

Task 3.4

Screening germplasm for adaptation to drought and temperature



Participants

- CSIC, Consejo Superior de Investigaciones Científicas
- IFV, Institut Français de la Vigne et du Vin
- INRA, Institut National de la Recherche Agronomique
- ISA, Instituto Superior de Agronomia, Portugal
- UNIVR, University of Verona, Italy

Expectations under a climate change scenario

- Higher heat accumulation during the growing season
- Increasing drought episodes

Effects on viticulture

- Altered phenology
- Altered ripening profiles
 - Concentration of sugars
 - Acid levels reduction
 - Aromas reduction
 - Uncoupling between technologic and phenolic maturity

- Strategies to cope with climate change:
 - Water management
 - Canopy management
 - Use of more efficient varieties and rootstocks

Development of more efficient varieties in grapevine

- Newly bred varieties
 - Identification of suitable parental lines
 - Identification of responsible genetic loci for MAS
 - Acceptance
- Improved current varieties
 - Clonal selection
 - Responsible genetic variation for clone identification
 - Availability of desired traits

Goals within Task 3.4

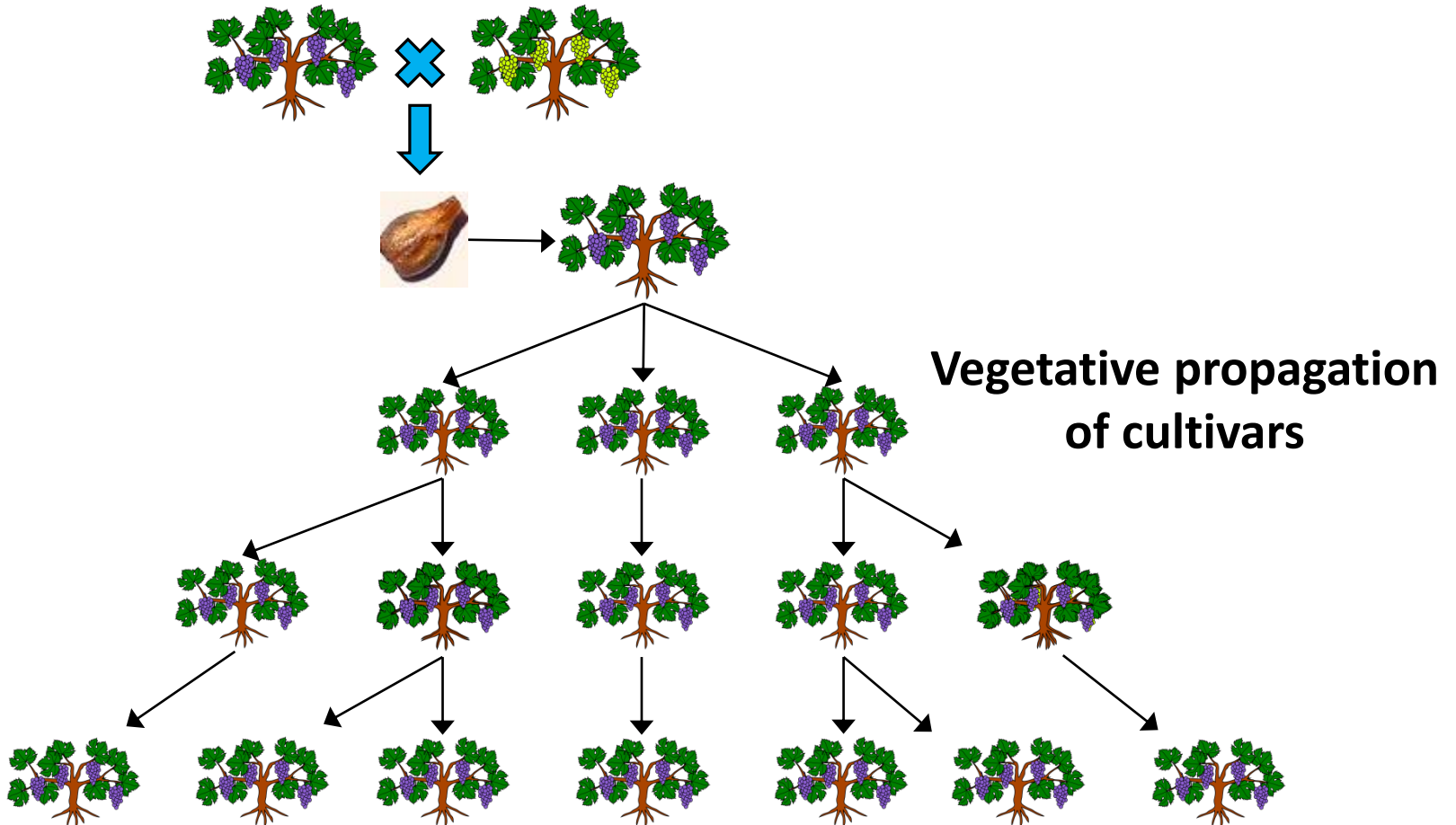
- Screening of genetic resources for **genetic diversity** (berry **quality** and environmental **stress adaptation**)
 - Evaluation of **cultivar core collections** (INRA, IFV)
 - Evaluation of **clone collections** of Tempranillo/ Aragonez (ISA, CSIC)
- Characterization of **genes** and **gene variants** involved:
 - **Cultivars** with different adaptation strategies to **water stress** (UNIVR)
 - **Clonal** variation in **stress adaptation** (ISA, CSIC)

