

CHARACTERIZATION OF DELETIONS CAUSING BERRY COLOR VARIATION IN GARNACHA AND TEMPRANILLO

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ICGBG

XII International Conference on
GRAPEVINE BREEDING and GENETICS
July 15-20, 2018
Bordeaux FRANCE



Instituto de
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ESTACIÓN DE VITICULTURA Y ENOLOGÍA
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eta Toki Administrazioko Departamentua

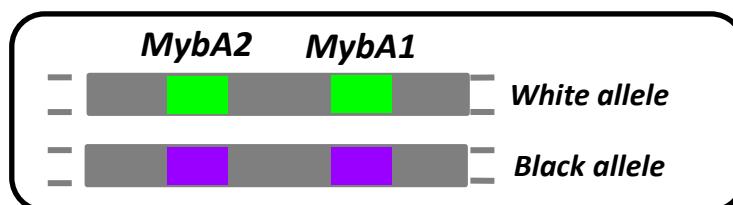
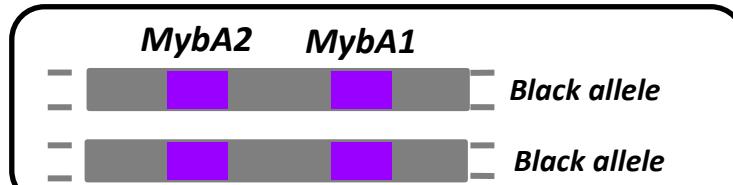


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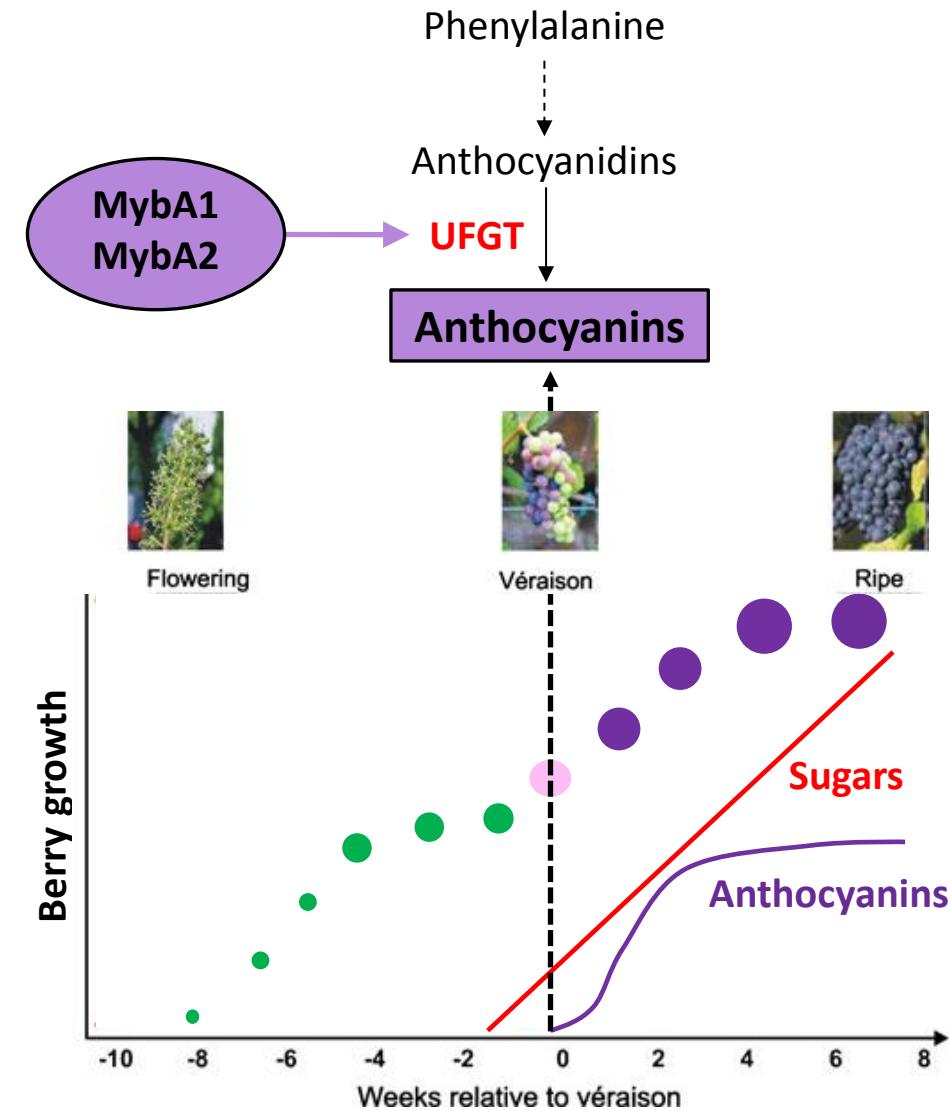
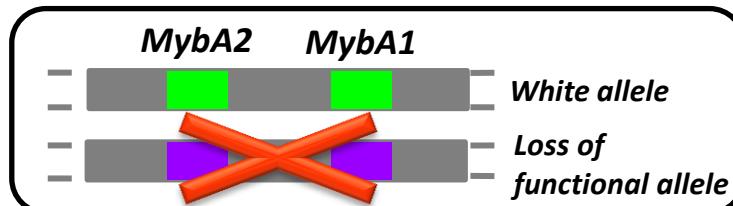
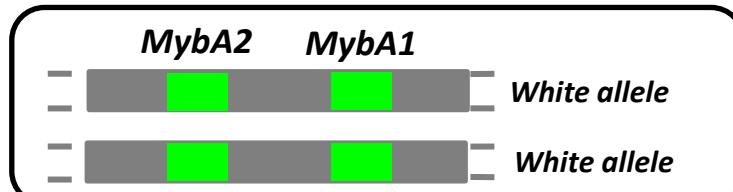
Grape ripening and anthocyanin accumulation



