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Grapevine genome and beyond

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To cite this version:

Anne-Francoise Adam-Blondon. Grapevine genome and beyond. 3. International Conference on Omics and Biotechnology in Fruit crops, Oct 2012, Nanjing, China. hal-02803277

HAL Id: hal-02803277

https://hal.inrae.fr/hal-02803277v1

Submitted on 5 Jun 2020

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The Grapevine Genome and Beyond

B. Londinsky

A-F Adam-Blondon

Unit of research on Plant Genomics (URGV)

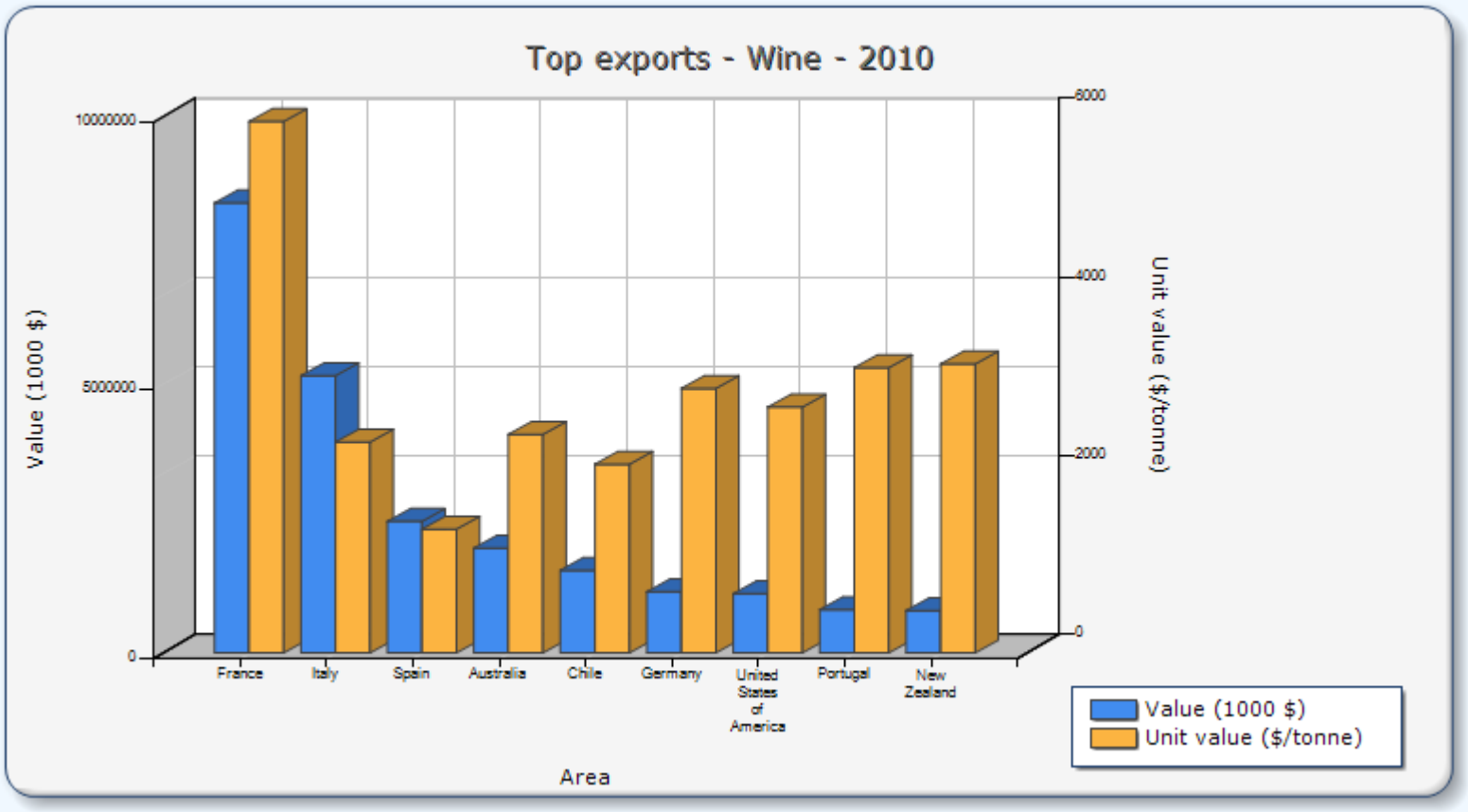
Unit of research on Genomics-informatics (URGI)

3rd Int Conf on Omics and Biotech in Fruit crops, Nanjing, 2012 October 28th

Grapevine is a crop with a high economic value

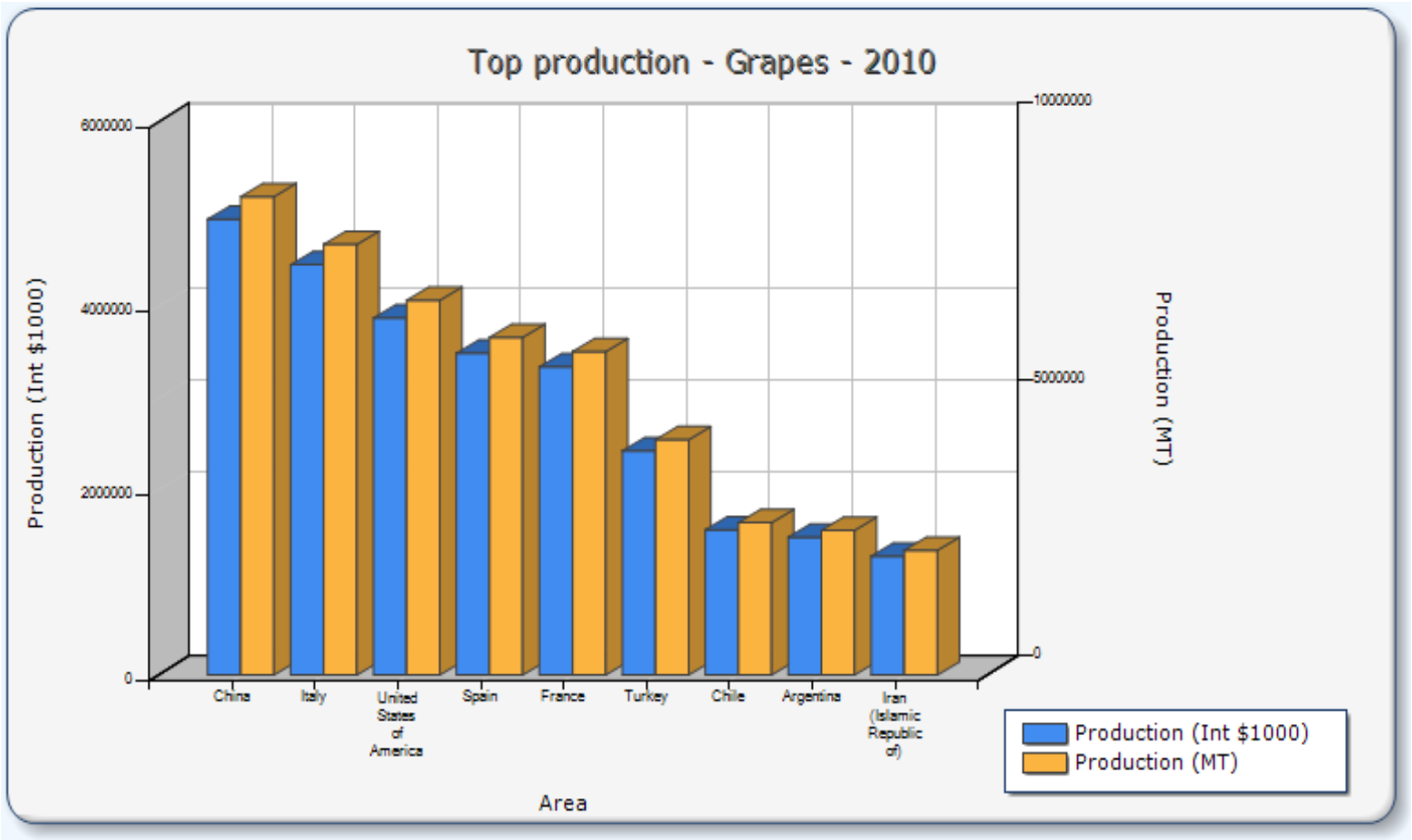
- 27.9 billion $ value for worldwide wine exports (8.4 billon $ for France) in 2010
- 4,601 million $ for worldwide grape and grape juices exports in 2006

http://faostat.fao.org/



Grapevine is a crop with a high economic value

- Grape producers in all continents

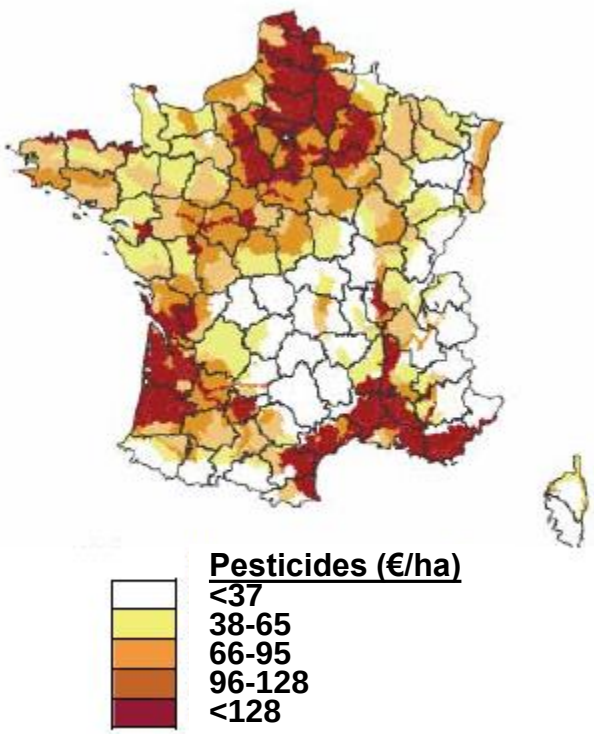


- Grapevine is adapted to poor soils and low water availability

Viticulture in Europe faces major challenges
1- Decrease the use of pesticides

- The viticulture has to reduce its use in pesticides because of:
  - Citizen awarness of fungicide effects on health and environment
  - EU policies aiming at reducing the sources of water pollution
  - State policies aiming at reducing the environment and health risks

- In France (2006 studies: « Ecophyto R&D », « Pesticides »):
  - France was the third worldwide consumer of pesticides even though a decrease has been observed since 2001.
  - 14% the pesticide value (20% of active compounds) was used in vineyards although they represent 3% of the cultivated surface
  - 13 to 21 treatments/years, from which 10 to 18 were fungicide applications (control of mildews and botrytis)



Viticulture in Europe faces major challenges
1- Decrease the use of pesticides

- High variability of practices for disease control among the vineyards:
  - possibility to spead the best one
  - Possibility to develop better methods to survey and control epidemics
- Improvement of the machines used for spraying: only 40-60% of the sprayed products end on leaves
- Development of highly qualitative resistant varieties:
  - Construction of durable resistance while maintaining high quality
- Combine everything to increase the security of the producers and the durability of the resistances

Viticulture in Europe faces major challenges
1- Decrease the use of pesticides

Construction of durable resistances

Phenotyping for disease resistance

Genomic tools for the study of the evolution of the pathogen populations

Marker Assisted Breeding at a genomic scale

Diverse phenotyping

Identification of sources of resistance and characterisation of their effect on pathogens

Run1, Ren1, Rpv1 to 8, Pdr1a, Pdr1b, ..

