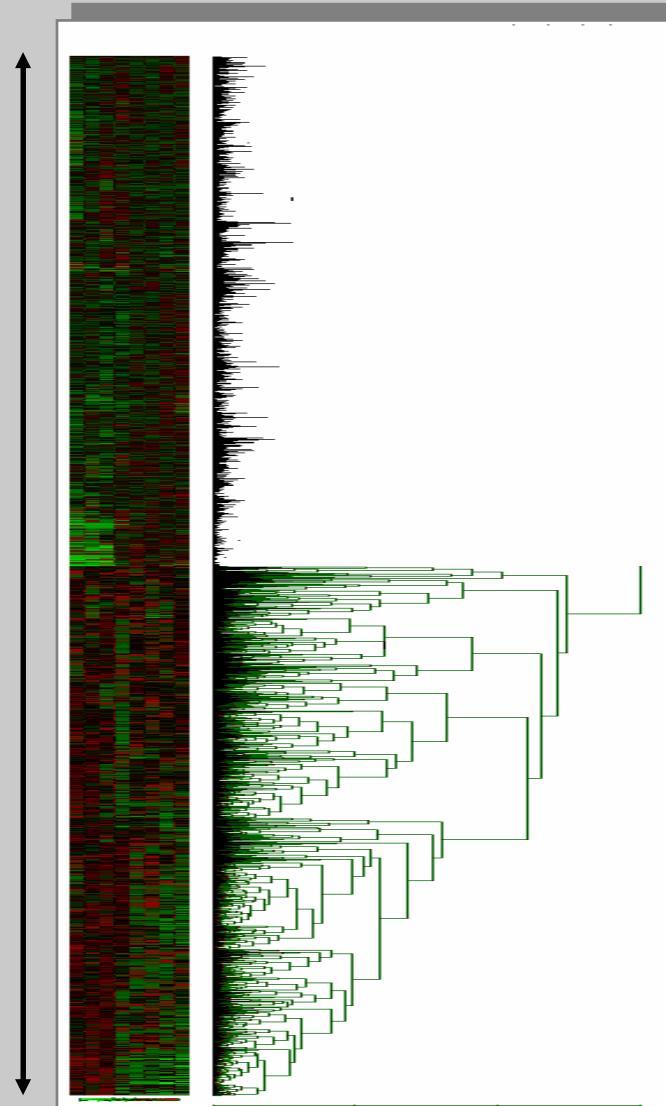
X=8 berry developmental stages



Regrouping of expression profiles
by hierarchical clustering

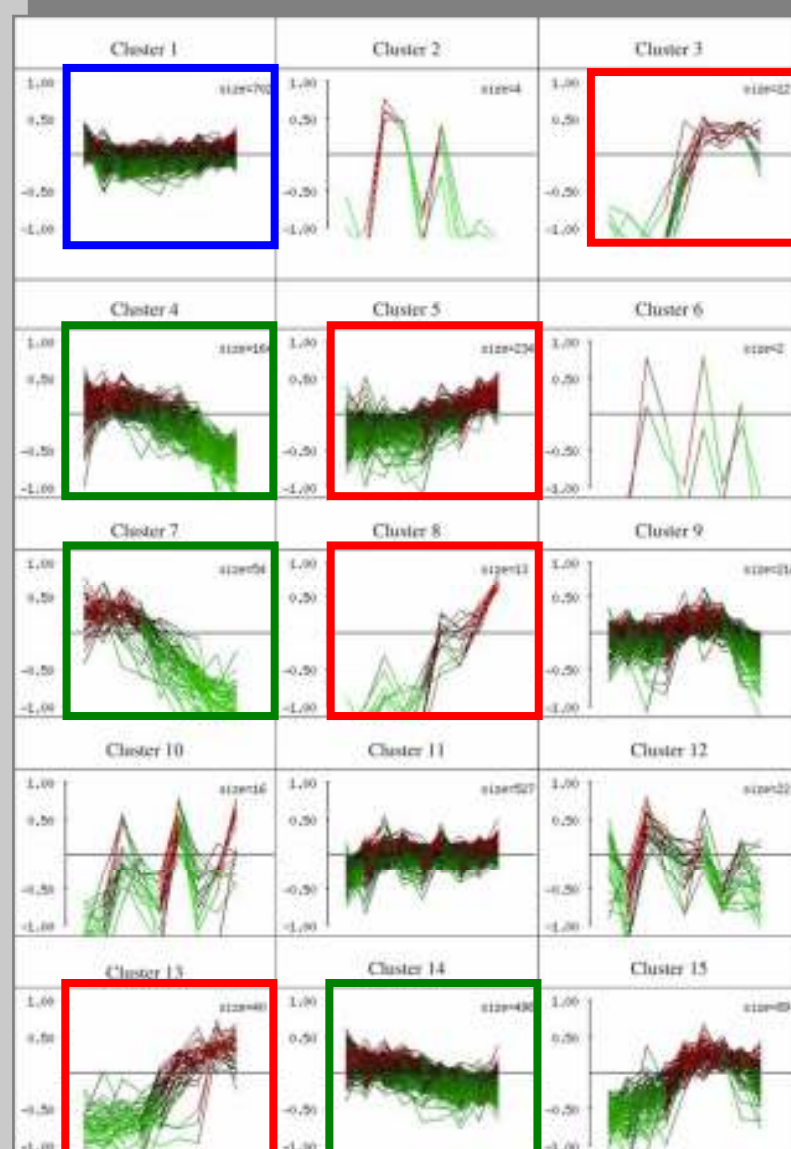
Y=3021 unigenes

Differential expression



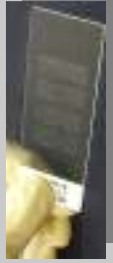
Chardonnay
cultivar

