



Caffè Scientifico

Il miglioramento genetico della vite:
quando tradizione e innovazione si
incontrano

Clizia Villano

Division of Grape and Wine Sciences, Avellino
University of Naples Federico II
Department of Agricultural Sciences

<http://www.viticulturaenologia.unina.it/>

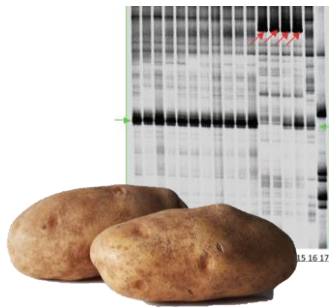
22/09/2021

Just few words about myself



2009

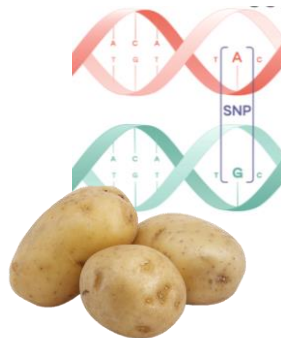
Bachelor's degree in Plant Biotechnology



Molecular markers in potato

Master's degree in Crop Biotechnology

2011

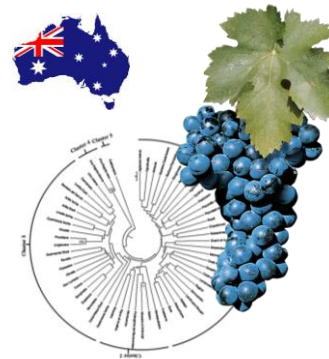


Allele mining for chr dosage in potato



2015

PhD in Plant Breeding and Genetics



Discovery and genotyping of DNA sequence variation in grape

RTDA AGR07
Division of Grape & Wine Sciences,
Avellino

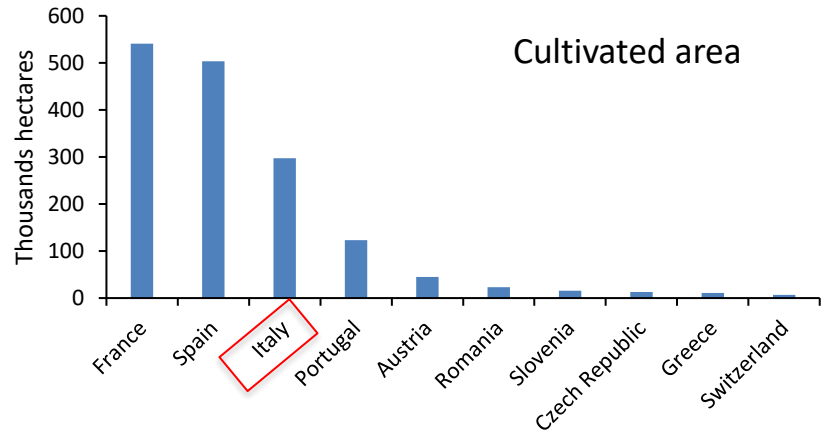
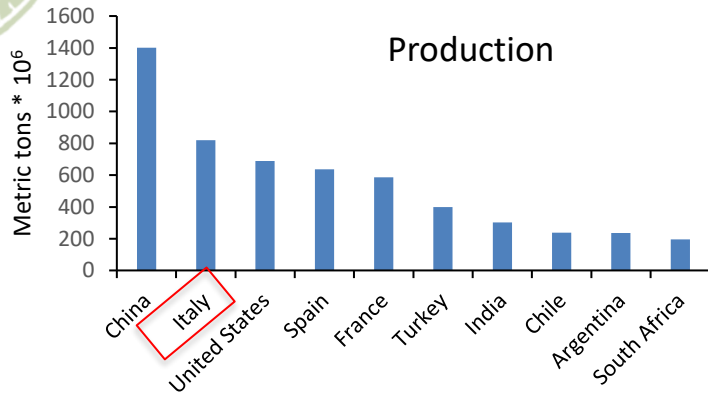
2017



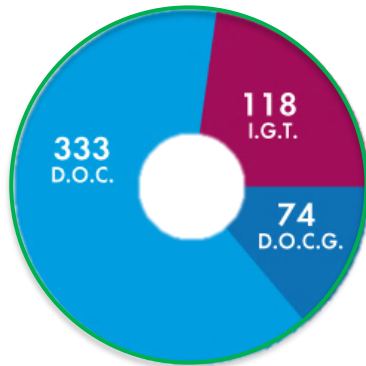
Grape biodiversity
NGS
Resistance to fungi