CSIC

GOAL:

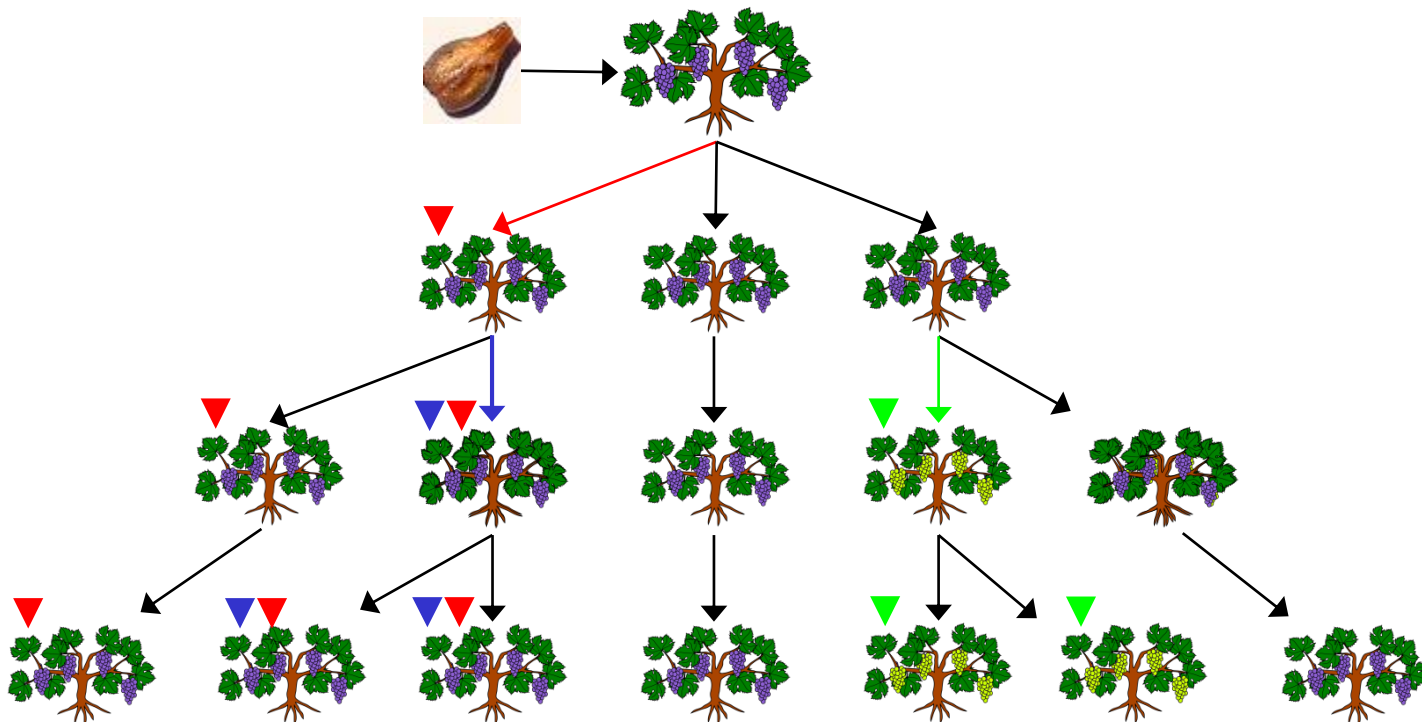
Search for **ripening** somatic **variation**
adaptable to **climate change** in
Tempranillo cultivar

Screening germplasm for adaptation to drought and temperature



Somatic mutation

Origin of clones or lineages (intra-varietal diversity)



HYPOTHESIS

- Long ripening cycle somatic variants (low sugar accumulation rate) could be more **adapted** to climate change conditions