Organization of the genes according to their expression pattern





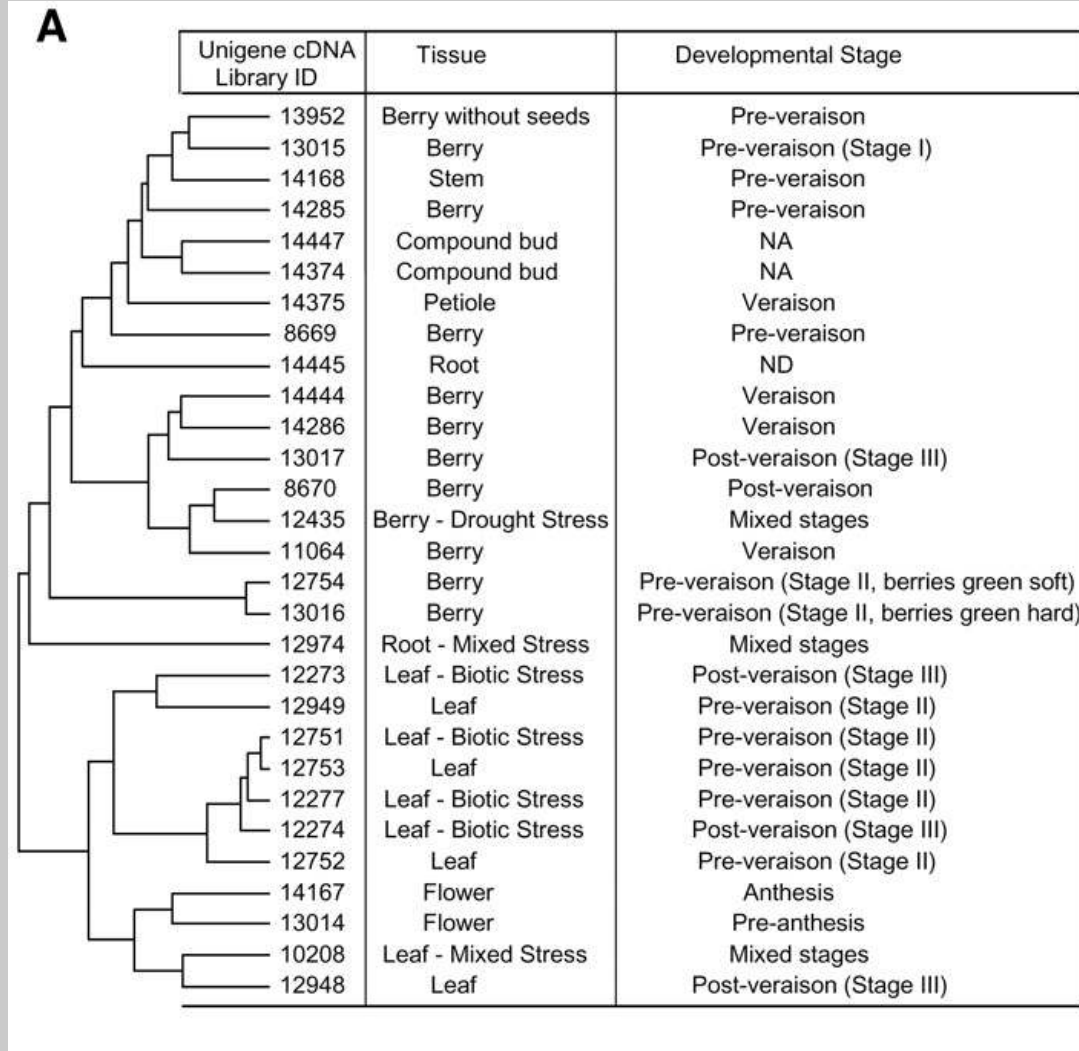
Transcriptomic : On-going research



- UMR 6161, Univ. Bdx1, INRA de Montpellier, CIVC, ITV, ADAR
Identification of genes involved in grape berry maturity (Cabernet franc, Cabernet Sauvignon et Pinot meunier).
- UMR 6161, Univ. Bdx2, Univ. Vérone