Assessment of durabity

Brewer&Milgroom 2010
Peressotti et al 2010

Pyramiding into a *Vitis vinifera* background in order to construct a durable resistance

The combinations of quantitative and qulitative resistances are more difficult to overcome by the pathogen (**Palloix et al New Phytol 2009**)

Breeding for quality, adaptation to environment

Viticulture in Europe faces major challenges
1- Decrease the use of pesticides

Construction of durable resistances

Phenotyping for disease resistance

Genomic tools for the study of the evolution of the pathogen populations

Marker Assisted Breeding at a genomic scale

Diverse phenotyping

Identification of sources of resistance and characterisation of their effect on pathogens

Pyramiding into a *Vitis vinifera* background in order to construct a durable resistance

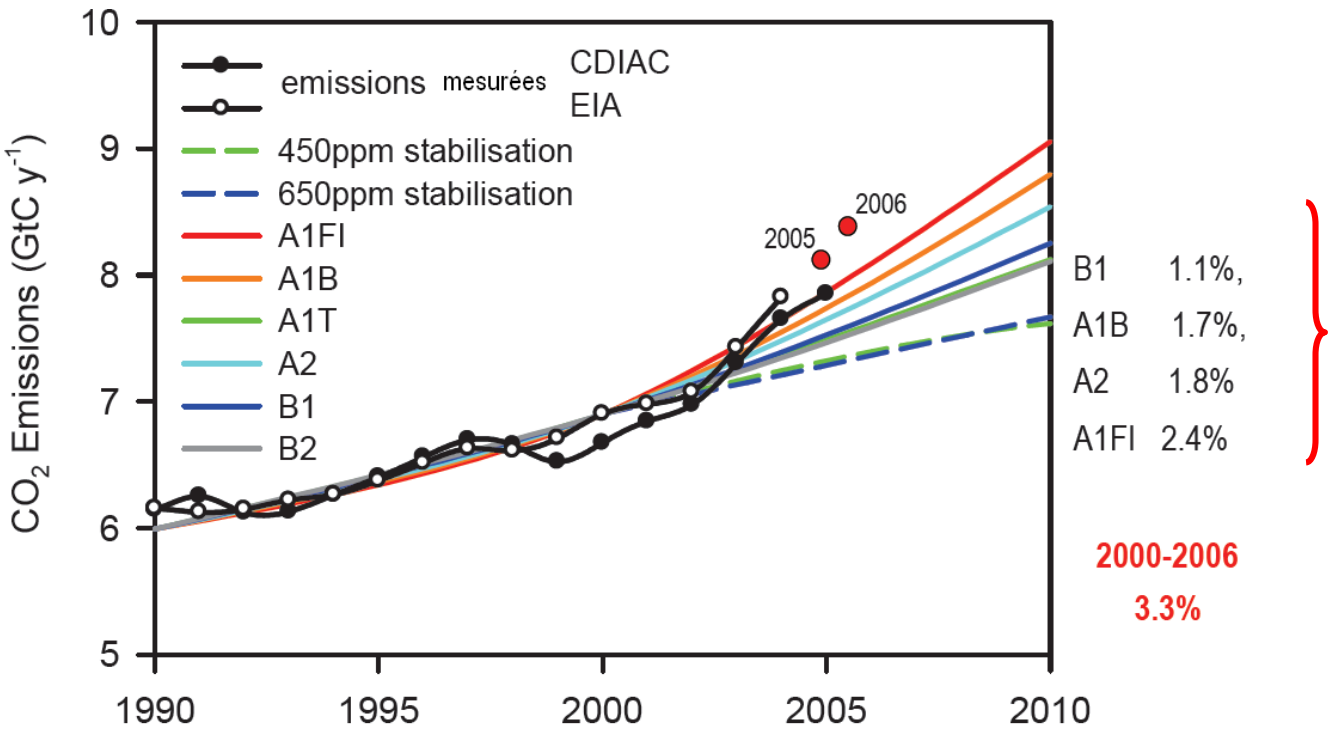
Breeding for quality, adaptation to environment

Assessment of durabity

Access and exchanges of GR limited and complex (biology, sanitary issues, international treaty)

How can we manage the durability of the resistance at the level of a landscape/region?

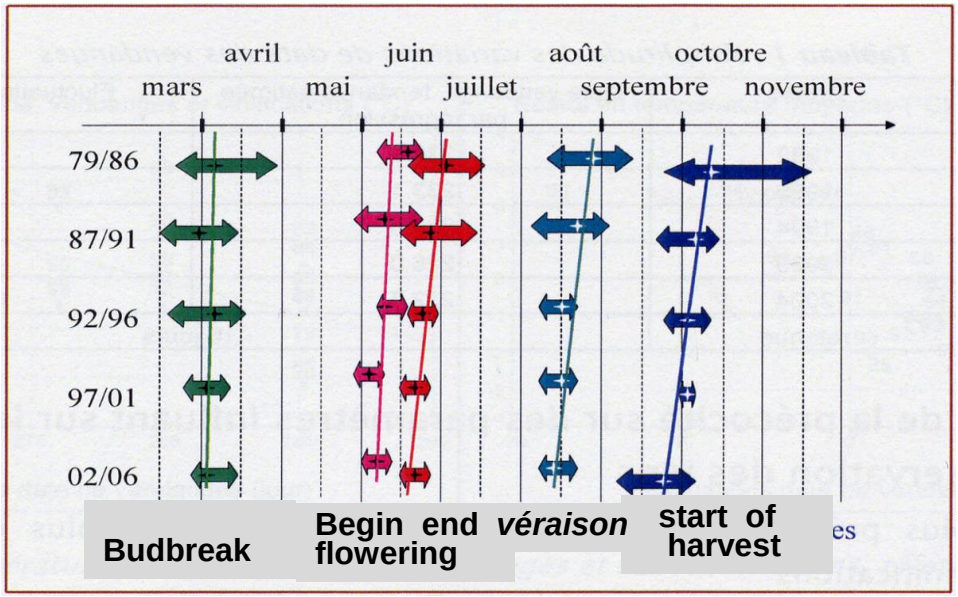
Viticulture in Europe faces major challenges
2- Adaptation to climate change



Hypothesis :
Constant rate of increase over 50 ans

From S. Delrot

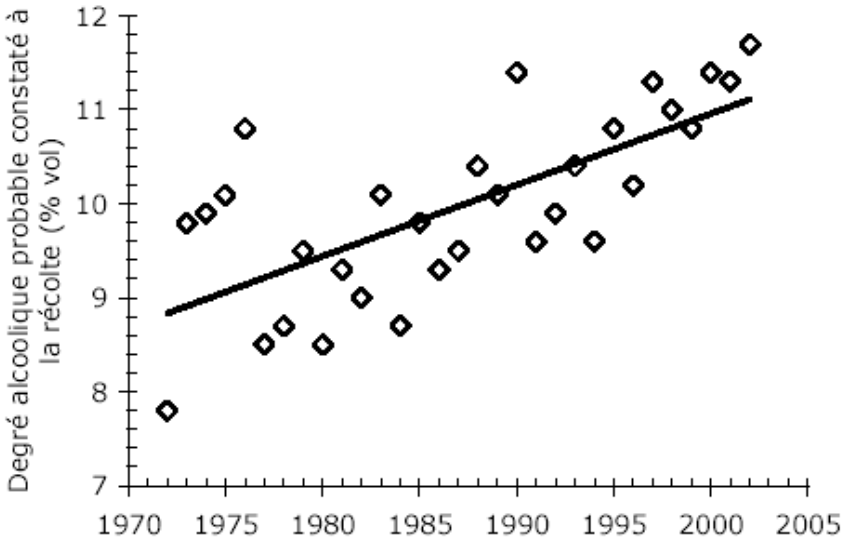
Viticulture in Europe faces major challenges
2- Adaptation to climate change



Phenological changes observed in the Cognac area (Ugni Blanc)

- Shortening of the period between budbreak and harvest
- Earlier phenological stages
- Ripening phase switched to warmer summer periods

(G.Snakkers, BNIC 2007)

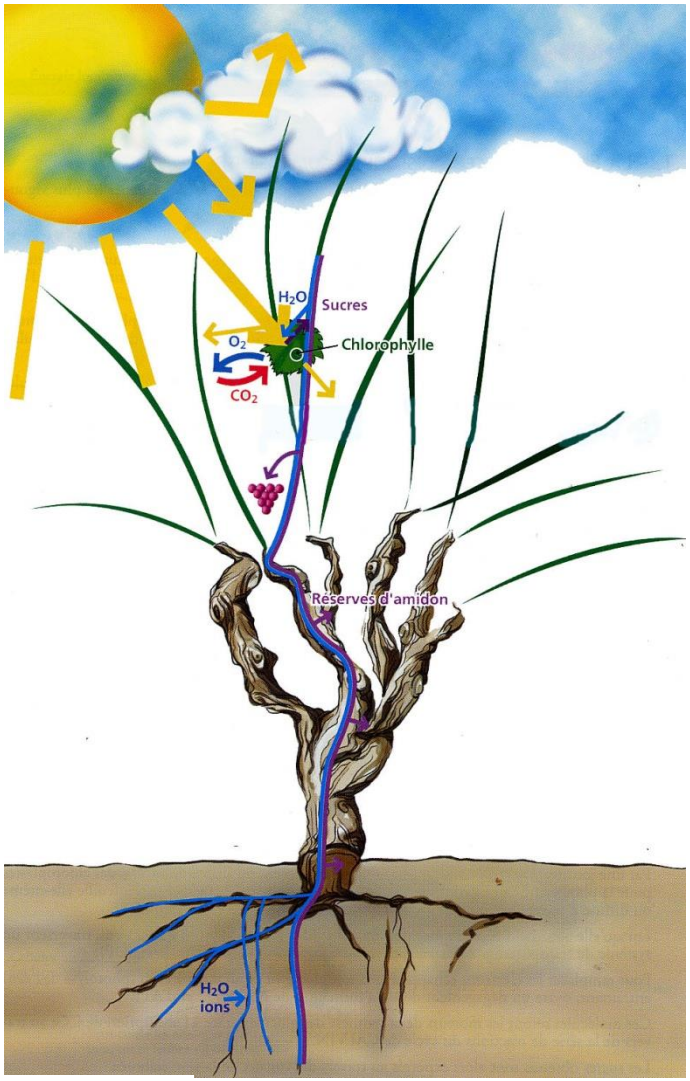


Ethanol content in wines from the Alsace area (Riesling)

- Over 2 degrees gained in 35 years
- However there are detrimental effects of the heat on the aromatic quality of wine in some areas.

