

Genetic variation for grapevine reproductive development

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Outline

- Introduction
 - Flowering induction/fertility
 - Cluster development
 - Berry development
- Understanding allelic variation
 - Fertility
 - Berry shape
 - Stenospermocarpy
- Conclusions



Grapevine reproductive development

Determines yield

- Fertility (60%)
- Cluster size (30%)
- Berry number and weight (10%)

Impacts berry and wine quality

- Cluster structure and compactness
- Berry diseases
- Berry ripening
- Skin to flesh ratio

Specific developmental features

- Two seasons flowering
- Tendril vs inflorescence
- Flower sex





Regulatory circuitry controlling flowering time in Arabidopsis



Blümel et al. Current Op. Biotech. 2015

Genes contributing to natural variation (QTL) for flowering time in Arabidopsis



Blümel et al. Current Op. Biotech. 2015



Reproductive development in grapevine

Flower induction and flower development take place in two consecutive growing seasons



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Coombe and Iland, 2004; Carmona et al., 2008

Factors controlling flowering induction in grapevine



Modified from Li-Mallet et al., Botany 2016

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Regulation of grapevine reproductive development

- Reproductive behavior and environmental interactions
- Genome sequence and annotation
- Transcriptional analyses of reproductive developmental processes

- Grapevine gene homologs
- Consistent expression patterns
- Limited genetic and molecular evidence:
 - Specific biological functions
 - Pathways and molecular mechanisms
 - Contribution to natural variation



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FT-TFL1 family

QTL analyses of flowering time



- Flowering time (FT) is independent from flowering initiation
- Moderate variation for FT
- Genotype x Environment interactions
- Frequently correlated with other phenological traits

Parent 1	Parent 2	LG	Reference
Italia	Big Perlon	1, 2, 6	Costantini et al. 2008
Riesling	Gewürztraminer	7, 14	Duchene et al. 2012
Syrah	Pinot Noir	7	Grzeskowiak et al. 2013
Schiava Grossa x Riesling	Börner	1, 10, 11, 14, 16, 17, 19	Fechter et al., 2014
Bacchus weiss x Seyval	Villard Blanc	1, 4, 8, 14, 17, 19	Fechter et al., 2014

Does flowering time in grapevine have the same meaning as in Arabidopsis



Genetic analyses of fertility

- Fertility Index: Cluster number per cane
- Ranges from 0,4 to 2,2 in cultivar collection
- Different genetic architecture in wine vs table grapes



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Parent 1	Parent 2	LG	Reference
Cabernet Sauvignon	Gloire de Montpellier	2, 18	Marguerit et al. 2009
Dattier de Beyrouth x 75 Pirovano	Alphonse Lavallée x Sultanine	5	Doligez et al. 2010
Olivette noire x Ribol	Muscat of Hamburg	5, 14	Doligez et al. 2010
Muscat of Hamburg	Sugraone	5, 14	Carreño Ruiz 2012
Syrah	Pinot Noir	3, 18	Grzeskowiak et al. 2013
V. rupestris x V. arizonica	Seedless table grape	1, 5, 6, 7, 12 13, 14, 19	Viana et al. 2013
Dominga	Autumn Seedless	50	Cabezas et al. (unpublished)
Red Globe	Crimson Seedless	5, 6, 10, 14	Diestro et al. (unpublished)



A role for gibberellins supported by Pinot Meunier somatic mutation in *VviGAI*

Boss and Thomas, Nature 2002

Regulation of cluster structure

- Wide variation for cluster size, shape and compactness
- Rachis length and branching pattern
- Flower number and fruit set
- Berry size
- Environmental factors and management practices



Genetic analyses of cluster traits

Ruby SDL x Sultanina F₁

Trait	LG	
Peduncle length	5a	
Rachis length	9, 17, 18b	
Rachis weight	5b	
Number of nodes main axis	14a, 18c	
Total number of berries	8, 14b, 18a	

Association analysis

Trait	LG
Compactness	7a
First bracnh length	7a, 12, 18
Total number of berries	7b
Iotal number of berries	/b

Tello et al Theor. Appl. Genet. 2016

Correa et al. Theor. Appl.Genet. 2014

GWAS for Cluster Weight on LG 13 (Laucou et al., *PLoS ONE* 2018)