Transcriptomic of berries submitted to ABA treatment.

Grape berry transcriptomic approaches 1

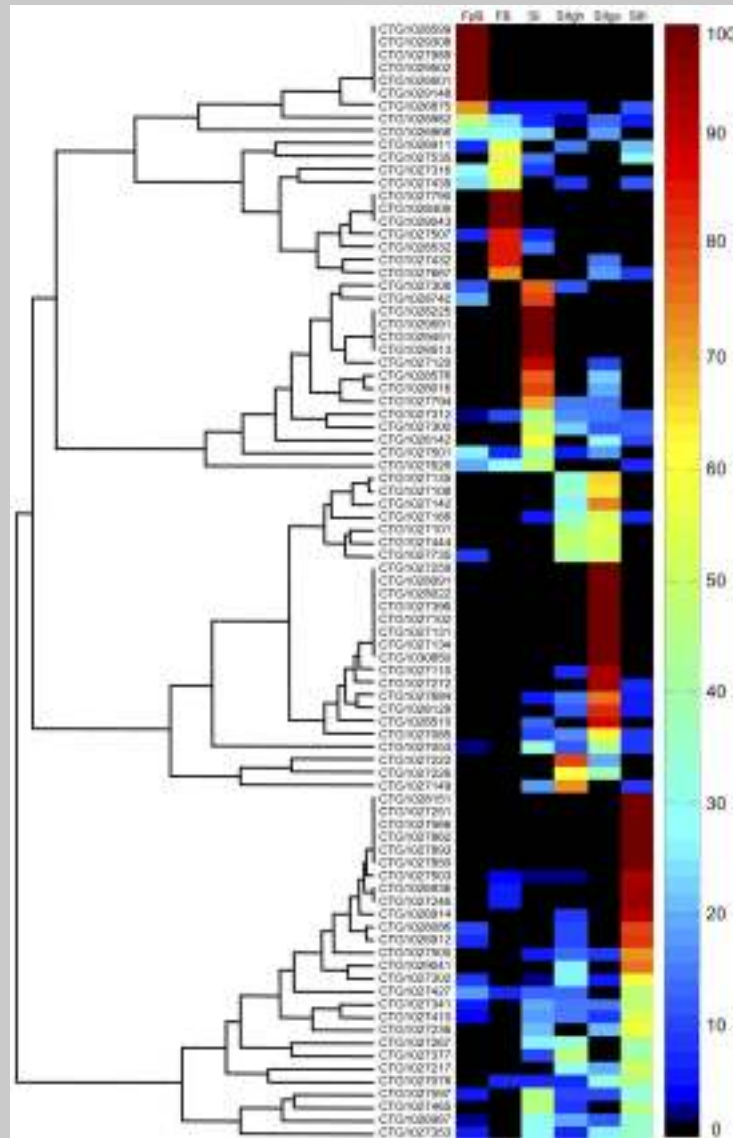
In silico characterization of the Grape Transcriptome



29 cDNA libraries (> 1000 ESTs)

Analysis based on the ESTs distribution of 665 TCs differentially expressed.