(SIVA statistics)

Viticulture in Europe faces major challenges
2- Adaptation to climate change



From S. Delrot

- Combination of environmental parameters affected
  - CO_2 level
  - Température
  - Hygrometry
  - Soil water content
- Plant processes affected (that may interact with each other)
  - Phenology
  - Yield potential
  - Fruit composition
  - Response to pathogens
- Major physiological targets that may be affected
  - Photosynthesis/Respiration
  - Stomatal regulation
  - Hydraulic conductance
  - Growth of organs and carbon allocation
  - Development
  - Ion uptake and assimilation
  - Primary and secondary metabolism

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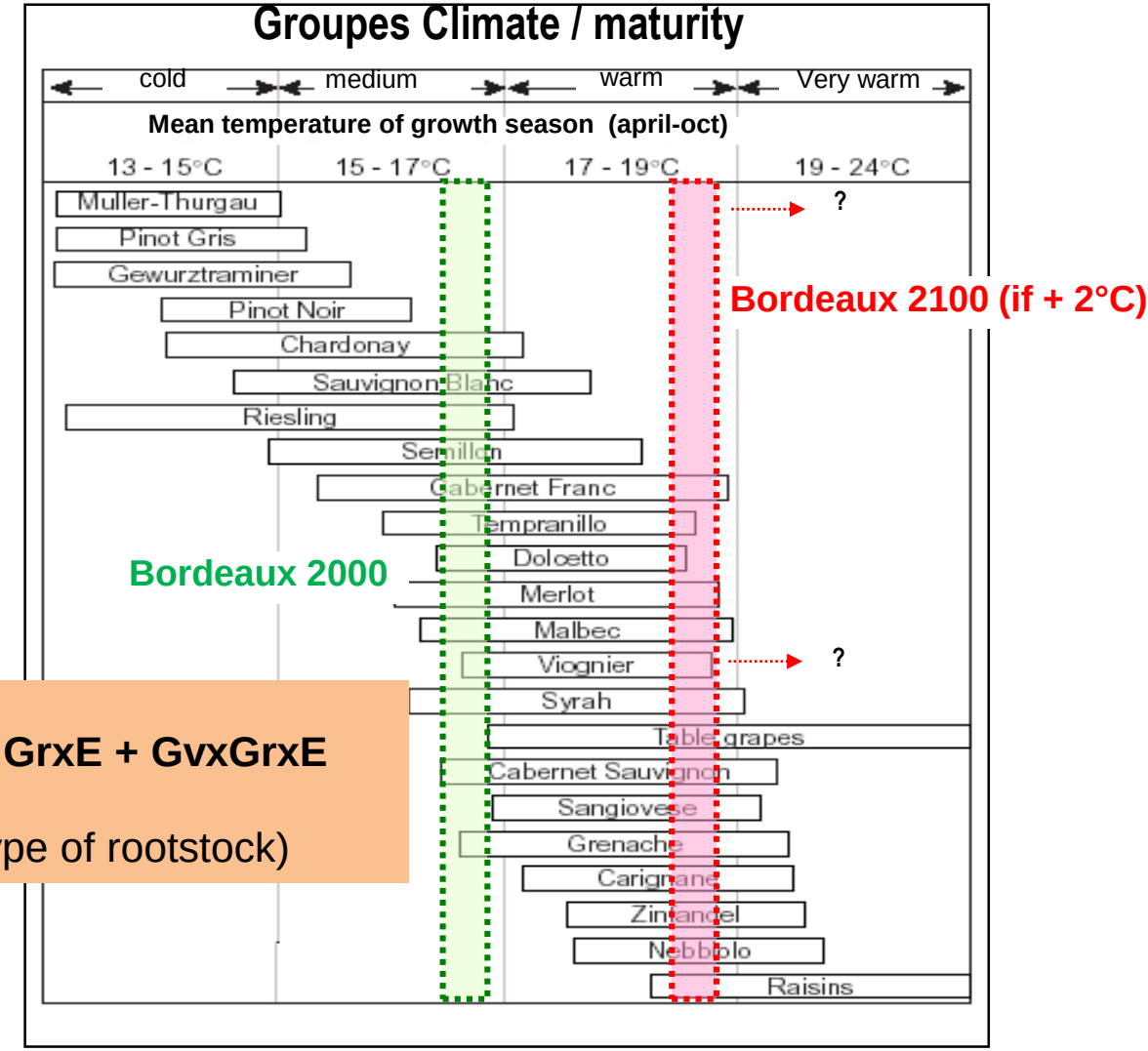
Viticulture in Europe faces major challenges
2- Adaptation to climate change

Genetic variability for adaptation to environment



- Phenotype = **G** + **E**+ **G×E**
- **P= Gv + Gr + E + GvxGr + GvxE + GrxE + GvxGrxE**

(Gv = genotype of variety; Gr = genotype of rootstock)

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BORDEAUX AQUITAINE

From S. Delrot

(from Jones, 2006)

Viticulture in Europe faces major challenges
2- Adaptation to climate change

From S. Delrot

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- How to predict a phenotype from the informations available on the genotype and its interactions with the environment? > How will the existing varieties respond, and what are the new phenotypes we are looking for (ideotypes)?

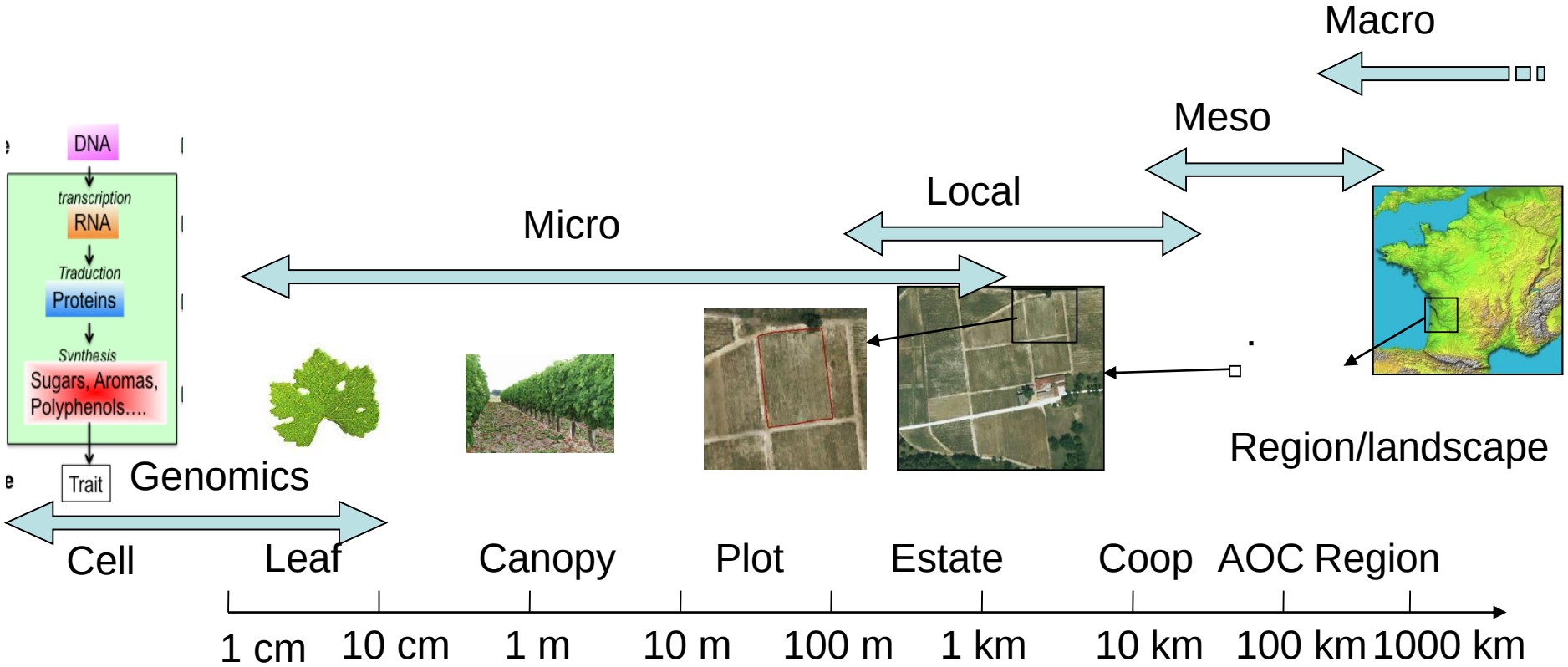
→ **Modeling at different scales**

Tardieu&Tuberosa 2010
Marguerit et al 2012

Cell Organ Plant Vineyard Region/landscape

Viticulture in Europe faces major challenges
2- Adaptation to climate change

Phenotyping at different scales



Genomics to help understand the underlying processes

From S. Delrot

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Sequencing the grapevine genome