The importance of grapevine



FAOSTAT, 2019



Italian DOCG, DOC and IGT wines



Campania DOCG, DOC and IGT wines



**Wines' variability derive from
plant variability**





The viticulture in the world



The international varieties tend to replace autochthonous ones

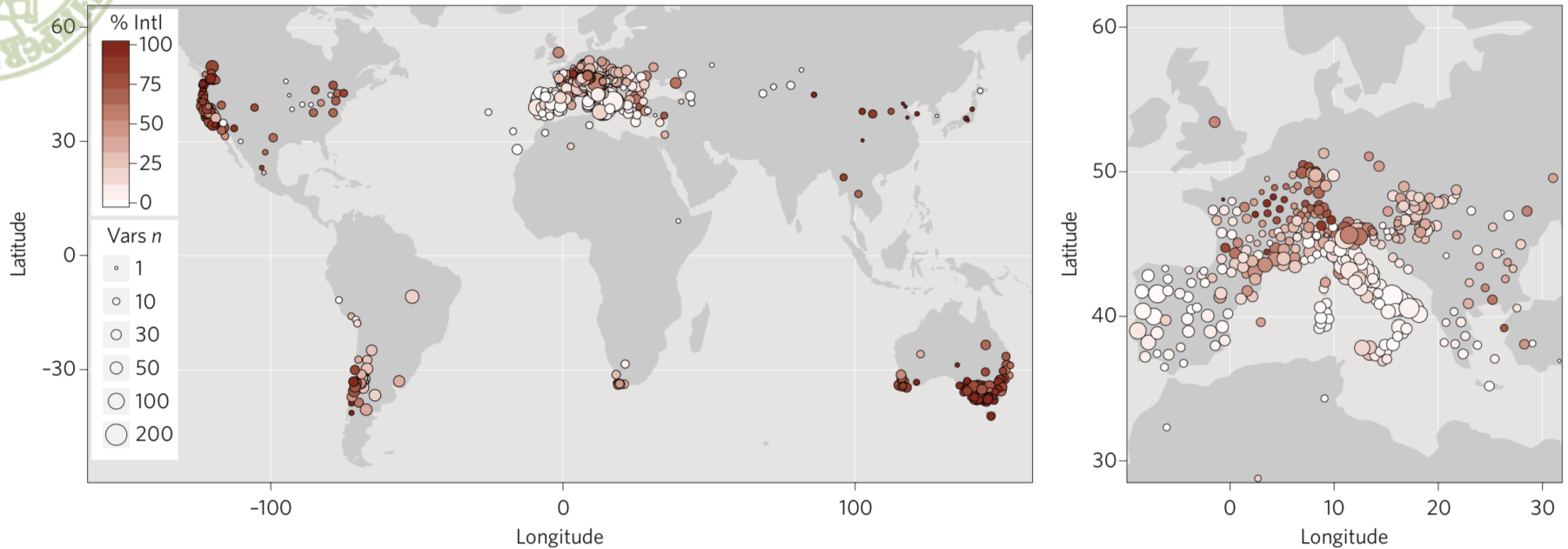


Fig. 1 | Current planted diversity of wine grapes. The number of varieties ('Vars n ') by region, and the percentage of each region's hectares planted with common 12 varieties ('% Intl', called international varieties) varies across the globe, with Europe growing the greatest number of different varieties (largest circles) and New World wine regions growing the greatest proportion of international varieties (darkest circles). Data from ref. ⁴⁷.

Wolkovich et al. 2018

Our varieties need to be protected!