In silico characterization of 87 genes differentially expressed during grape berry development



X = 6 cDNA libraries corresponding to the major grape berry developmental stages

Analysis based on the ESTs distribution of 87 genes differentially expressed

8 clusters

- Pathogenesis related
- Abiotic stress
- Primary metabolism
- Secondary metabolism
- Berry growth and water relations
- Ethylen metabolism
- Seed specific proteins
- Other proteins

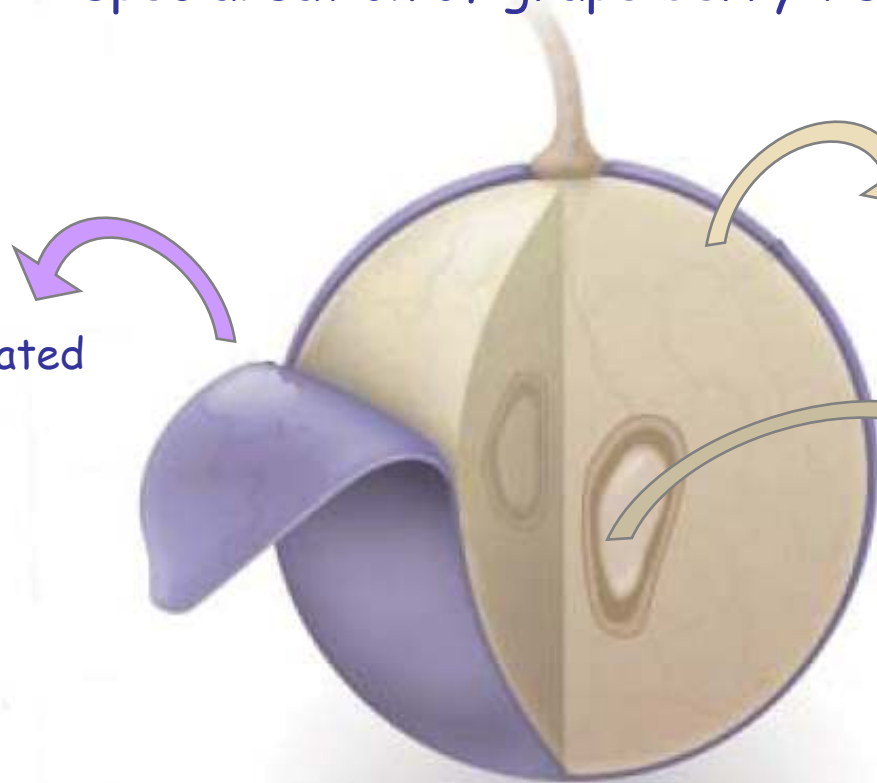
Tissue-specific mRNA expression patterns within grape berries and other tissues

Nevada University (Grant R. Cramer and John C. Cushman)



Increase in transcripts abundance correlated with specialisation of grape berry tissues

- Pathogen related
- Cell wall modification
- Flavonoids biosynthesis



- Cell wall
- Transport processes

- Phenylpropanoid synthesis
- Storage protein
- Embryogenesis proteins

Transcriptomic : On-going research (Grant R. Cramer and John C. Cushman)



•Water deficit response

➔ Increase in transcript expression of

- *Genes involved in water and ions transport*
- *Genes involved in Phenylpropanoid biosynthesis*
- *Genes involved in stress response*

•Study of berry development for two different cultivars, Cabernet Sauvignon and Chardonnay

➔ Between 27 to 34% of the 14,650 gene probes represented on the microarray show differential expression across berry development in both cultivars.

- *Transcript abundance for the phenylpropanoid pathway (affecting berry phenolics and color) was significantly different between the two cultivars and affected by water stress.*

Transcriptomic : On-going research (Peter Hayes and Mark R. Thomas)

•Berry development of Cabernet sauvignon over 3 seasons grown in 3 different regions that range from mild to hot growing conditions (Clare, Willunga and Mildura).

+ normal irrigation and partial deficit irrigation

Thousands of genes changed their level of expression at veraison as expected, but also in both Pre and Post-veraison stages.