Reference genome sequence of *V. vinifera* as a basic tool

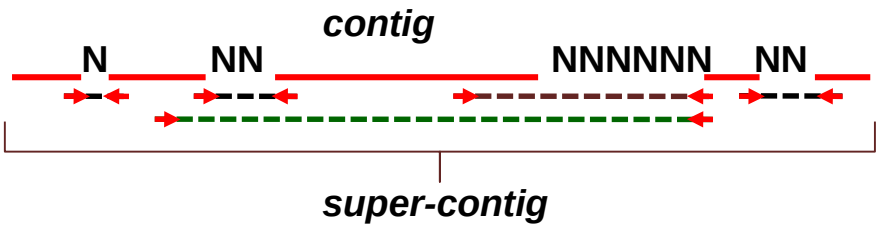
- Access to the about 30,000 genes and their order along the genome in the *Vitis* genus for genetic and functional analysis
- Possibility to quickly develop markers in any genome region in the *Vitis* genus

8X version of the genome published in 2007 (Jaillon et al 2007)

Current status: 12X.0 assembly

Library	Average insert length	Number of reads	Number of mate pairs	Coverage
BAC	100k	120091	57,606	0.12x
Fosmids	40k	251425	117,869	0.34x
Plasmids	10k	2848550	1,387,612	3.77x
Plasmids	3k	5518286	2,705,911	7.68x
Total		8738352	4,268,998	11.91x

Sanger shotgun sequencing approach



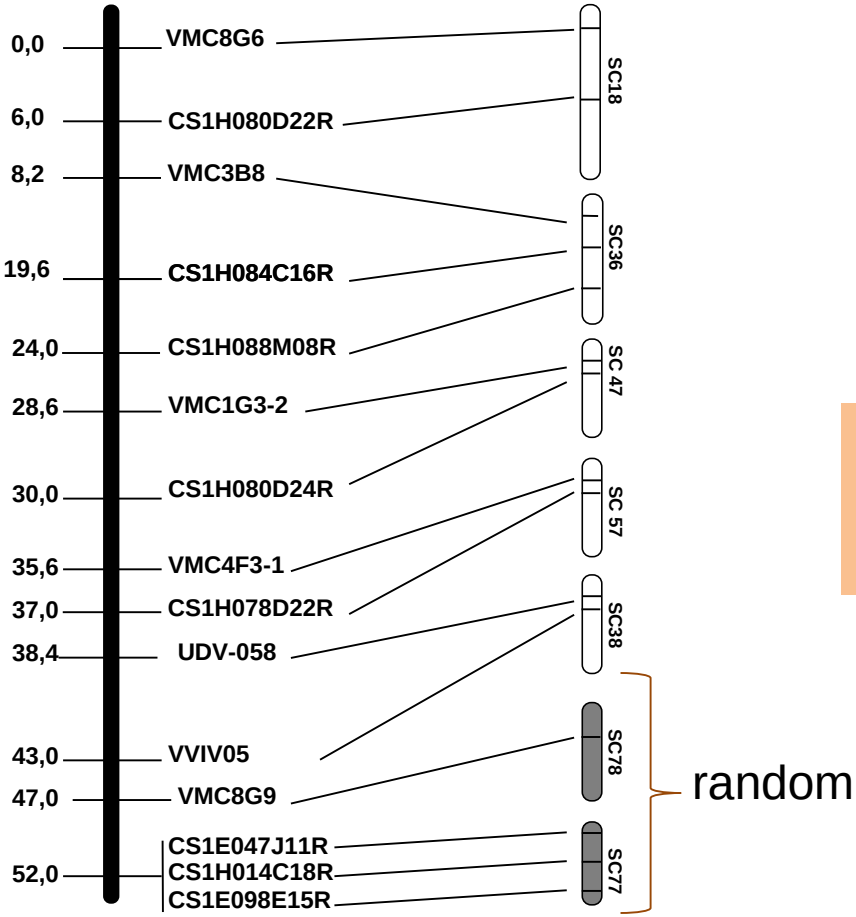
	8X	12X
Contigs Nb	19,578	17,196
Longest Ctg (kb)	557	774
Ctg N50 (kb)	65	100
Total size	467	481
Super-contig Nb	3,515	2,888
Longest sctg (kb)	12,675	13,101
Sctg N50 (kb)	2,065	3,300
Total size	487	499

Adam-Blondon et al (2011) Genome Sequence Initiatives. *in:* A-F Adam-Blondon, JM Martinez-Zapater, Chittaranjan Kole (eds) *Genetics, Genomics and Breeding of Grapes.* Science Publishers and CRC Press. pp 211-234

Sequence built of the 19 chromosomes

Genetic map : chromosome 12

Super-contigs of sequence



http://urgi.versailles.inra.fr/

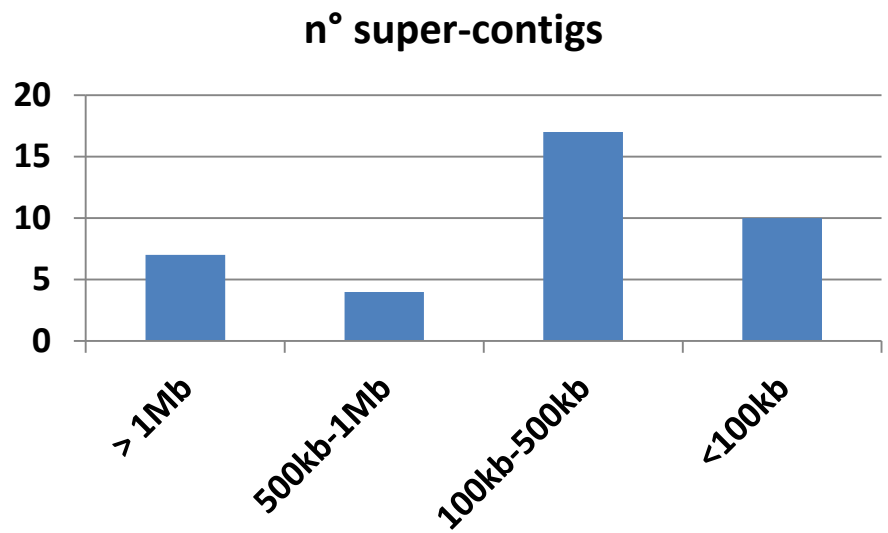
12X.0 version (consensus map from 2 largers pop; 514 unique SSR markers):

- **91%** of the draft sequence is anchored on the genetic map
- **87%** is anchored and oriented

Cipriani et al (2011) Molecular Linkage Maps: Strategies, Resources and Achievements. *in:* A-F Adam-Blondon, JM Martinez-Zapater, Chittaranjan Kole (eds) *Genetics, Genomics and Breeding of Grapes.* Science Publishers and CRC Press. pp 111-136

12X.0 : characteristics of the remaining « random » sequence

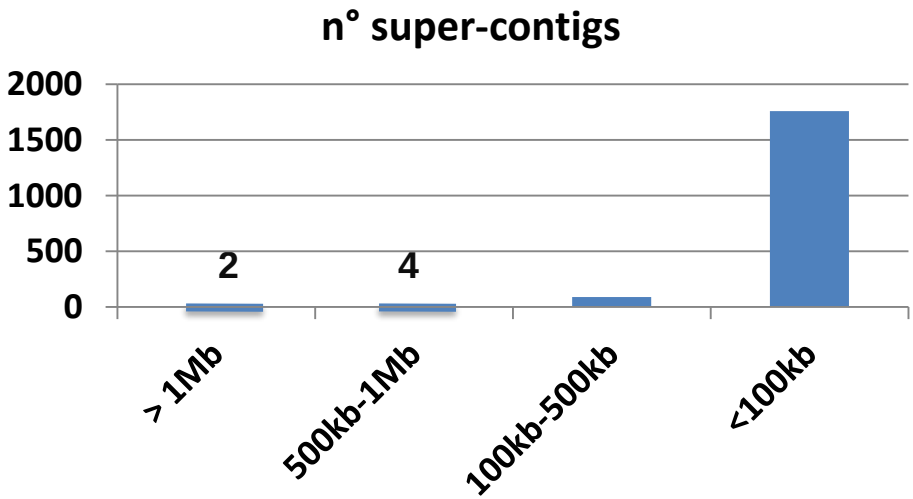
Anchored to a chromosome but not oriented : 38 sgtg; 16.8 Mb



4.33% of 'N'

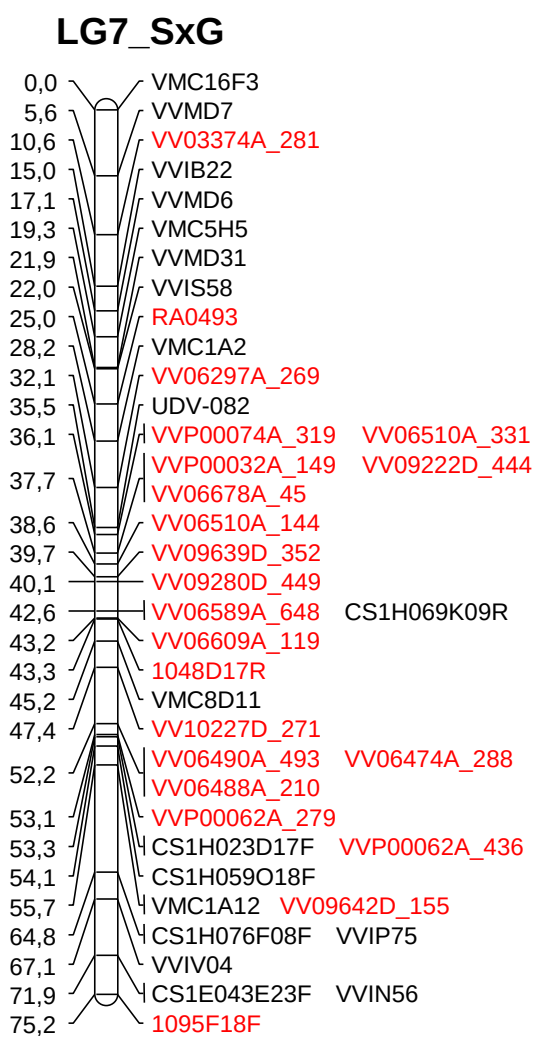
Anchored and oriented sequence : 2.33% of 'N'