Grapevine varieties: a priceless treasure



Genetic analysis

Molecular breeding to improve traits of interest

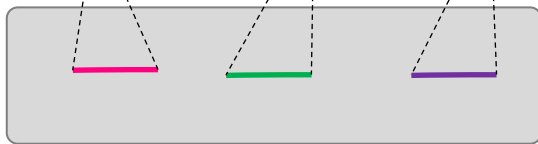
PLANT
SAMPLING



DNA
EXTRACTION



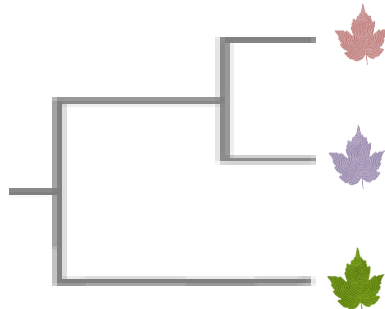
MOLECULAR
MARKERS



RESULTS
ANALYSES



PEDIGREE
RECONSTRUCTION

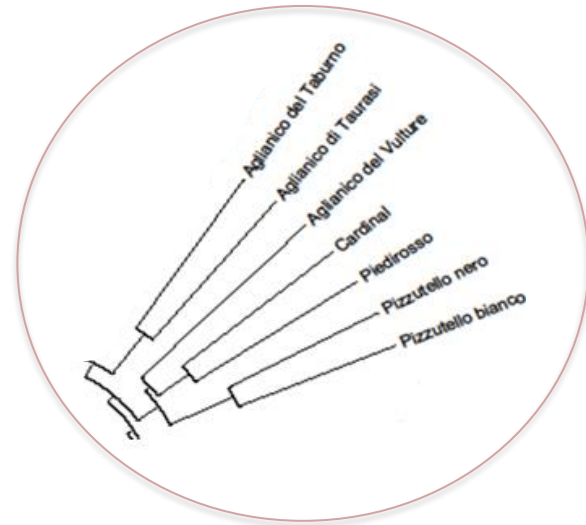
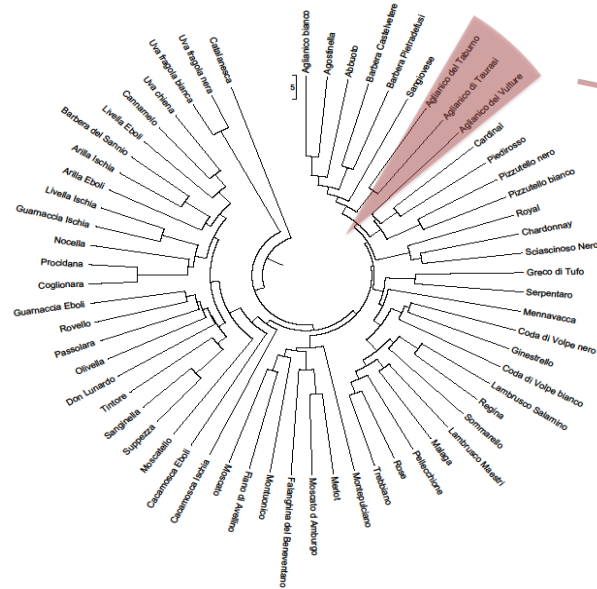




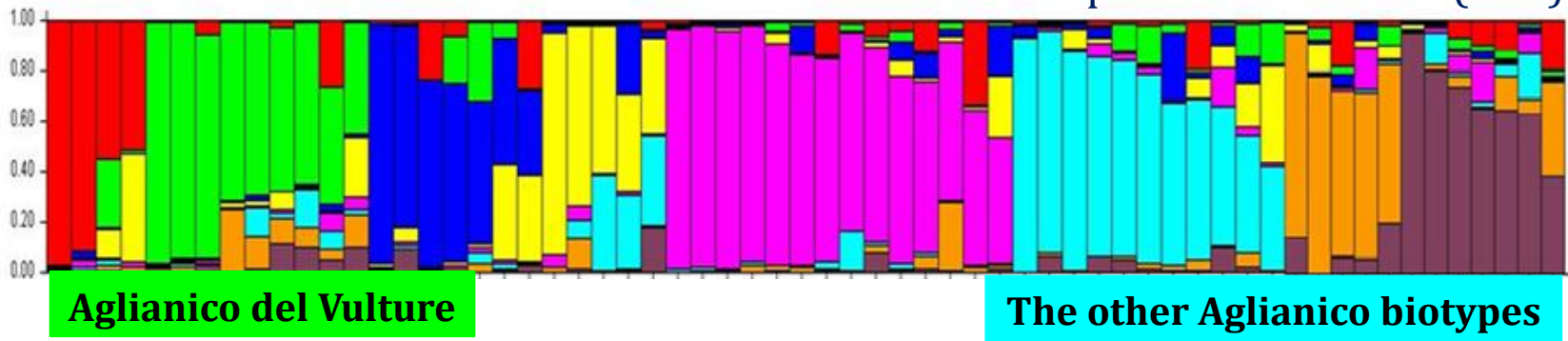
Analysis of grape populations



Phylogeny



Population structure (K=8)