Chromosome 2



Chromosome 2

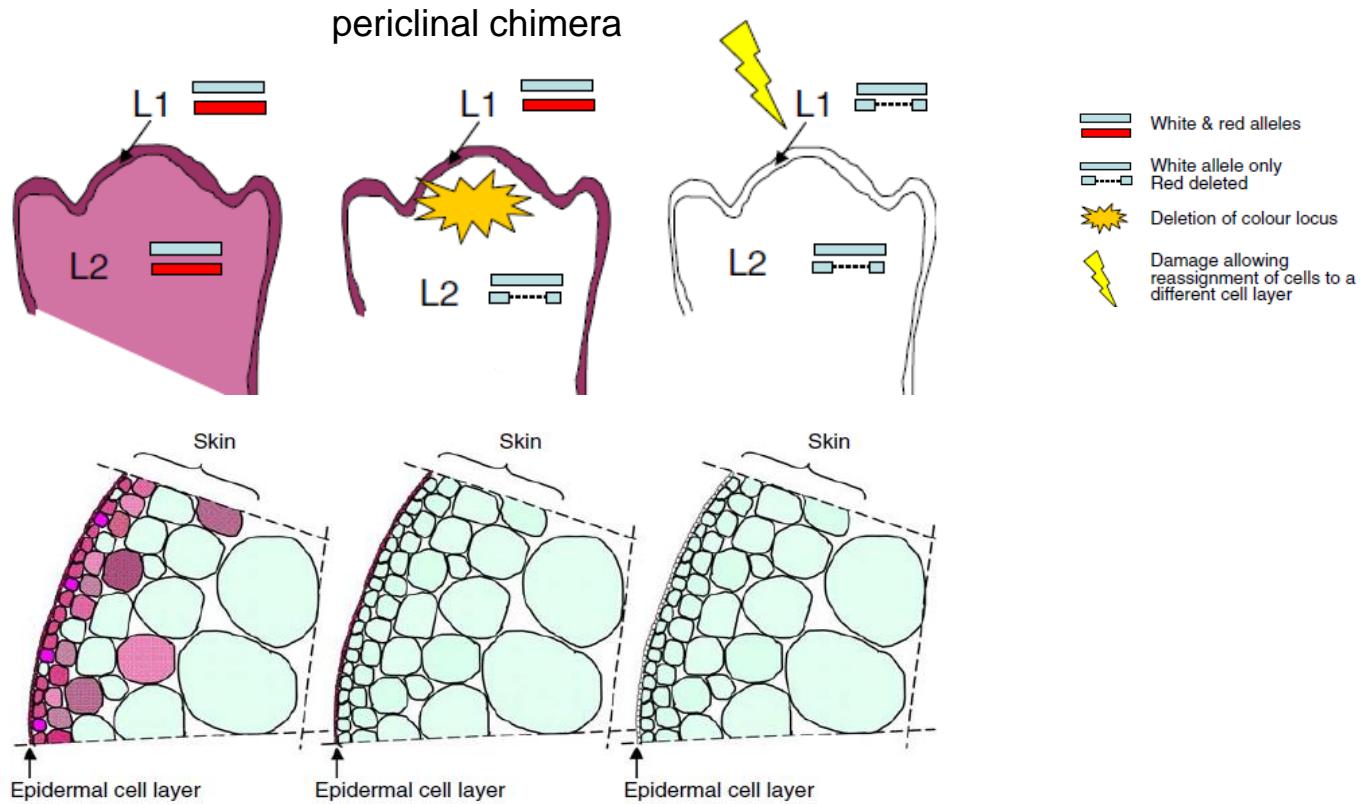


Background

Somatic Variation