SPECIFIC EXPERIMENTAL OBJECTIVES

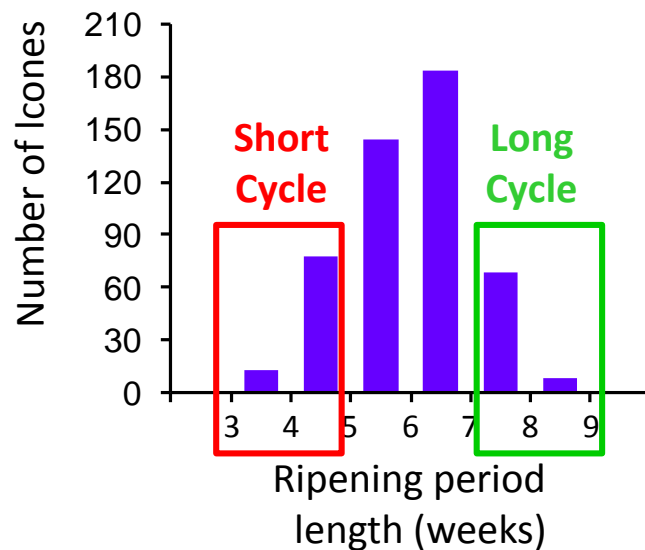
- Identify Tempranillo clones **contrasting** in **ripening cycle**
- Identify the **responsible genetic variation** for the contrasting variant phenotypes



- **Pre-selection screening:**
494 virus-free **Tempranillo** clones
 - **Phenology and production**
(3 years)



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Screening germplasm for adaptation to drought and temperature



- **Pre-selection screening:**
494 virus-free **Tempranillo** clones
 - **Phenology and production**
(3 years)



- **Selection evaluation**
10 **long cycle** and 9 **short cycle**
 - **Technological and phenological ripening time-course**
 - **Field clone bank**
 - **Fruit cutting**

6 clones Selected Deliverable M24
3 search of responsible variation

TRANSCRIPTOME ANALYSIS DESIGN: clone selection

Cycle	Clone
Short	1048
Long 1	807
Long 2	1084

- Consistent **ripening cycle** length (field and cuttings)
- Balanced **yield**
- Medium-High **anthocyanin** content

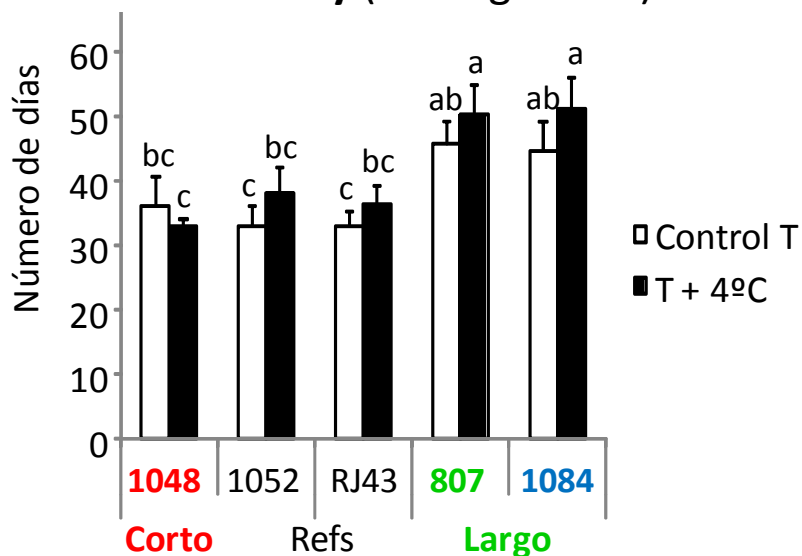
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Veraison-maturity (cuttings 2015)



Veraison-maturity (field 2015)

Cycle	50% veraison	Veraison-Maturity (days)
Short	31/07/2015	21
Long 1	30/07/2015	41
Long 2	31/07/2015	42

TRANSCRIPTOME ANALYSIS DESIGN:

- Samling:**

X 3 clones

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Long 2	1084



28/07/2015

(~20% *véraison* date)
~90% coloured surface
RNA from pericarp



3 Biological replicates

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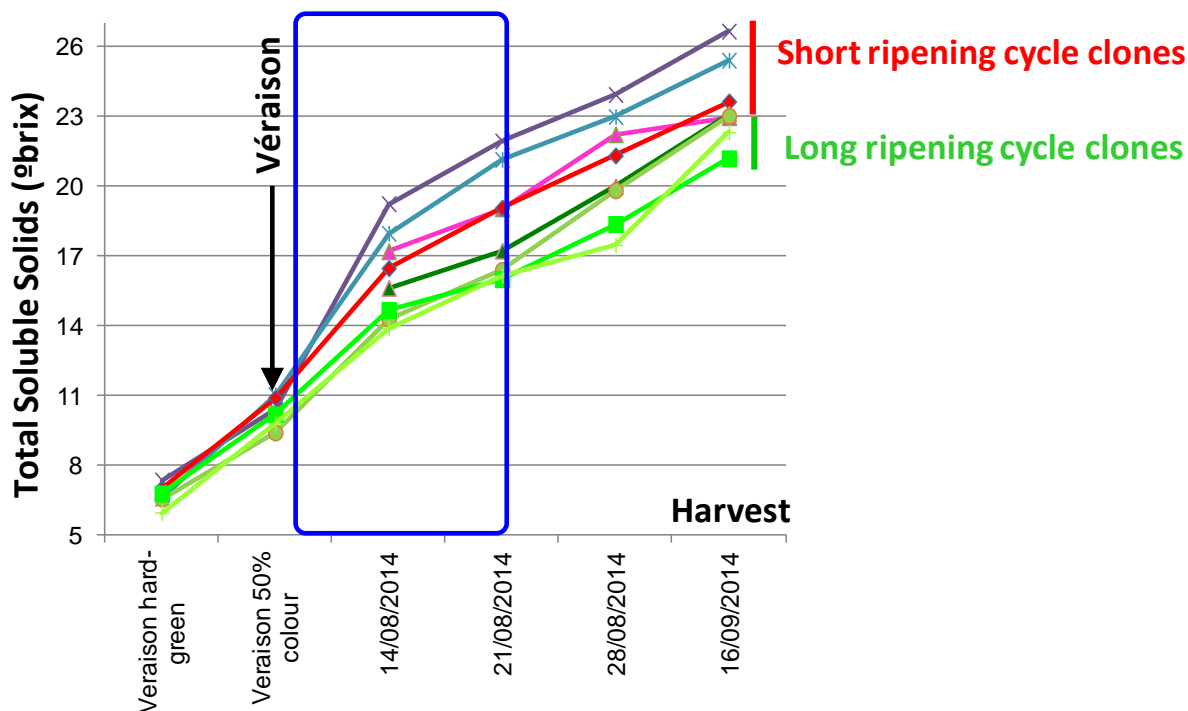


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3 Biological replicates



- **RNA-seq:**

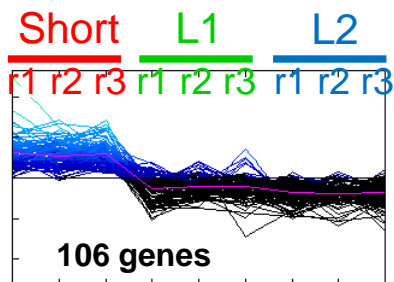
- 9 cDNA libraries (200 million of 125 bp paired-end reads)



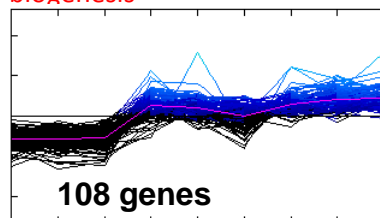
- **Analysis:**

- **DEGs** between the three clones (altered processes)
- **Candidate** responsible molecular variation:
 - **Regulatory mutations:** allele-specific over-expression (ASE)
 - **Polymorphism affecting protein sequence** (SNP/INDEL)

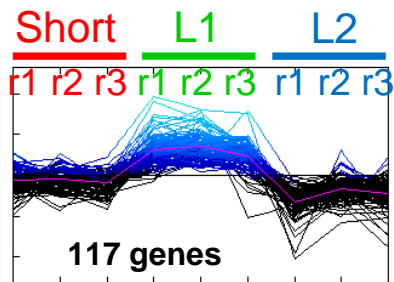
Differentially expressed genes (852): profiling and functional analysis



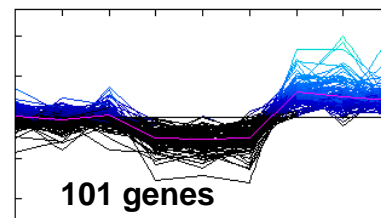
- Hormone signalling
- Carotenoid biosynthesis
- ABA biosynthesis
- Auxin signalling
- Cell growth
- Oil body organization and biogenesis



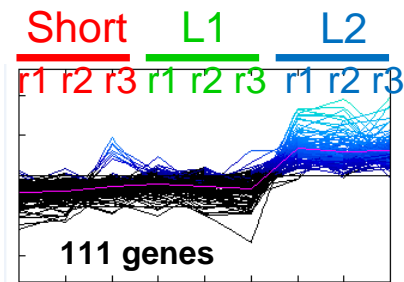
- Thylakoid targeting pathway
- Photosystem II
- Flavonoid biosynthesis
- Photosynthetic-chain phosphorylation
- Reaction center pigment biosynthesis
- Iron assimilation