Analysis of grape populations

Mol Biotechnol
DOI 10.1007/s12033-014-9780-y

RESEARCH

Use of SSR and Retrotransposon-Based Markers to Interpret the Population Structure of Native Grapevines from Southern Italy

Clizia Villano · Domenico Carputo ·
Luigi Frusciante · Xenia Santoro · Riccardo Aversano

- Catalanesca resulted unique;
- Procidana = Coglionara;
- Among 3 biotypes, Barbera del Sannio is genetically different;



Analysis of grape populations

Mol Biotechnol
DOI 10.1007/s12033-014-9780-y

RESEARCH

Use of SSR and Retrotransposon-Based Markers to Interpret the Population Structure of Native Grapevines from Southern Italy

Clizia Villano · Domenico Carputo ·
Luigi Frusciante · Xenia Santoro · Riccardo Aversano

**Availability of a SSR
markers database**



**WE CAN INVESTIGATE ON
GENETIC INTER- AND INTRA-
VARIETAL DIVERSITY**



New projects and collaborations



DICOVALE

in collaborazione con altre UO del DiA



SUVAI

Studio sull'uso dei vinaccioli in ambiente agro-industriale

in collaborazione col Prof. Forino

RINNOVALA

Ripopolamento Innovativo
Vitigno dell'Aglianico Lasco
dell'Arianese



Team:
Prof. Blaiotta, Prof. Aversano,
Prof. Basile, Prof. Forino, la
prof.ssa Piombino e la
Prof.ssa Gambuti

INDIGENA

Camaiola and Barbera del
Sannio



Team:
Prof. Aversano, Prof.
Blaiotta, Prof. Basile, Prof.
Vecchio, Prof. Caracciolo di
Torchiarolo e la Prof.ssa
Gambuti

Exploiting our varieties!



ELSEVIER

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Metabolic and RNA profiling elucidates proanthocyanidins accumulation in Aglianico grape

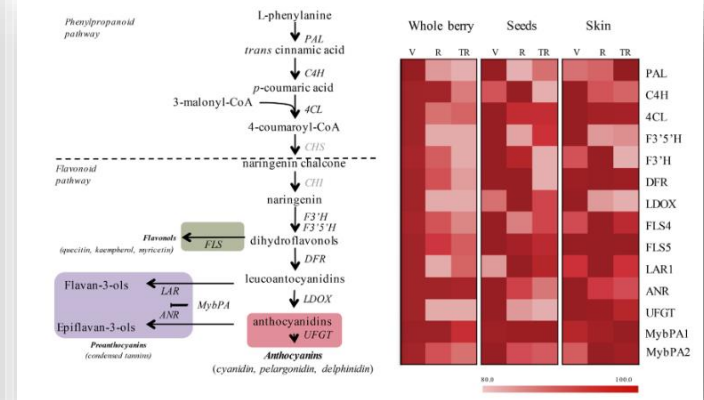
Alessandra Rinaldi^{a,b,1}, Clizia Villano^{c,1}, Carmine Lanzillo^c, Angelo Tamburrino jr^c, Michael Jourdes^d, Pierre-Louis Teissedre^d, Luigi Moio^a, Luigi Frusciante^c, Domenico Carputo^c, Riccardo Aversano^{a,*}

^aUniversità degli Studi di Napoli Federico II, Department of Agricultural Sciences, Division of Grape and Wine Sciences, Viale Italia, 83100 Avellino, Italy

^bBiolaflort, 126 Quai de la Souys, 33100 Bordeaux, France

^cUniversità degli Studi di Napoli Federico II, Department of Agricultural Sciences, Division of Plant Genetics and Biotechnology, Via Università, 100, 80055 Portici, Italy

^dUniversité Bordeaux Segalen, USC 1366, EA 4577, Oenologie INRA-UBS-IPB, ISVV, 210 chemin de Leysotte, CS 50008, 33882 Villenave d'Ornon Cedex, France



Aglianico seed extracts can induce apoptosis in a largely dose and time-dependent manner in mesothelioma cell lines.