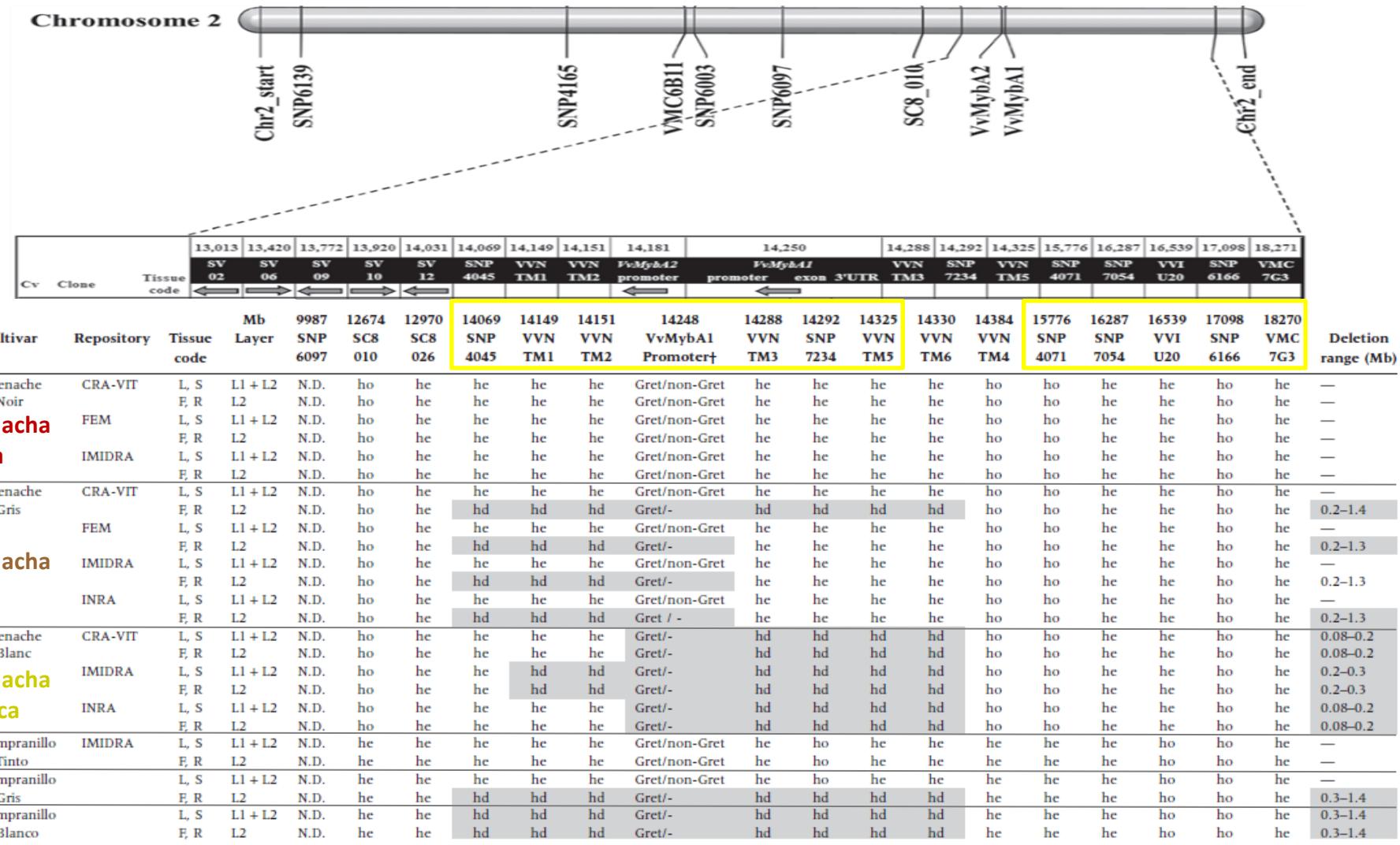


SAM



(Adapted from
Walker *et al* 2006)