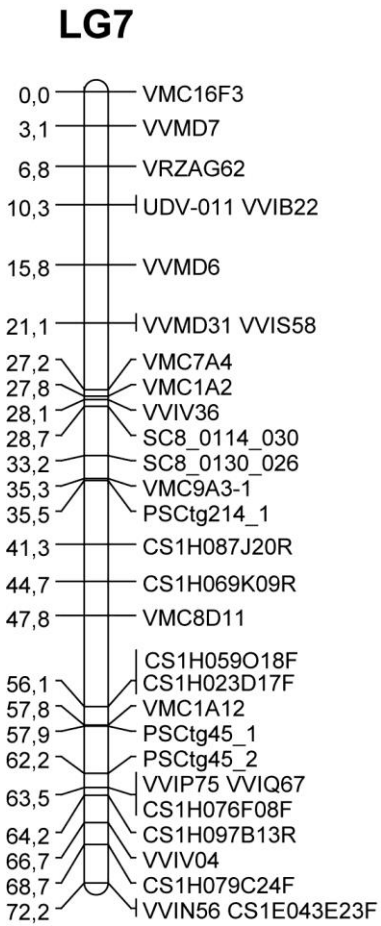
Not anchored to a chromosome : 44 of them >200kb; 15.6 Mb



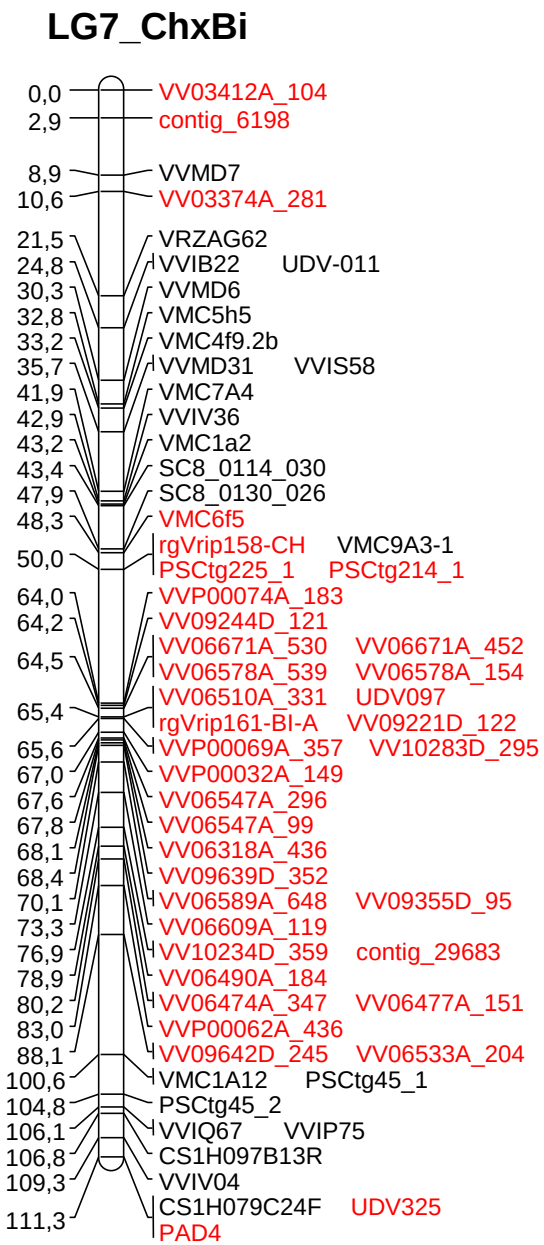
10.22% of 'N'
2064 gene models (7.8%)

Improvement of the genetic map





Integrated SxG and ChxBI (Cipriani et al 2011)

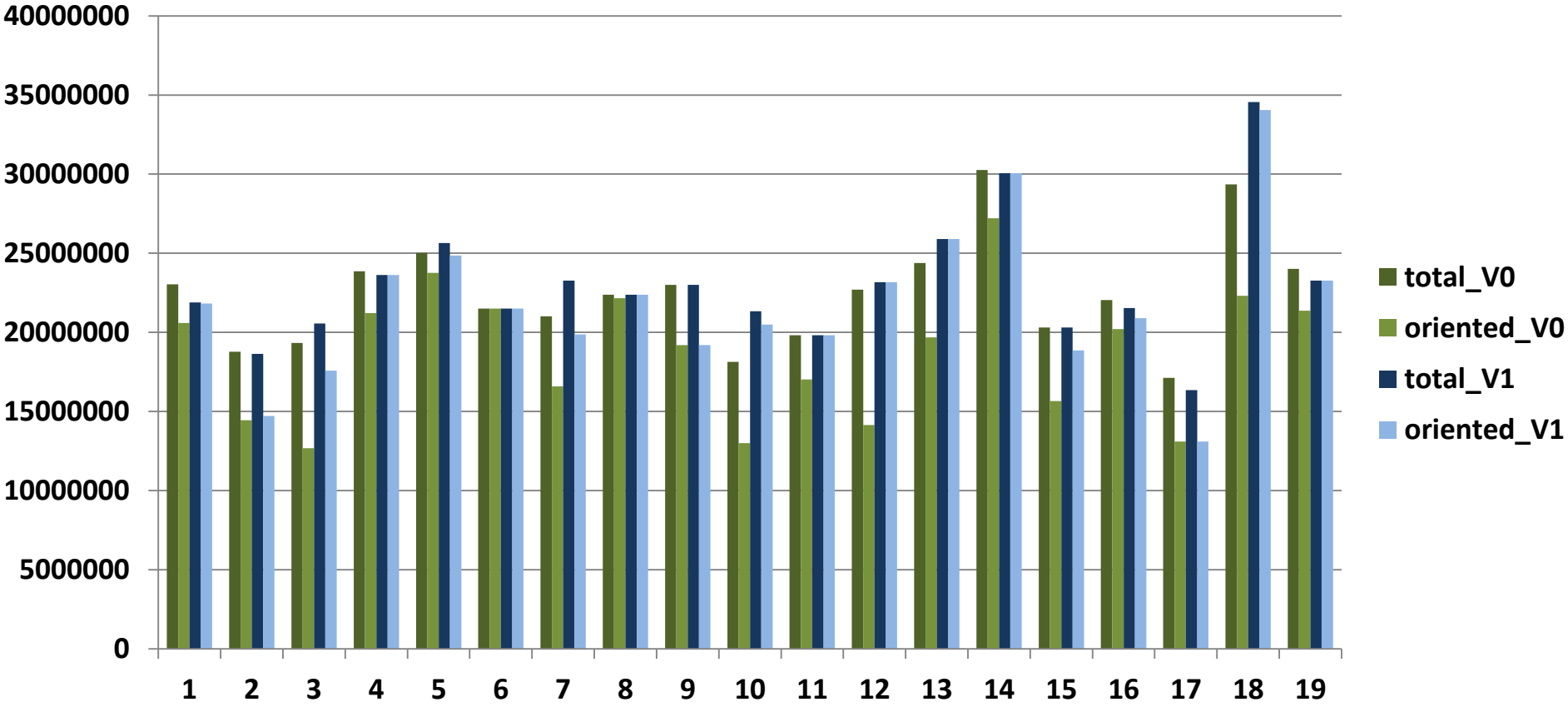


A. Canaguier

Anchoring the scaffolds using the new genetic map

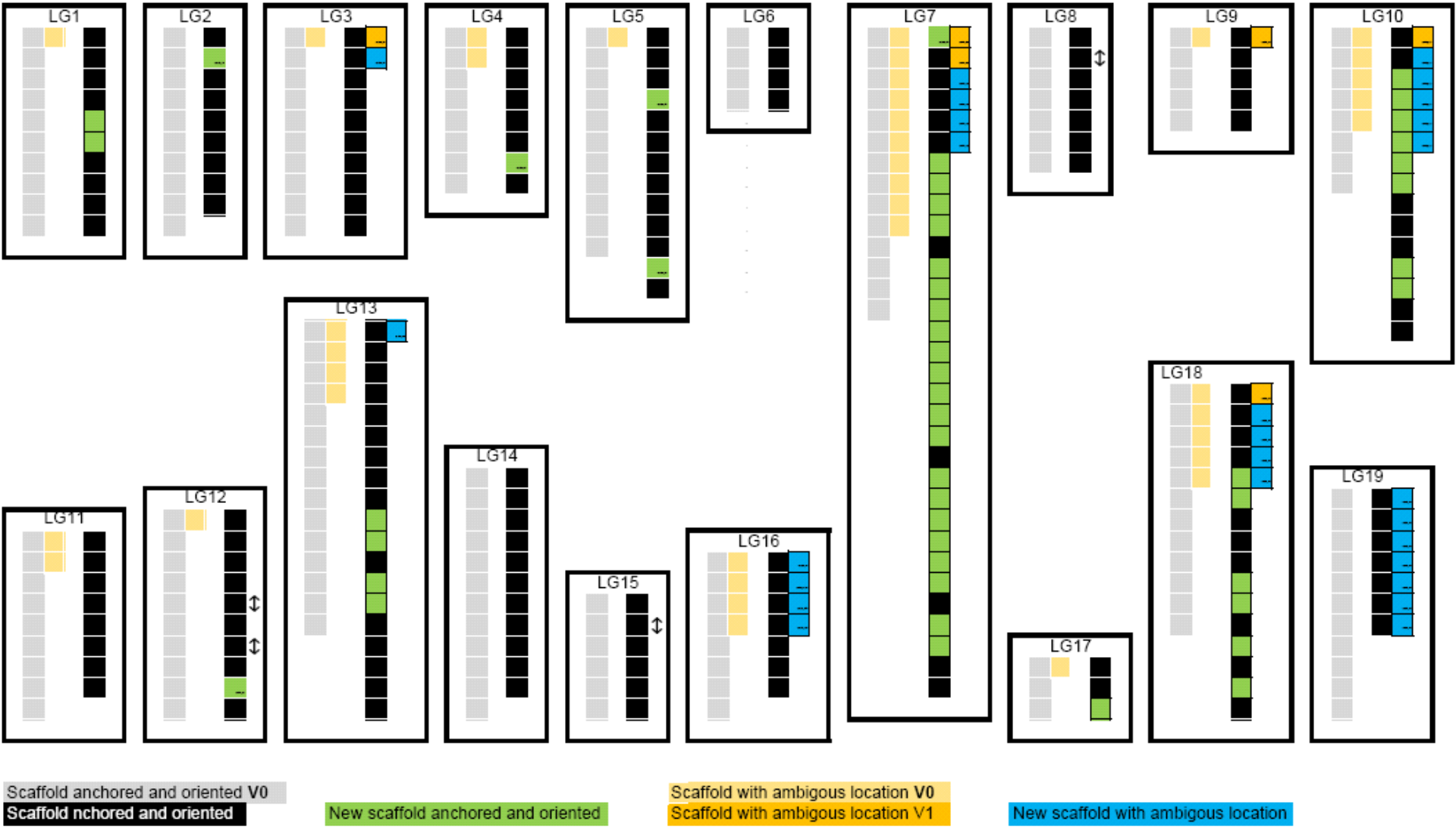
V0: 426 Mb anchored (85% genome) -> V1: 436.8 Mb anchored (87% genome)