Journal of Functional Foods 61 (2019) 103515



ELSEVIER

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff



Anti-cancer activity of grape seed semi-polar extracts in human mesothelioma cell lines

Francesco Di Meo^{a,b}, Riccardo Aversano^{c,*}, Gianfranco Diretto^d, Olivia Costantina Demurtas^d, Clizia Villano^c, Salvatore Cozzolino^b, Stefania Filosa^{a,c}, Domenico Carputo^c, Stefania Crispi^{a,*}

^aInstitute of Biosciences and BioResources-UOS Naples CNR, via P. Castellino, 111-80131 Naples, Italy

^bDepartment of Biology, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, via Cinthia, Naples, Italy

^cDepartment of Agricultural Sciences, University of Naples Federico II, Via Università 100, Portici, Italy

^dDepartment of Sustainability (SSPT), Biotechnology Laboratory, ENEA, Casaccia Research Center, Via Anguillarese 301, Rome, Italy

*IRCCS Neuromed, Pozzilli, IS, Italy





Food traceability



Analysis of food fraud relevant keywords of 984 papers with a word cloud generator.



Visciano and Schirone, 2021

Wine adulteration

- Intrinsic → addition of water, sugar, colouring, flavouring
- Extrinsic → fraudulent misrepresentation of the cultivar and its geographical origin



Holmberg, 2010



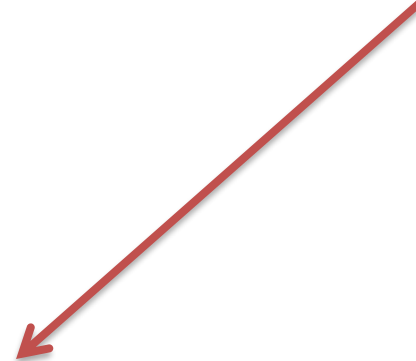
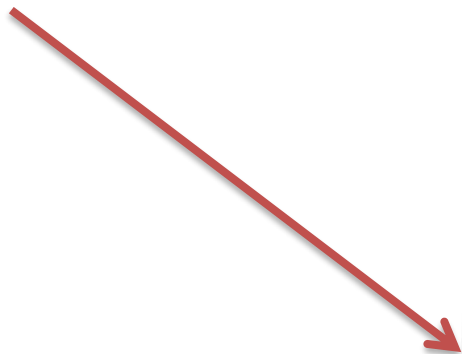
Wine varietal authentication



VOLATILE COMPOUNDS

PHENOLIC COMPOUNDS

MOLECULAR MARKERS





Wine varietal authentication



Food Control 80 (2017) 1–10

Contents lists available at [ScienceDirect](#)



Food Control

journal homepage: www.elsevier.com/locate/foodcont

Review

Wine varietal authentication based on phenolics, volatiles and DNA markers: State of the art, perspectives and drawbacks

[Clizia Villano](#)^{a,1}, [Maria Tiziana Lisanti](#)^{b,1}, [Angelita Gambuti](#)^b, [Riccardo Vecchio](#)^b, [Luigi Moio](#)^b, [Luigi Frusciante](#)^a, [Riccardo Aversano](#)^{b,*}, [Domenico Carputo](#)^a



VOLATILOME

PHENOLIC COMPOUNDS

MOLECULAR MARKERS



Take-home message:
Wine varietal authentication is possible!

DNA sequencing: encoding the code!