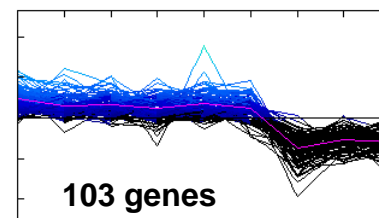
- Electropotential-gated transporters



none



- Wounding response
- Abiotic stress response



none

CANDIDATE GENES:

Evidences of *cis*-regulatory mutations: allele-imbalanced over-expressed DEGs

- Dominant over-expression somatic variant

the plant journal

The Plant Journal (2010) 61, 545–557

doi: 10.1111/j.1365-3113X.2009.04090.x

Transposon-induced gene activation as a mechanism generating cluster shape somatic variation in grapevine

Lucie Fernandez^{1,2}, Laurent Torregrosa³, Vincent Segura⁴, Alain Bouquet^{3,1} and José M. Martínez-Zapater^{1,2,*}

The Plant Journal (2013)

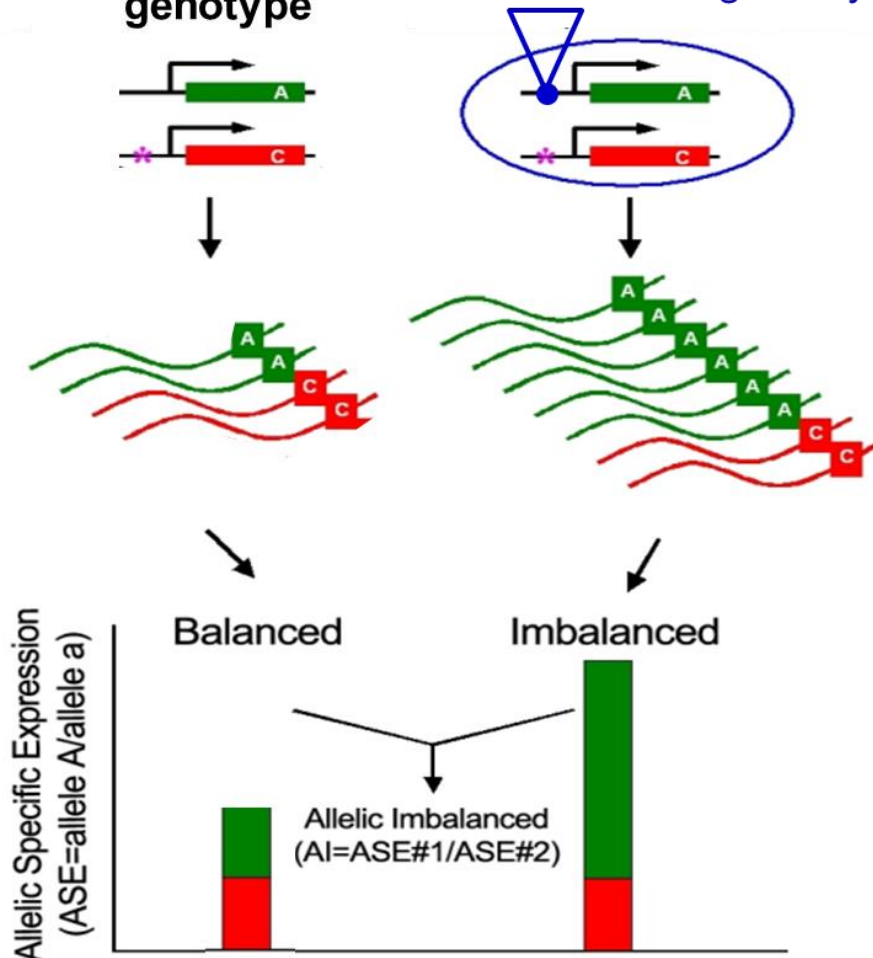
doi: 10.1111/tpj.12083

Mis-expression of a *PISTILLATA*-like MADS box gene prevents fruit development in grapevine

Lucie Fernandez^{1,2,1,1}, Jamila Chaib^{3,1}, José-Miguel Martínez-Zapater¹, Mark R. Thomas^{3,8} and Laurent Torregrosa^{2,*8}