A role for *VviTFL1A* is supported by cluster somatic variants

Carignan somatic variant RRM



Fernandez et al. Plant J. 2010





Similar phenotypes detected in Ugni Blanc and Garnacha



Fernandez et al unpublished

VviTFL1A position (LG 6) not detected in genetic analyses



Berry size and shape



2-4 times cell number

- Wide variation (1-10g)
- Many interacting components:
 - Pistil size and shape
 - Carpel number
 - Cell division and expansion after fruit set
 - Seed development
 - Seed content



1 cm



Houel et al. AJGWR 2013

Genetic analyses of berry size (weight)

- Berry size traits highly correlated with each other
- Many QTL analyses focused on seedless table grape

Parent 1	Parent 2	LG	Reference
Italia	Big Perlon	1, 12, 18	Costantini et al. 2008
Dominga	Autumn SDL	15, 18b, 18	Cabezas et al., 2006
Ruby SDL	Sultanina	18	Mejía et al. 2007
Dattier de Beyrouth x 75 Pirovano	Alphonse Lavallée x Sultanine	11, 17, 18	Doligez et al. 2013
Grenache	Syrah	8, 13, 17, 18b	Doligez et al. 2013
Picovine	Ugni Blanc	7	Houel et al. 2015
Red Globe	Crimson SDL	5, 8, 18, 19 [Diestro et al. (unpublished)

Interesting GWAS results on LG17 and other loci presented by Timothée Flutre and col.



Understanding allelic variation for reproductive traits



Could fertility be related with berry size and shape?



Fertility Index (clusters/cane)

RG

100



Red Globe (RG) Crimson SDL (CS)

292 F₁ segregants



In this progeny, Fertility Index is negatively correlated with Berry Volume, Berry Weight, Berry Length and Berry Shape Index



90 80 70 60 50 40 30 20 10 0,9 1 1,1 1,2 1,3 1,4 1,5 1,6 1,7 1,8

Berry shape index (length/diameter)

A major QTL on LG5 explains up to 50% of variation in Fertility Index



3 QTLs explaining 30% of total variance. LG **5**, 6 and 10 3 QTLs explaining 55% of total variance. LG **5**, 10 and 14

- Detected in three genetic maps (both progenitors and consensus)
- Co-localized QTL in six table grape progenies
- Non identified in two wine grape progenies

Consensus Map:



A major QTL on LG5 explains up to 20% of variation in Berry Shape



Red Globe: Crimson Seedless: Consensus map: 3 QTLs explaining 18.2% of total variance. LG **5**, **8** and **19** 5 QTLs explaining 24.0 % of total variance. LG 1, **5**, **8**, **10** and **18** 3 QTLs explaining 41.0 % of total variance. LG **1**, **5** and **8**

SHAPE and FER QTLs co-localize



Transcriptional analyses of contrasting fertility phenotypes





Transcriptional analyses of contrasting berry shape phenotypes

Sampling



G-H stage flowers RNA from 18 selected RG x CS F_1 siblings:





9 Elliptical-berried vs 9 Spherical-berried





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Transcriptional analyses of contrasting phenotypes



t-test *P*-value <0.05 ≥2-fold change

Significant DE transcripts: 104 Low fertility up: 77 / 12 in LG 5 / 5 in FER CI High fertility up: 27 / 8 in LG5 / 4 in FER CI

Four upregulated transcripts in low fertility FER and elliptical SHAPE are coincident



 Significant DE transcripts: 55
Elliptical Up: 23 / 6 in LG5 4 in SHAPE CI Spherical Up: 32 / 12 in LG5 / 6 in SHAPE CI



No hit, no candidate gene?