The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla

The French-Italian Public Consortium for Grapevine Genome Characterization*

2007

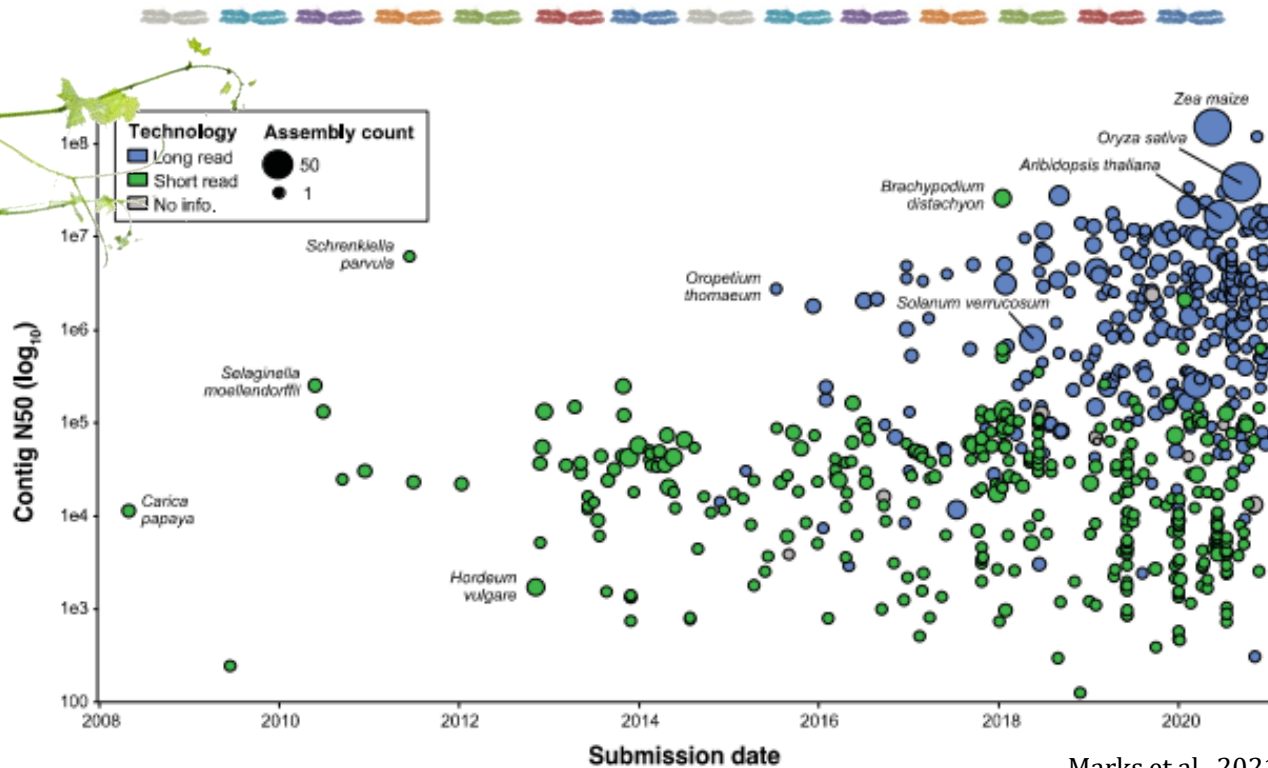
The analysis of the first plant genomes provided unexpected evidence for genome duplication events in species that had previously been considered as true diploids on the basis of their genetics¹⁻³. These polyploidization events may have had important consequences in plant evolution, in particular for species radiation and adaptation and for the modulation of functional capacities⁴⁻¹⁰. Here we report a high-quality draft of the genome sequence of grapevine (*Vitis vinifera*) obtained from a highly homozygous genotype. The draft sequence of the grapevine genome is the fourth one produced so far for flowering plants, the second for a woody species and the first for a fruit crop (cultivated for both fruit and beverage). Grapevine was selected because of its important place in the cultural heritage of humanity beginning during the Neolithic period¹¹. Several large expansions of gene families with roles in aromatic

All grapevine varieties are highly heterozygous; preliminary data showed that there was as much as 13% sequence divergence between alleles, which would hinder reliable contig assembly when a whole-genome shotgun strategy was used for sequencing. Our consortium therefore selected the grapevine PN40024 genotype for sequencing. This line, originally derived from Pinot Noir, has been bred close to full homozygosity (estimated at about 93%) by successive selfings, permitting a high-quality whole-genome shotgun assembly.

A total of 6.2 million end-reads were produced by our consortium, representing an 8.4-fold coverage of the genome. Within the assembly, performed with Arachne¹², 316 supercontigs represent putative allelic haplotypes that constitute 11.6 million bases (Mb). These values are in good fit with the 7% residual heterozygosity of PN40024 assessed by using genetic markers. When considering only



DNA sequencing: encoding the code!