Loss Of Heterozygosity around color locus on chr2

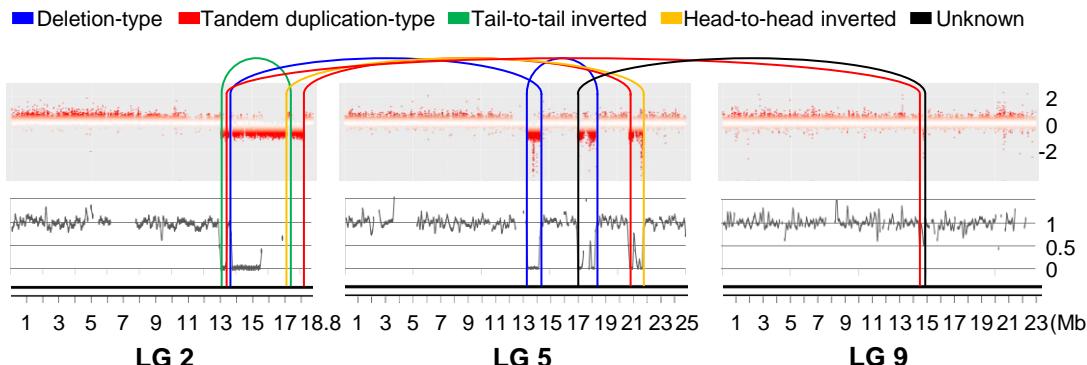


(Adapted from Vezzulli *et al* 2012 , Migliaro *et al* 2014)

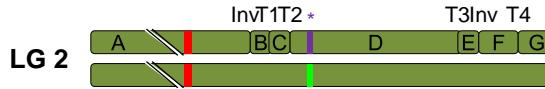
Tempranillo Tinto and Blanco Whole Genome Sequence

A

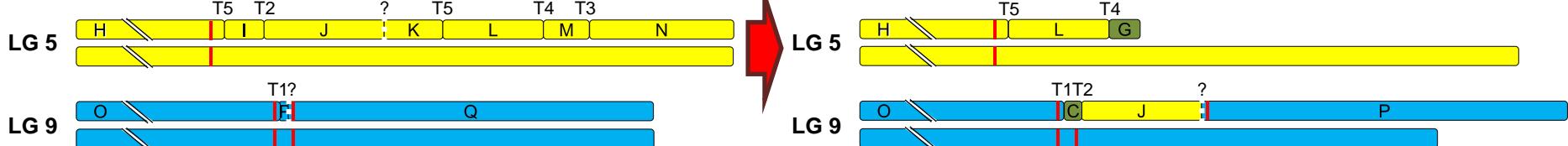
Structural rearrangement graph:

**B**

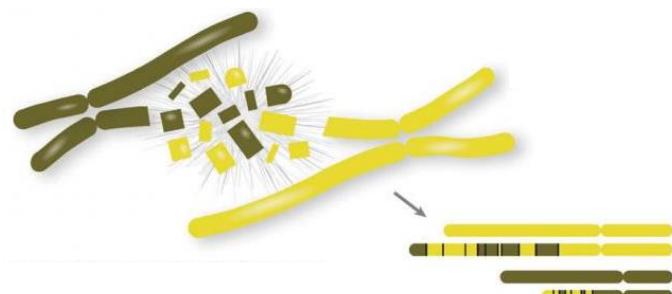
Original chromosomes



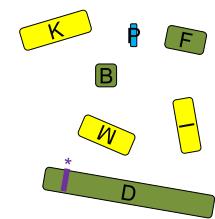
Rearranged chromosomes



Chromothripsis



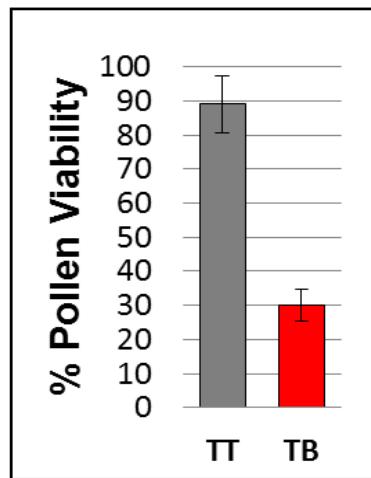
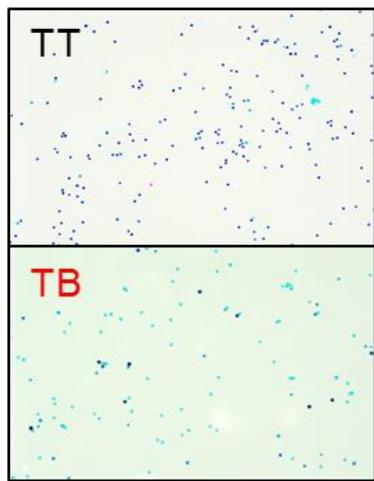
Lost fragments