Expression of *No hit-2* in siblings with contrasting berry shape phenotypes and QTL genotypes





Common upregulated genes within the QTL belong to the same gene family





BUCENTAUR protein family

- Widespread distribution in eukaryotes
- Molecular function
 - Component of yeast chromatin remodeling complex SWR1-C
 - Known as Swc5 in yeast
 - Displacement of H2A/H2B by H2A.Z/H2B dimers in nucleosomes
- Biological function
 - Yeast defective mutants are viable
 - Essential for metazoan embryo development
 - Unknown function in plants



Sun and Luk, Nucleic Acids Res. 2017



Biological function of SWR1 Complex in plants

- Mutants altered in components of the SWR1 complex show pleiotropic phenotypes
- Involved in temperature regulation of flowering
- Consistent with the pleiotropic effects observed in grapevine





Completely hypothetical for the FER/SHAPE locus



Seedlessness

Seedlessness is a major trait in table grape breeding



Fruit set



Stenospermocarpy:

- Viable embryo development
- Seed coat development is incomplete
- Endosperm degeneration
- Aborted seeds remain as seed traces
- Berry size less affected than in parthenocarpy

Maturity





Stenospermocarpy first originated as a somatic mutation in Sultanina

Most seedless varieties derive from Sultanina

Control by a major dominant locus *SDI* interacting with three recessive loci (Bouquet and Danglot, 1996) Stenospermocarpy behaves as a quantitative trait





Bouquet and Danglot, *Vitis*Doligez et al. *Theor. Appl. Genet.*Cabezas et al. *Genome*Mejía et al. *Am. J. Enol. Vitic.*Costantini et al. *BMC Plant Biol.*Mejía et al. *BMC Plant Biol.*Carreño Ruiz et al. PhD Thesis 2012

Doligez et al. *BMC Plant Biol.*Di Genova et al. *BMC Plant Biol.*Wang et al. *Mol. Genet. Genomics*Ocarez and Mejia *Plant Cell Rep.*Wang et al. *BMC Genomics*Malabarba et al. *J. Exp. Bot.*



Stenospermocarpy is determined by a major QTL on LG18



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Royo et al., Plant Physiol. 2018



Expression analyses of candidate genes

RNA-seq: screening for candidate SDI mutations

Red Globe × Crimson Seedless F_1 Seeds from pea-size fruits 4WAF



No DEG detected among the 14 candidate genes of the interval



Expression analyses of *VviAGL11*

- Tested for expression between seeds and seed traces
- Analyzed for the presence of specific allelic expression imbalance



Expression of AGL11 alleles in RG×CS F_1 :





Sequence variation of candidate genes

RNA-seq: screening for candidate *SDI* mutations

- Sixty eight SNV specific of the *Sdi* haplotype within the 14 gene interval
- Six missense amino acid substitutions in 4 genes
- Three predicted deleterious amino acid substitutions in two genes
 - VviPPAT2 Phospho-pantethein-adenylyl transferase (2 SNV)
 - VviAGL11 Vitis homolog of Arabidopsis Seedstick (1 SNV)



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Sequence variation at *VviPPAT2*

VviPPAT2 SNV were sequenced in 93 varieties (73 seeded, 20 seedless)





Sequence variation at VviAGL11

VviAGL11 gene plus 2-kb upstream sequence re-sequenced in 132 accessions (111 seeded, 21 seedless)





Seeded variants of Sultanina have lost the seedless specific *VviAGL11* mutation



Some accessions of Sultanina could still be chimeric somatic variants for seedlessness



Possible biological function of *SDI* in grape seeds

Oil palm domestication:

missense mutation in AGL11/SHELL reduces coconut lignification



Singh et al., Nature 2013

Grape stenospermocarpy: Defects in endotesta lignification



Pinot Noir Sultanina Malabarba et al., *J Exp Bot* 2017



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Conclusions

Complexity of reproductive development Phenotypic variation Sequence variation Focus on understanding natural variation Integrate genetics with genomics Integrate information and resources

Acknowledgements

VITIGEN Group

Pablo Carbonell-Bejerano Carolina Royo Rafael Torres-Pérez Javier Ibáñez Nuria Mauri Jérôme Grimplet **Javier Tello** Nieves Diestro Lara Pereira











Elisa Baroja Enrique García-Escudero Juana Martínez



Juan Carreño José A. Cabezas Laurent Torregrosa Cécile Marchal Natalie Ollat



Instituto Nacional de Investigación

y Tecnología Agraria y Alimentaria

Collaborators



Manuel Tornel

Lucie Fernández

Thierry Lacombe

Diego Lijavetzky

Serge Delrot



Thanks for your attention