➡ Identification of key genes involved in berry development

Brassinosteroids (Plant steroidal Hormones) have a role in berry development

➡ Transcript accumulation patterns consistent with increase in BR levels at the onset of ripening

Application of BRs to grape berries promote ripening

Transcriptomic interests

- Analysis of transcriptional regulation of genes associated to grape-berry quality

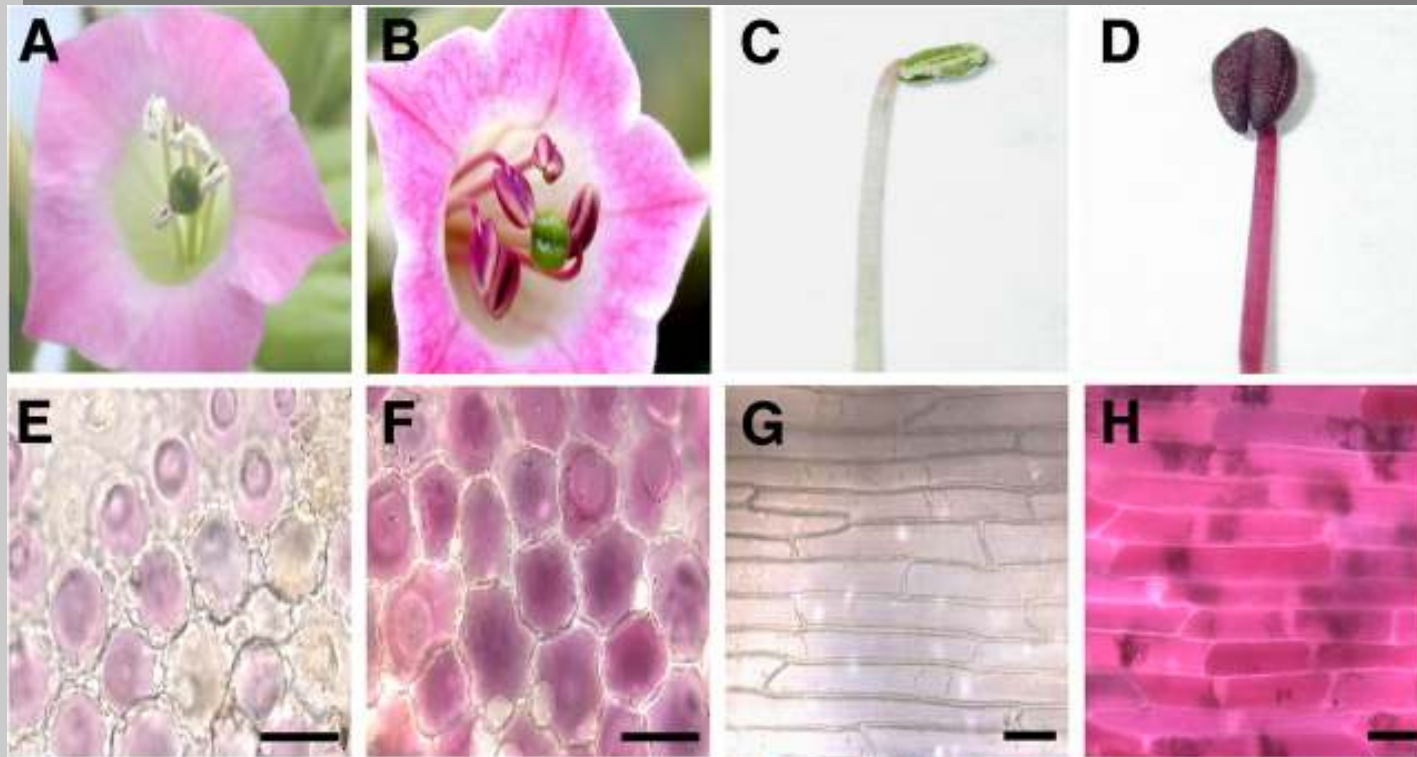
➔ Sugar, organic acids and flavonoid metabolism...

- Identification of genes involved in response to biotic and abiotic stress :

➔ Pathogenesis resistance, saline, water, cold stress....

Functional characterization of related genes

Accumulation of phenolic compounds in tobacco
overexpressing the *Vvmyb5* transcription factor



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