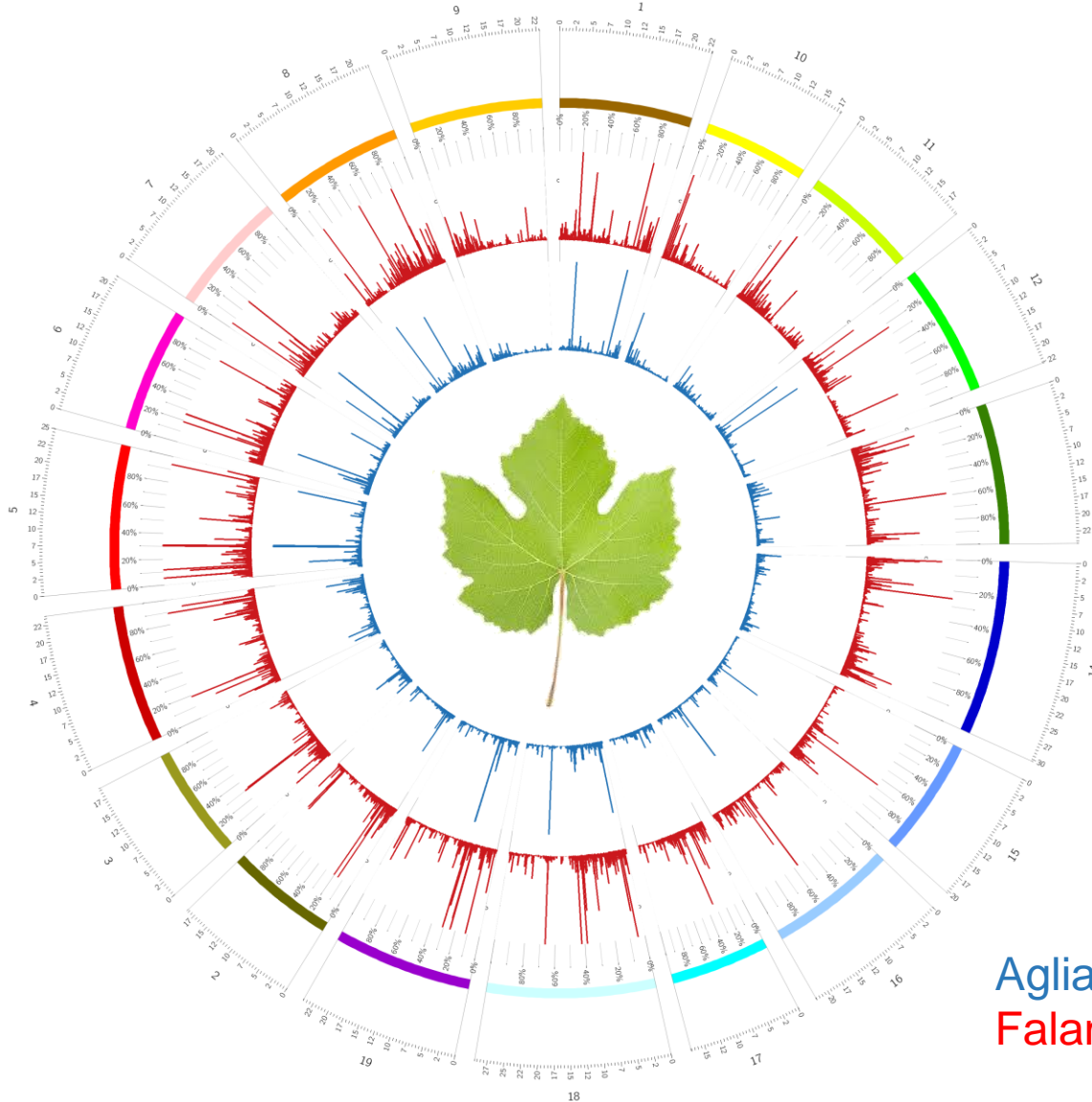
NGS in Aglianico and Falanghina



Genome sequencing



la **guardiense**



Aglianico
Falanghina



Grapevine varieties: a priceless treasure



Genetic analysis

Molecular breeding to improve traits of interest

PLANT
SAMPLING



DNA
EXTRACTION