- Ancestral genotype

- Somatic variant:
Mutation in regulatory region

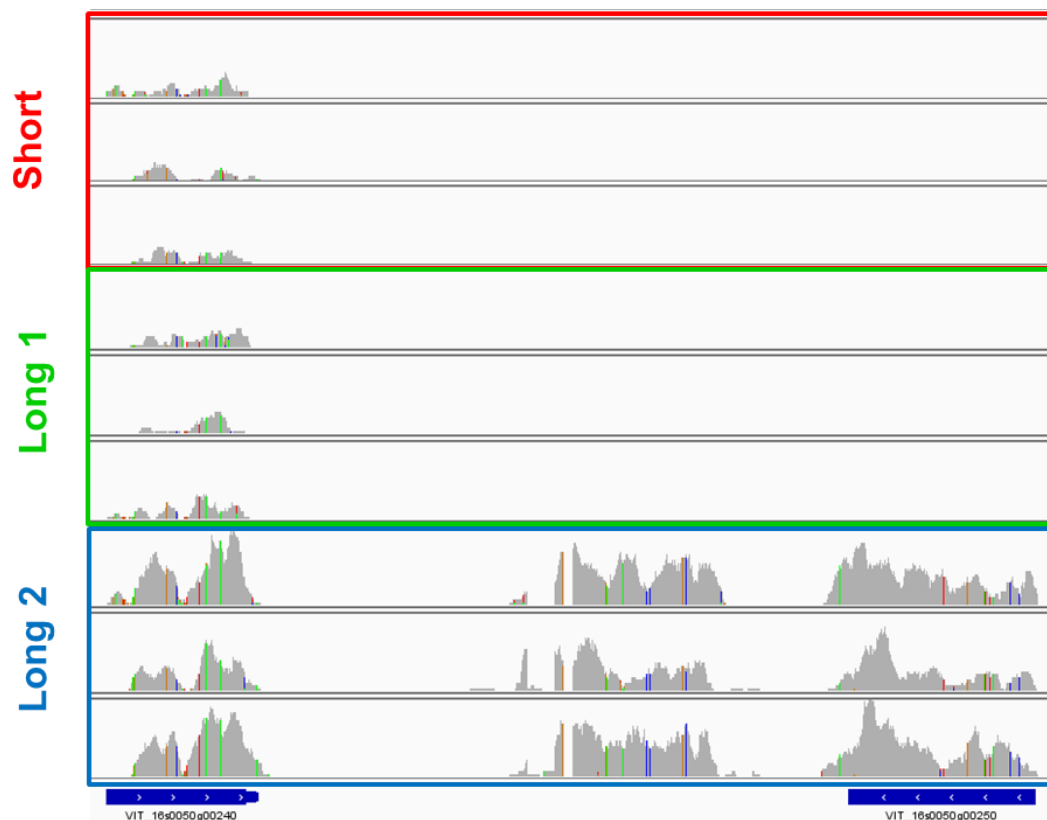


CANDIDATE GENES:

Evidences of *cis*-regulatory mutations: allele-specific over-expression

Long 2 Up

Unique ID	Functional annot	Short- FPKM	Long1- FPKM	Long2- FPKM
VIT_16s0050g00240	UDP-quercetin glycosyltransferase 88A4	0.6	0.6	2.6
Unannotated	LRR receptor-like	0	0	2.4
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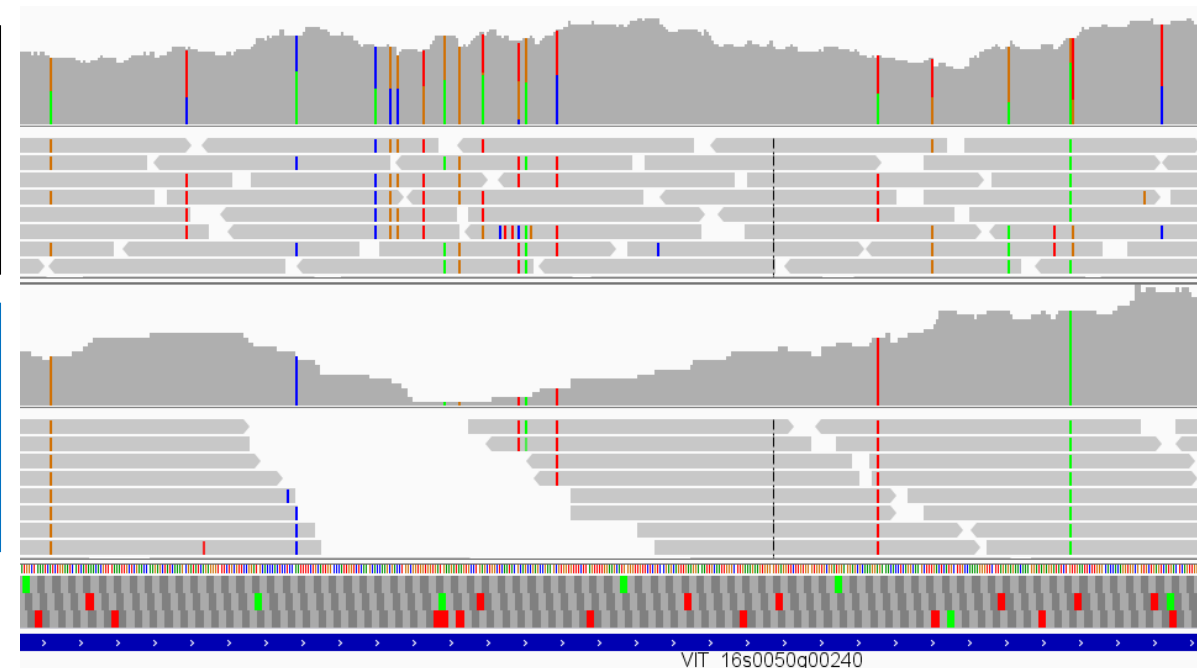
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Tempranillo
genome