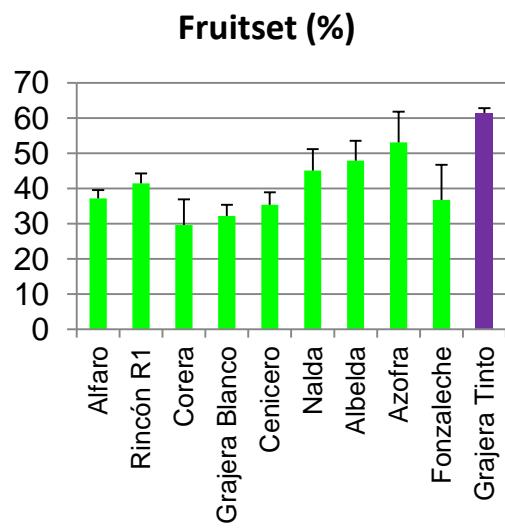
(Adapted from
Carbonell-Bejerano *et al* 2017)

Tempranillo Blanco Affected Traits

Rearranged chromosomes are gamete-lethal



Fruit set



Complex genome rearrangements cause side-effects in yield production

Tempranillo and Garnacha accessions

Tempranillo Tinto



6

Tempranillo Gris



9

Tempranillo Blanco



1

Garnacha Tinta



5

Garnacha Roja



2

Garnacha Blanca



60

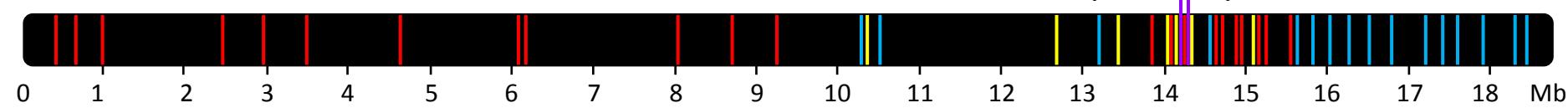
**I_cV_v**GB
21**LA RIOJA**

SNP genotyping along chromosome 2

Chromosome 2 LOH genotyping:
48 SNP chip:

- Heterozygous in Garnacha Tinta and Tempranillo Tinto
- Heterozygous in Garnacha Tinta
- Heterozygous in Tempranillo Tinto

Chromomose 2:



SNP selection from:

Lijavetzky et al., 2007, Carbonell-Bejerano et al., 2017, Laucou et al., 2018

Minor Allele Frequency (MAF) ≈ 0.5

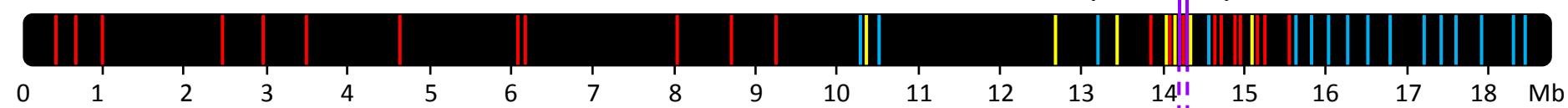
Loss Of Heterozygosity (LOH) delimits likely deleted fragments

Loss Of Heterozygosity in Tempranillo Gris

Chromosome 2 LOH genotyping:
48 SNP chip:

- Heterozygous in Garnacha Tinta and Tempranillo Tinto
- Heterozygous in Garnacha Tinta
- Heterozygous in Tempranillo Tinto

Chromomose 2:



Tempranillo Blanco WGS



Tempranillo Gris type 1 (1 accesión)



Tempranillo Gris type 2 (1 accesión)



Tempranillo Gris type 3 (3 accesión)



Tempranillo Gris type 4 (4 accesión)



Results obtained in roots = L2

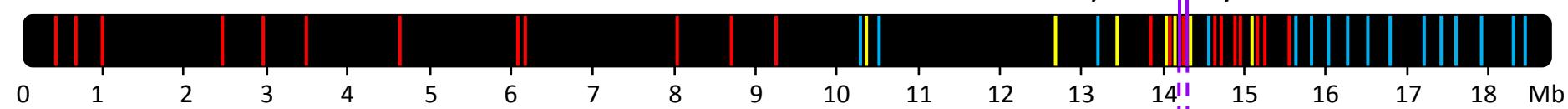


Loss Of Heterozygosity in Tempranillo Gris

Chromosome 2 LOH genotyping:
48 SNP chip:

- Heterozygous in Garnacha Tinta and Tempranillo Tinto
- Heterozygous in Garnacha Tinta
- Heterozygous in Tempranillo Tinto

Chromomose 2:



Tempranillo Blanco WGS

Tempranillo Gris type 1 (1 accesión)