V0: 355 Mb oriented (71% genome) -> V1: 415 Mb oriented (83% genome)



A. Canaguier, G. DiGaspero, S. Scalabrin

Anchoring the scaffolds using the new genetic map



Current Annotation from the CRIBI: V1 annotation

Input :

- 339,008 Vitis ESTs (NCBI)
- flcDNAs (99,828 reads from 5 libraries; URGV + Genoscope)
- Deep EST sequencing using Illumina-Solexa (175M reads from 4 libraries; IGA) and Roche-454
- V0 automatic annotation using the Gaze software (Genoscope)

↓

- 12X.0 annotation V1 : 29 971 gene models => used for the whole genome Nimbelgen transcriptome array (Univ. Verona)
- Repeat annotation (IGA + CRIBI)

Proposition of a stable automatic gene numbering based on the Arabidopsis system (AtXgZZZZZ):

VvXX	sYYYY	gZZZZ
n°chr	n°super-contig	n°gene on super-contig

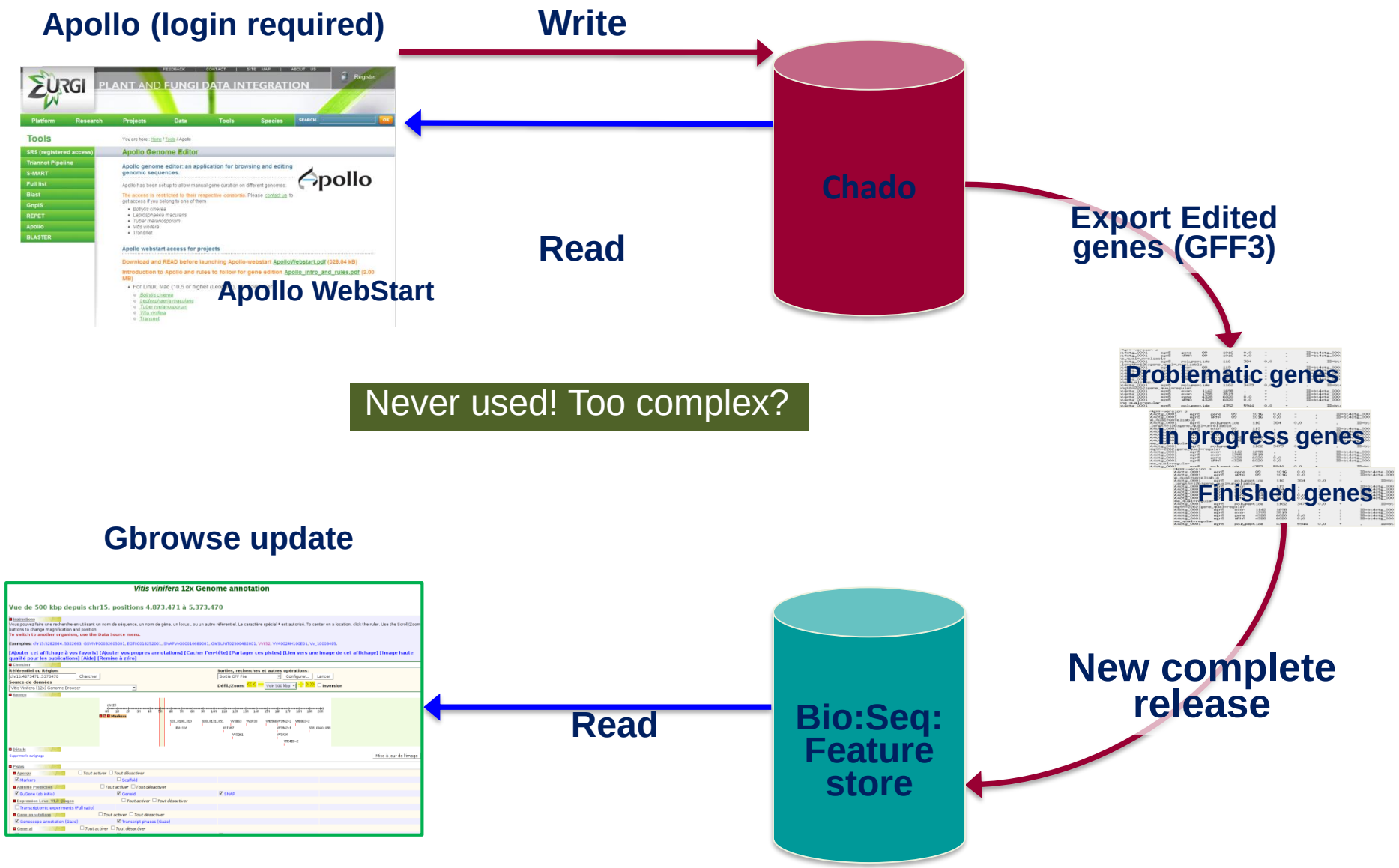
Improvement of the V1 annotation

- Several important gene families have been expertised, both at the level of the gene model and of the function:
  - 152 genes/pseudogenes of the terpene synthase gene family (Martin et al 2010; http://urgi.versailles.inra.fr/gb2/gbrowse/vitis_12x_pub)
  - 48 genes/pseudogenes of the CHS and stilbene synthase gene family (Vannozzi et al 2012; Parage et al 2012; http://urgi.versailles.inra.fr/gb2/gbrowse/vitis_12x_pub)
  - 829 genes/pseudogenes of the NSB-LRR gene family (unpublished yet)
  - 19 genes putative TF with a TIFY domain (Zhang et al 2010)
  - 17 PR10 related genes/pseudogenes (Lebel et al 2010)
  - 17 CDPK genes (see the presentation of Pr Cheng)
  - Etc…

- Toward a V2 annotation in the frame of a new EU COST action in collaboration with the tomato genomic consortium.

Improvement of the V1 annotation

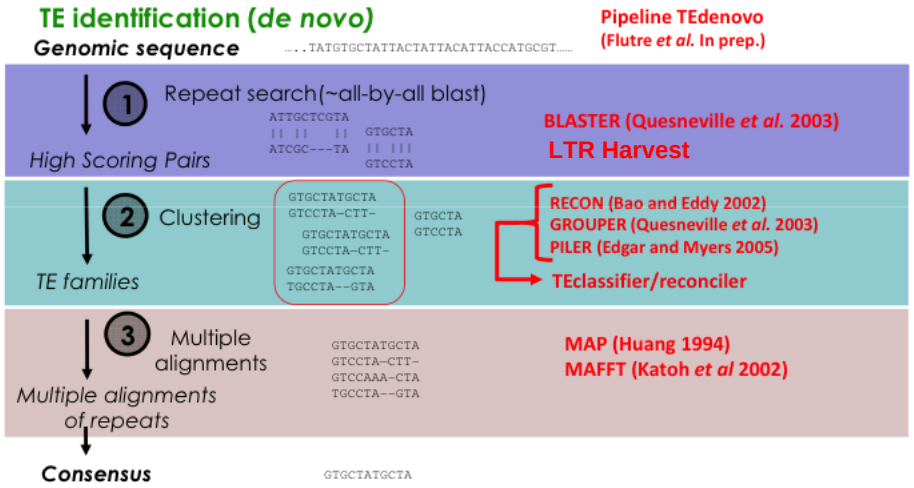
http://urgi.versailles.inra.fr/index.php/urgi/Species/Vitis/Resources/Genome-sequence-and-annotations

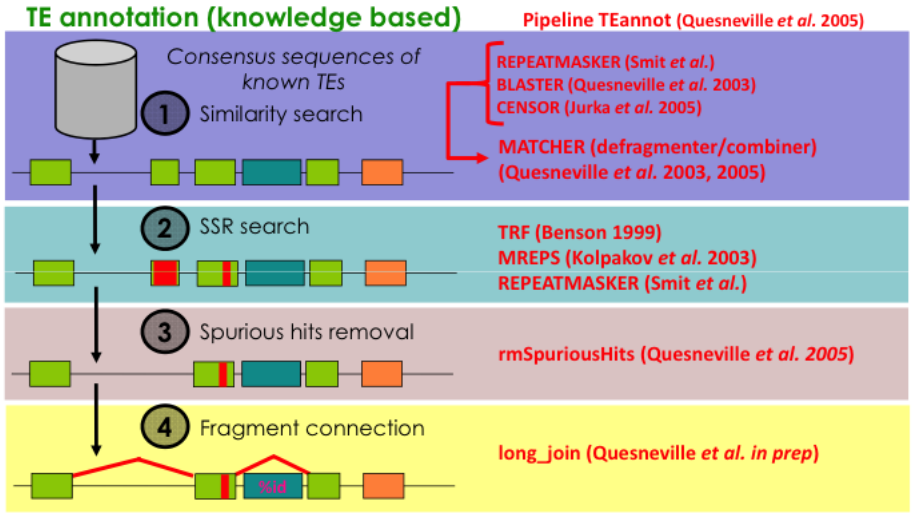


Improvement of the V1 annotation

- Automatic annotation by IGA (Udine, Italy)
- Curation of the Class II transposons (Benjak et al 2008, 2009)

REPET V2 (http://urgi.versailles.inra.fr)





Novel TE automatic annotation using REPET V2 (Flutre et al 2011)

First round: 65% genome = TE

41.4% Class I

8.7% ClassII

33.8% unclassified repeats

according to Wicker et al 2007

=>7822 consensus

Second round:

6738 consensus with at least one complete copy in the genome

=> 67.8% genome =TE

Manual curation on going

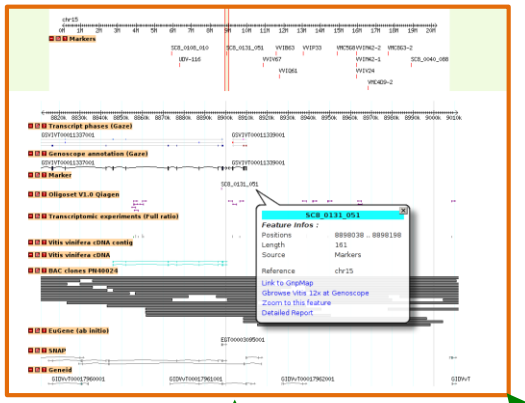
Where can you access all these informations?

http://urgi.versailles.inra.fr

Access to GnpIS portal

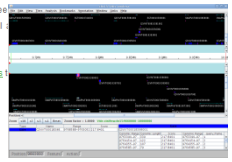
Access to Vitis resources

Markers

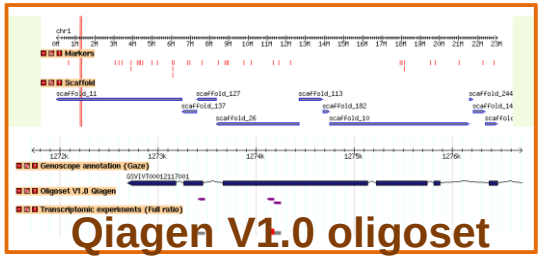


Vitis web pages

Apollo



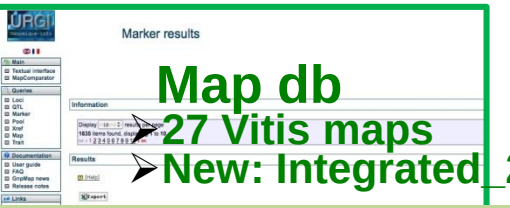
Transcriptomics data (GRASP project)



Qiagen V1.0 oligoset

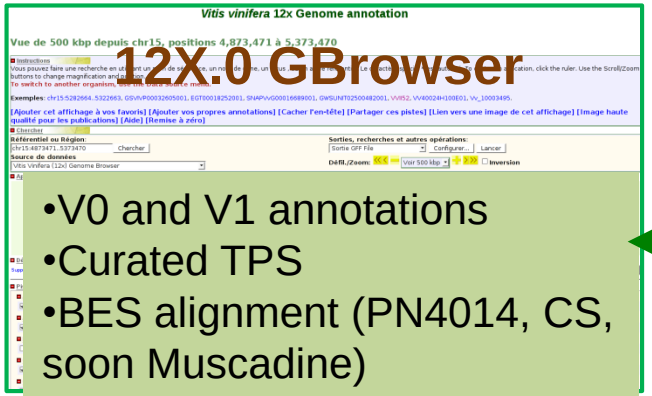
Map db

- 27 Vitis maps
- New: Integrated_2



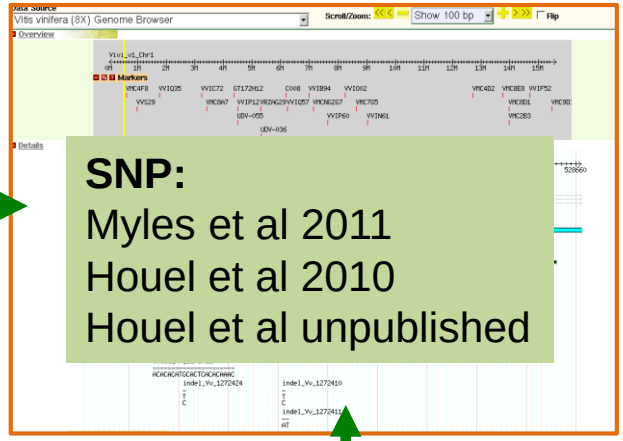
- 27 Vitis maps
- Integrated_2 (**Cipriani et al 2011)**

12X.0 GBrowser



- V0 and V1 annotations
- Curated TPS
- BES alignment (PN4014, CS, soon Muscadine)

Polymorphism data

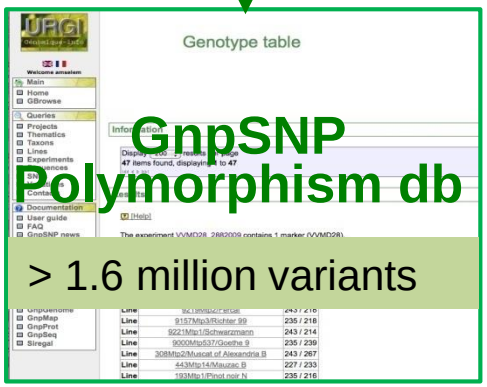


SNP:
Myles et al 2011
Houel et al 2010
Houel et al unpublished

SIREGAL
Genetic resources db

Passeport data of the grapevine National collection (> 7000 accessions)

GnpSNP
Polymorphism db



> 1.6 million variants

Molecular basis of trait variation and development of MAS at a genomic scale

Illumina re-sequencing of 47 grapevine genotypes

In the frame of EU KBBE GrapeReSeq project and of a french ANR project Muscares

Leaf harvest and DNA prep

20 diverse *Vitis vinifera*
4 *Vitis sylvestris*
3 *V. cinerea*
3 *V. berlandieri*
3 *V. aestivalis*
3 *V. labrusca*
6 *M. rotundifolia*

DNA quantification
Library prep
Illumina GAII sequencing

French National plateform for Genotyping (CNG), Evry, France

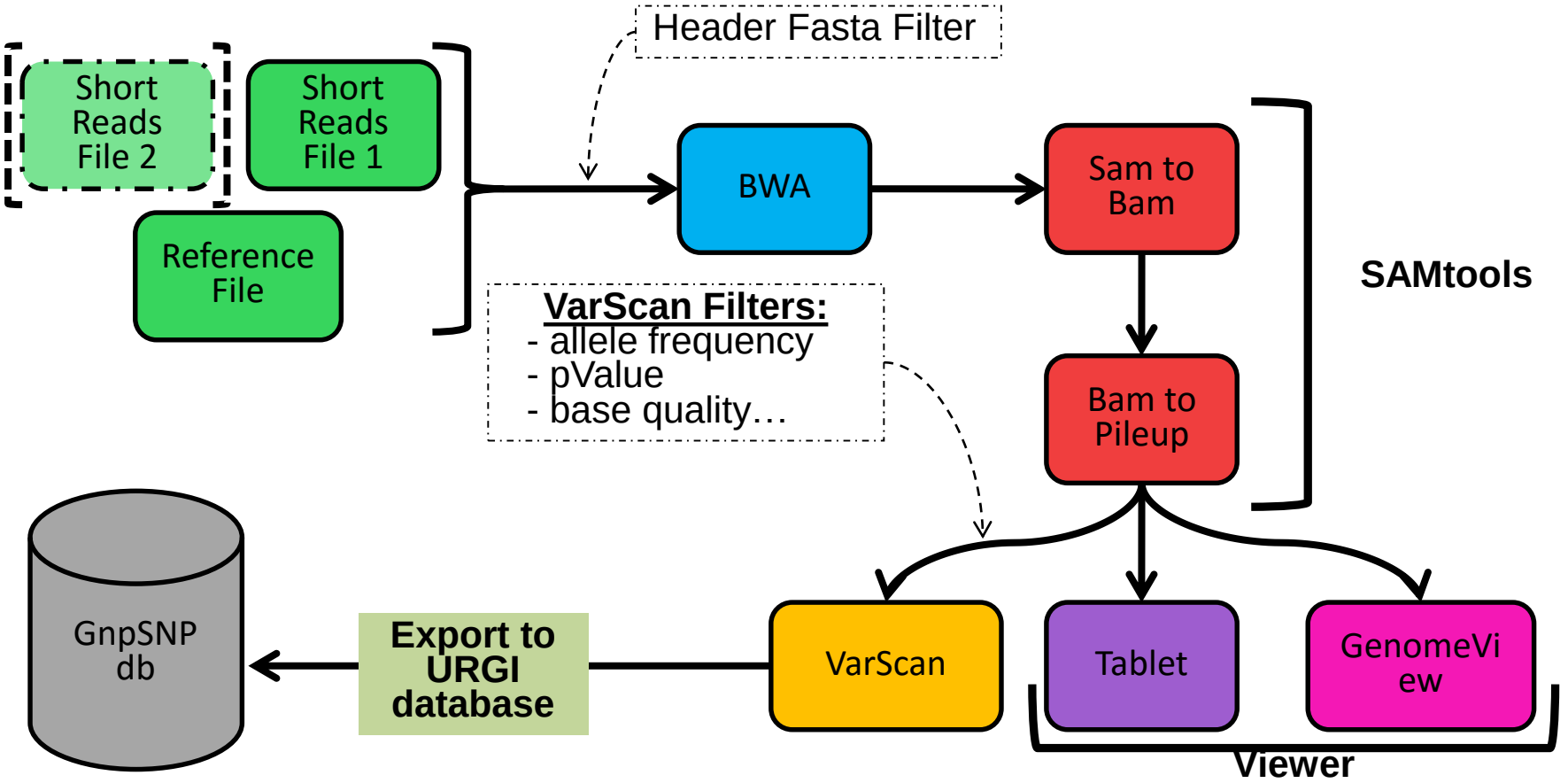
Triming for quality

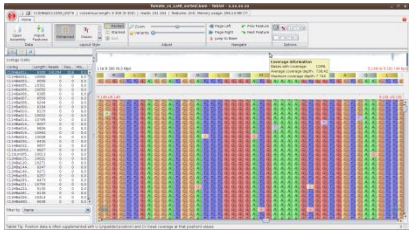
Alignment on the reference
Genome sequence
SNP detection

Results from the re-sequencing of 25 V. vinifera varieties at IGA (Udine, Italy)

Illumina 20K genotyping Chip (http://urgi.versailles.inra.fr => Vitis pages)

Pipeline for SNP detection: MAPHiTS
Mapping **A**nalysis **P**ipeline for **Hi**gh-**T**hroughput **S**equencing