Long 2
RNAseq

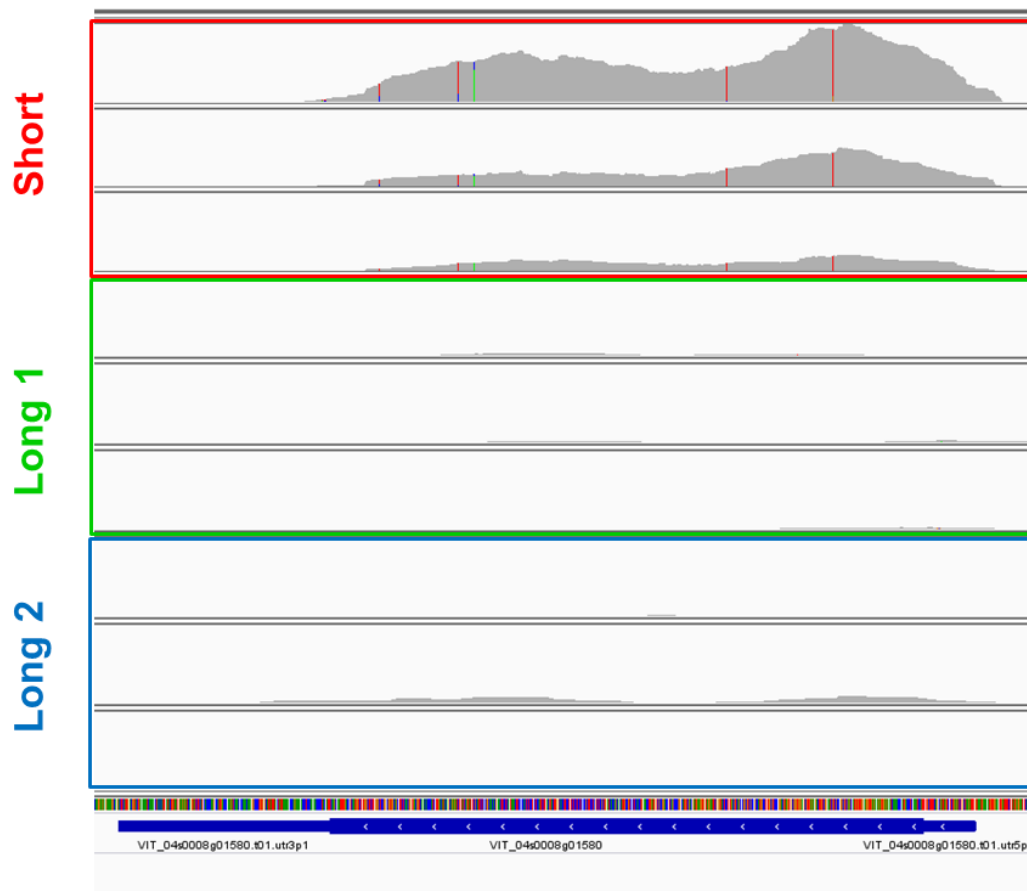


CANDIDATE GENES:

Evidences of *cis*-regulatory mutations: allele-specific over-expression

**Short cycle
clone Up**

Unique ID	Functional annotation	Short- FPKM	Long1- FPKM	Long2- FPKM	Observ
VIT_04s0008g01580	Heat shock protein	11.7	0.4	0.9	At-HSP17.6A is induced by heat, osmotic stress, and seed develop



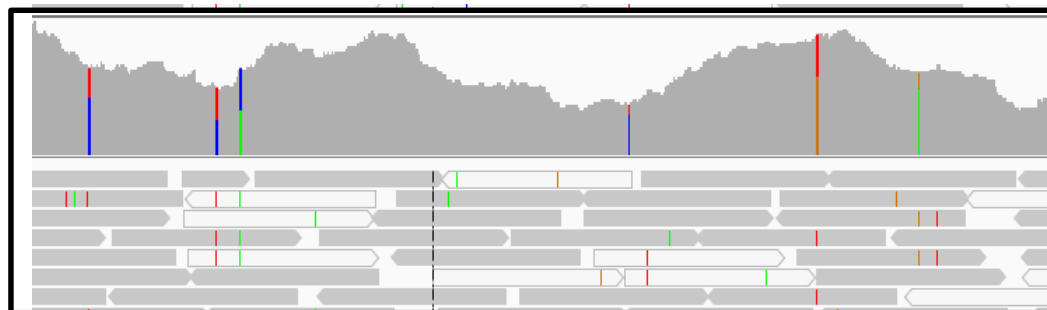
CANDIDATE GENES:

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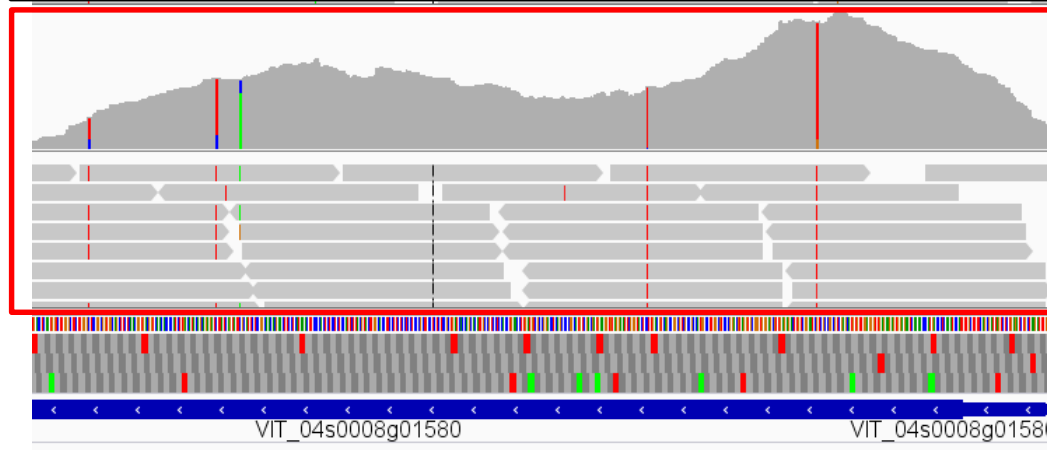
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**Tempranillo
genome**



**Short cycle
clone RNA-seq**

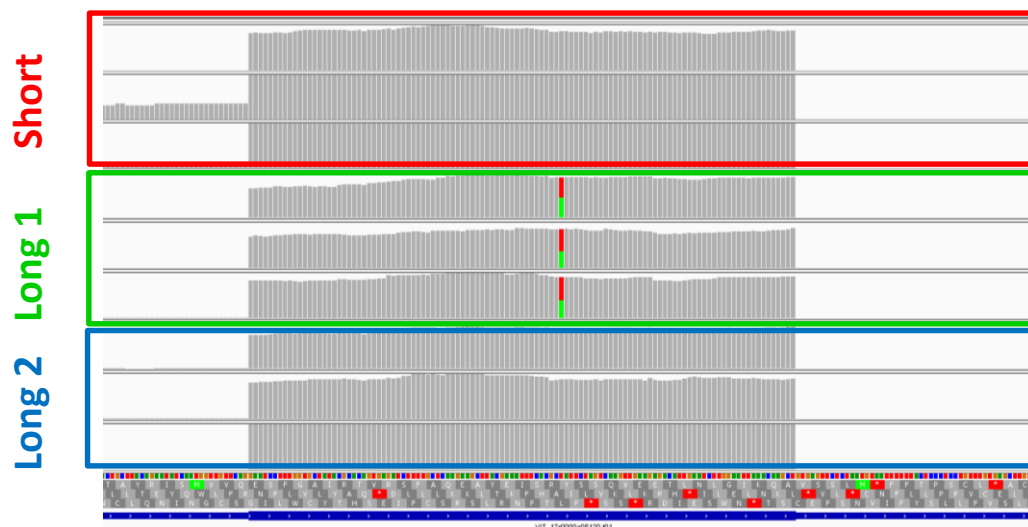


CANDIDATE GENES:

Candidate coding mutations: clone-specific polymorphisms

Only Heterozygous clone-specific SNPs detected

	Tempranillo clone		
	Short	Long 1	Long 2
SNPs	8	6	9



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Location	Gene	Gene annotation	Change	Prediction
Long 1-specific				
chr17:5623447	VIT_17s0000g05120	ABC TAP2 vacuolar AI-transporter	Tyr271Asn	Deleterious
Long 2-specific				
chr6:7807608	VIT_06s0004g07060	Ubiquitin activating enzyme E1	Phe227Leu	Deleterious

Conclusions

- **Somatic variation** generates new **ripening phenotypes**: clones potentially adaptable to climate change conditions.
- Transcriptomic analysis are useful to identify:
 - Sequence polymorphisms **distinguishing clones**.
 - Candidate **responsible alleles**: regulatory mutations and point mutations altering protein activity.
- Identified candidate genes involve molecular pathways previously **not related with ripening** control: putative gain of function mutations.

Thank you!!