Tempranillo Gris type 2 (1 accesión)

Tempranillo Gris type 3 (3 accesión)

Tempranillo Gris type 4 (4 accesión)

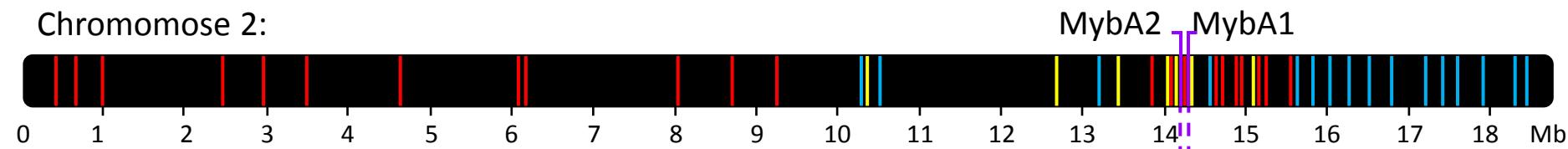
Tempranillo Blanco regeneration through somatic embryogenesis from Tempranillo Gris type 1

Loss Of Heterozygosity in Tempranillo and Garnacha

Chromosome 2 LOH genotyping:
48 SNP chip:

- Heterozygous in Garnacha Tinta and Tempranillo Tinto
- Heterozygous in Garnacha Tinta
- Heterozygous in Tempranillo Tinto

Chromomose 2:



Tempranillo Blanco WGS



Tempranillo Gris type 1



Tempranillo Gris type 2



Tempranillo Gris type 3



Tempranillo Gris type 4



Garnacha Blanca type 1 (21 accessions and 2 Garnacha Roja)



Garnacha Blanca type 2 (39 accessions)



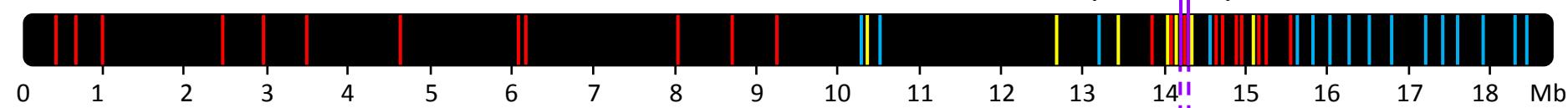
Loss Of Heterozygosity in Garnacha Blanca

Chromosome 2 LOH genotyping chip:

48 SNP

- Heterozygous in Garnacha Tinta and Tempranillo Tinto
- Heterozygous in Garnacha Tinta
- Heterozygous in Tempranillo Tinto

Chromomose 2:



Garnacha Blanca type 1:



Garnacha Blanca type 2:



Selection of two pairs of Garnacha for Whole Genome Sequencing (WGS)

Garnacha Whole Genome Sequence analysis in progress

Loss Of Heterozygosity (LOH)

Copy Number Variation (CNV):

Structural variation as deletions, insertions, and duplications.

*Plant Physiology**

- Catastrophic unbalanced genome rearrangements cause somatic loss of berry color in grapevine

Pablo Carbonell-Bejerano, Carolina Royo, Rafael Torres-Pérez, Jérôme Grimplet, Lucie Fernandez, Jose M. Franco-Zorrilla, Diego Lijavetzky, Elisa Baroja, Juana Martínez, Enrique García-Escudero, Javier Ibáñez, José M. Martínez-Zapater