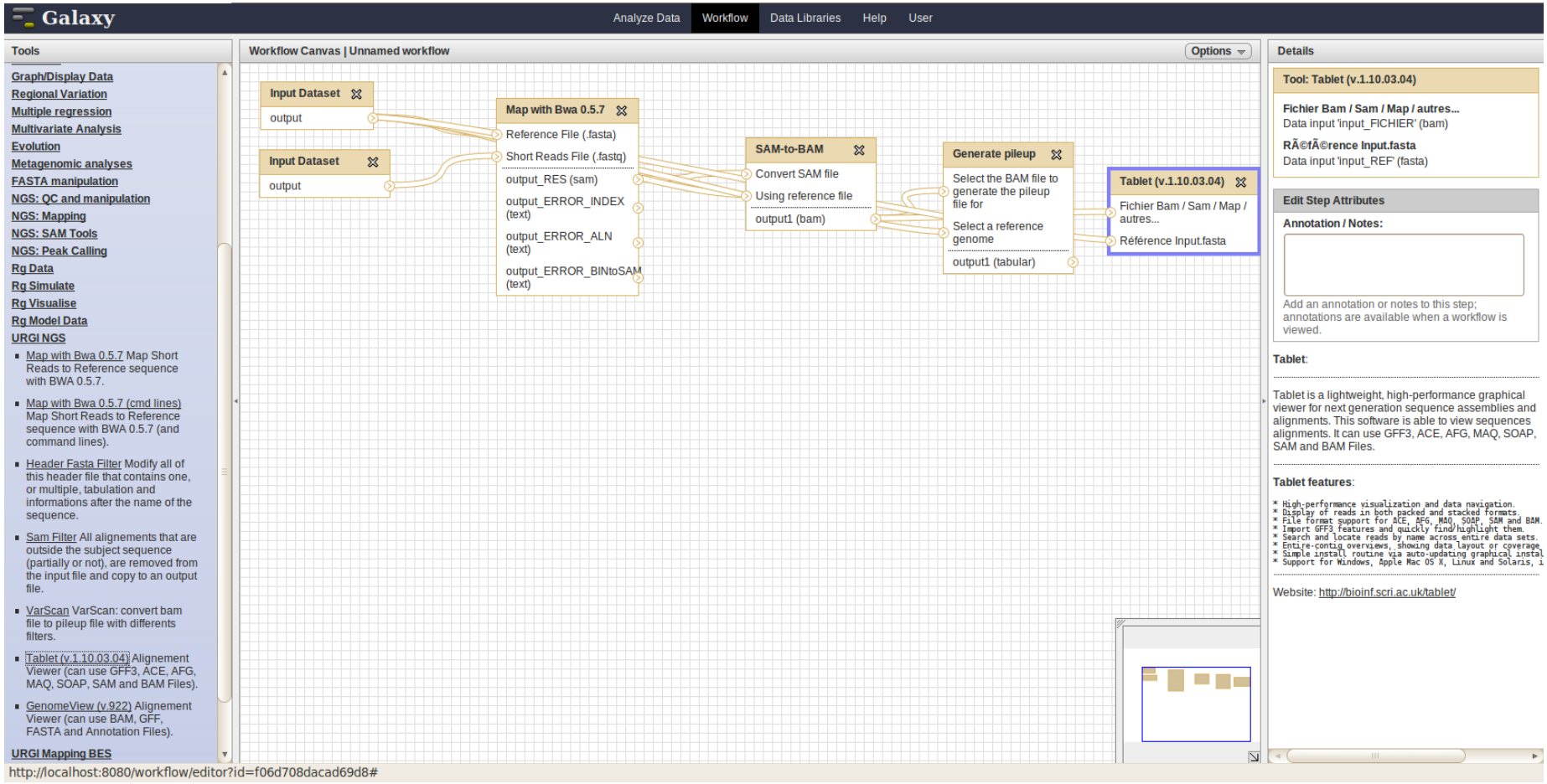




Calculations parallelized

MAPHiTS workflow in Galaxy

(a web-based platform for genomic research : http://usegalaxy.org)



Sequencing results for the 47 V. vinifera varieties

Read Length	Average	Standard dev	Min	Max
Read length (after trim)	94	6,38913	72	100
Bases after trimming	9 245 106 087	6 109 483 264	3 717 274 733	33 989 041 742
Genome coverage after trimming	19	12.6	8	70
Genome coverage with uniq reads	13	8.1	4	47
% of the genome covered	81%	5%	67%	93%
SNP detected	4 285 569	1 798 766	734 738	8 661 975
SNP after filters	926 880	336 957	162 806	1 545 045

•GA II and/or High Seq sequencing

• Filtering for position in known structural variants, in repeats, with too few or too many reads

20k Illumina GrapeReSeq genotyping chip

The GrapeReSeq_20K Illumina array:

- 15 022 SNP from *Vitis* vinifera
- 4 978 SNP from *Vitis* species

SNP chosen on the following criteria:

- Illumina score >= **0.9**
- SNP for Infinium type II array
- MAF criteria:
  - 90% SNP with MAF > 0.1 (85463 SNPs)
  - 10% SNP with 0.05< MAF < 0.1 (27631 SNPs)
- Heterozygosity (Vitis species SNP)
- Genome distribution

Aknowledgements

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N. Choisne
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N. Mohellibi
S. Reboux
D. Steinbach
D. Valdenaire
D. Verdelet
H. Quesneville

AGAP – INRA Montpellier

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SVQV – INRA Colmar

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URGV – INRA Evry

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Aknowledgements

IGA/ University of Udine, Italy
Michele Morgante
Gabriele Di Gaspero
Simone Scalabrin

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Mario Pezzotti
Massimo Delledonne

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Giorgio Vallé
Nicola Vitulo

JKI, Germany
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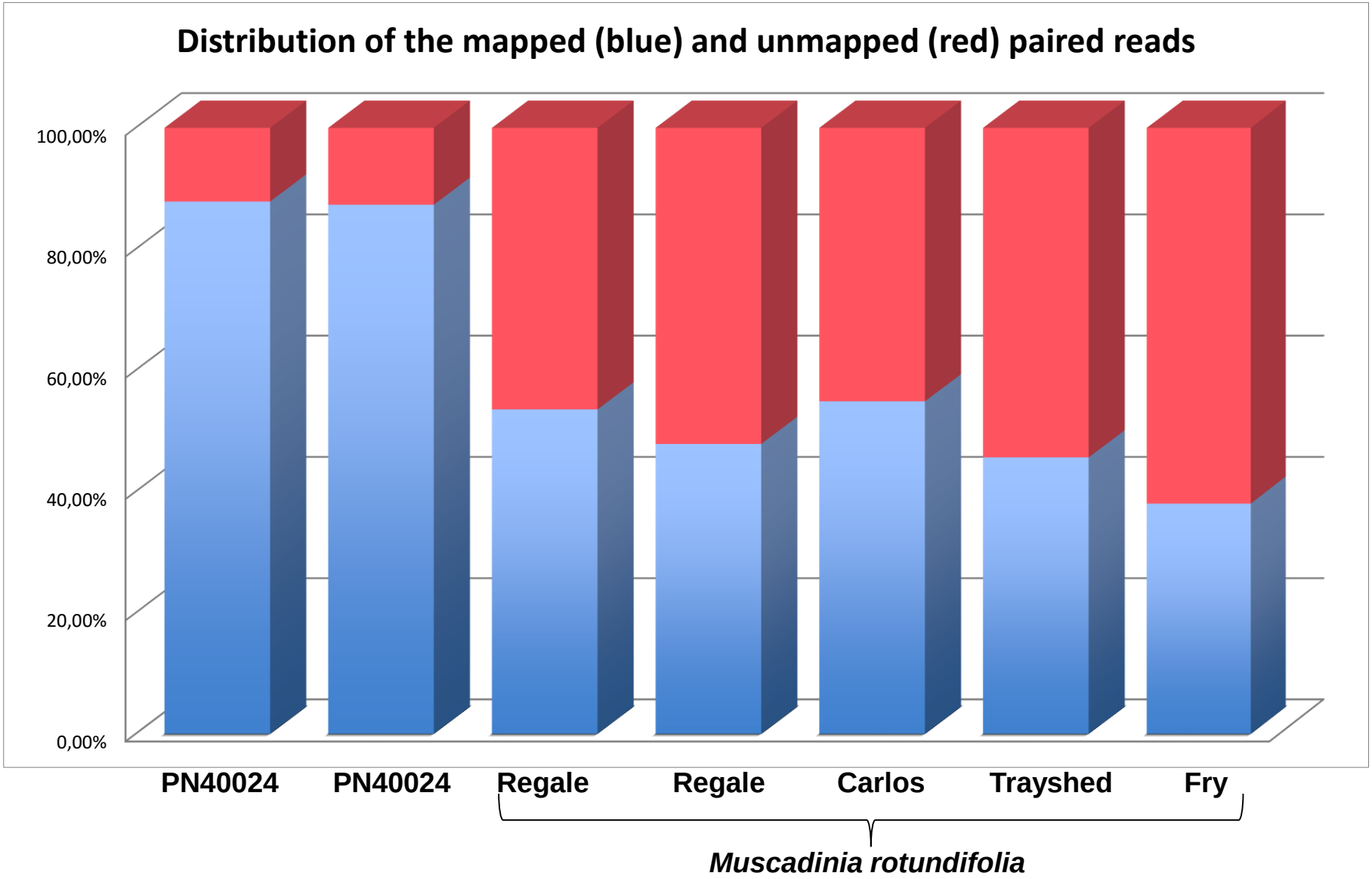
Thank you!

Mapping with 99% of identity



Anchoring the scaffolds using the new genetic map

Nearly ready to provide a new chromosome sequence

Improvement of the genetic map

- Design of 200 SNP markers from the sequence of 37 non oriented scaffolds and 40 large non anchored scaffolds
- Recovery of SNP genotyping data from Vezzulli et al 2008
- Recovery of SSR genotyping data from Doligez et al 2006
- Recovery of SSR genotyping data from Cipriani et al 2011

↓

- 1022 SSR primer pairs/SNP loci used on two populations:
  - 535 markers in the Syrah x Grenache population (193 indiv)
  - 730 markers in the Chardonnay x Bianca population (358 indiv)

A. Canaguier

System Biology of adaptation to biotic and abiotic stresses

- Need to combine modeling at different scales and subjects and taking into account genetic variation

- Need connected databases with different layers of information

- System biology of two organisms in interaction…
  => Need genomic tools for the pathogens (genome sequence)

- …or may be more (wood diseases, new decay syndromes…)
  => Metagenomics approaches