Published August 2017. DOI: <https://doi.org/10.1104/pp.17.00715>

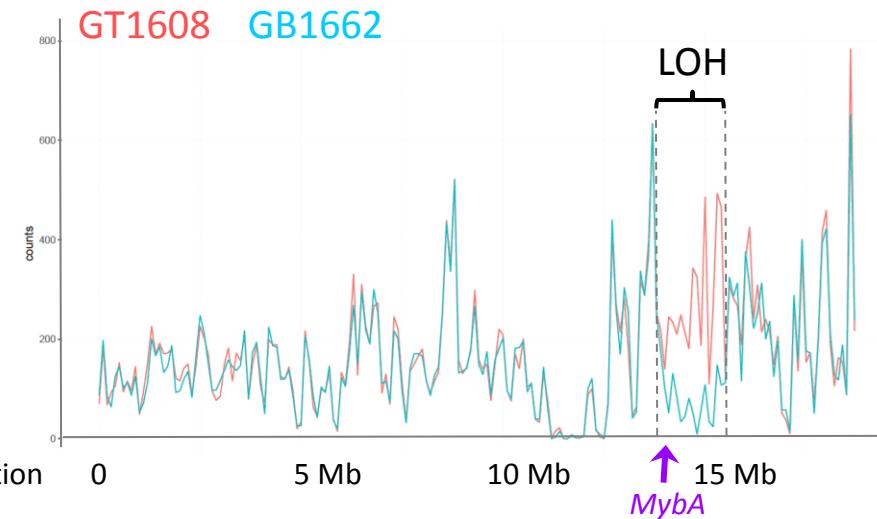
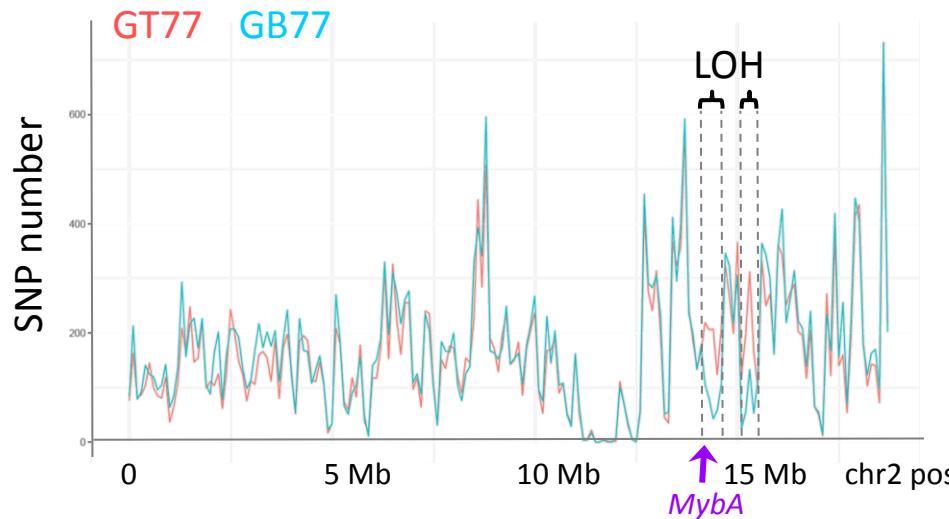


Garnacha WGS: Heterozygosity on chromosome 2

Garnacha Blanca type 1



Garnacha Blanca type 2



SNP genotyping:

chr2:14123701-14661047

chr2:14983912-15130499

chr2:14123701 -15130499

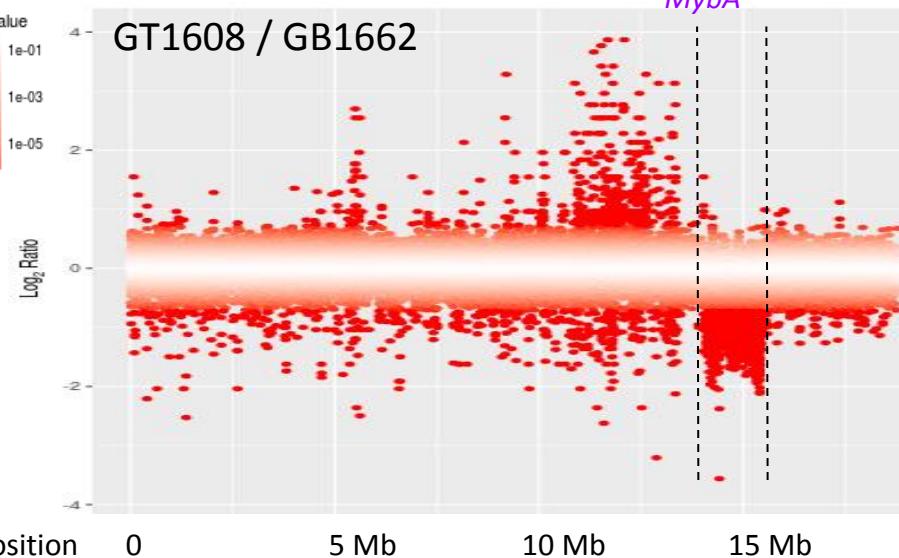
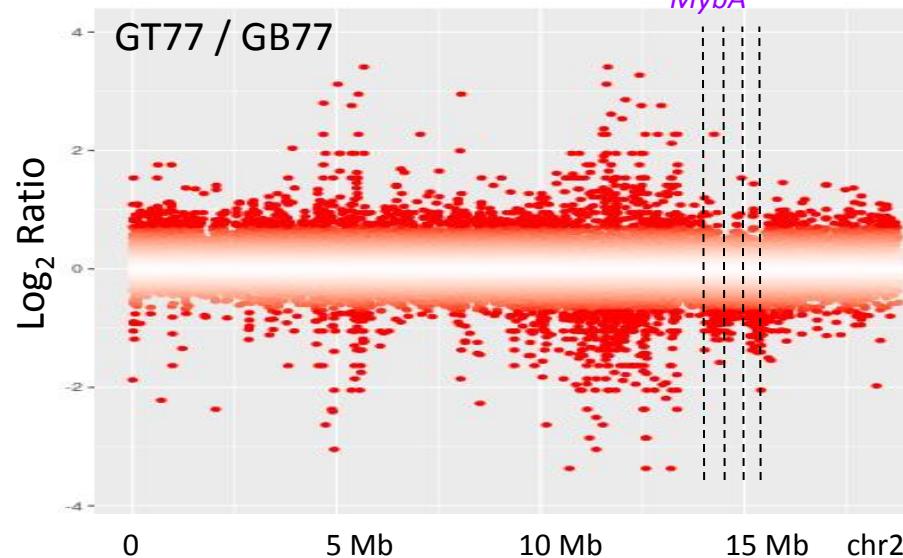
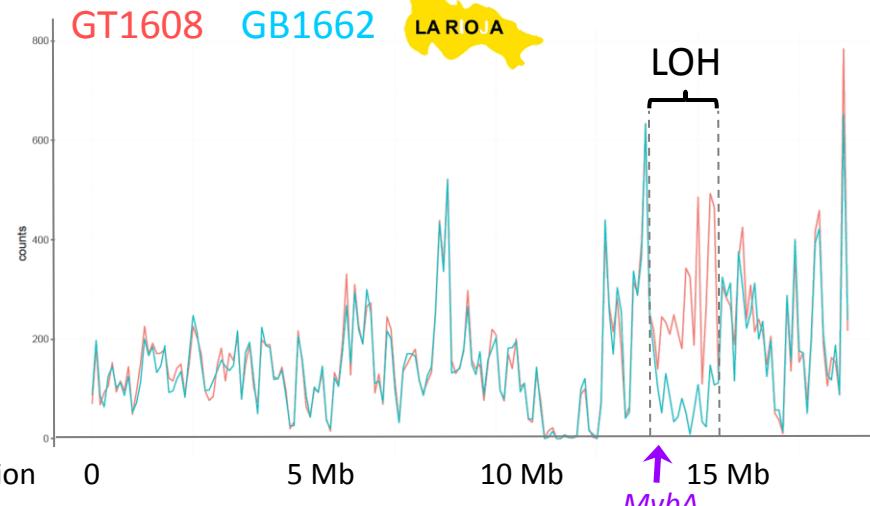
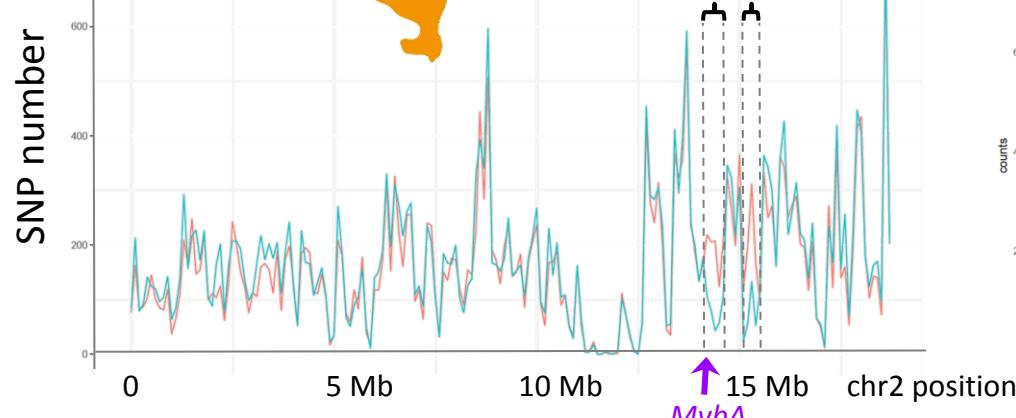


Garnacha WGS: LOH and Copy Number Variation on chromosome 2

Garnacha Blanca type 1



Garnacha Blanca type 2





Conclusions

1. The genotyping tool that we design proved useful to delimit deletions on chromosome 2.
2. The loss of color is related to hemizygous deletions at the color locus in all the variants studied by whole-genome sequencing.
3. In Tempranillo, multiple independent SV types were detected, including minimal deletion lines suited for the development of improved clones of Tempranillo Blanco.
4. In Garnacha Blanca, the results indicate the likely presence of recurrent SV fragile sites.



ACKNOWLEDGEMENTS



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Carolina Royo



THANKS FOR YOUR ATENTION



Carolina Royo