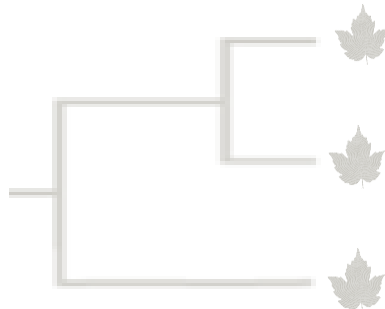
MOLECULAR
MARKERS



RESULTS
ANALYSES



PEDIGREE
RECONSTRUCTION

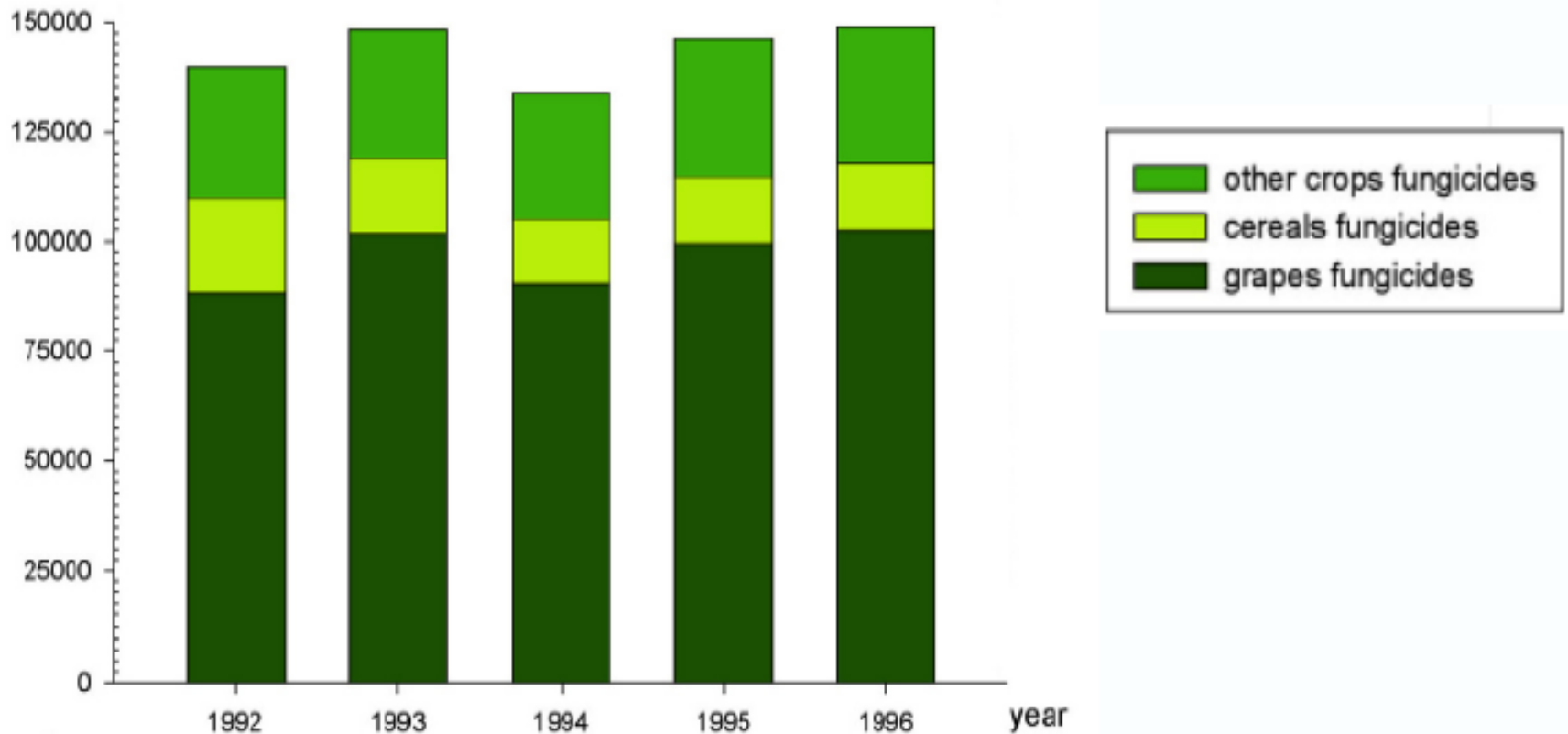




Fungicide use in viticulture



- In Europe, 8% of cultivated area is used for viticulture and 70% of fungicide are used only for grape production.

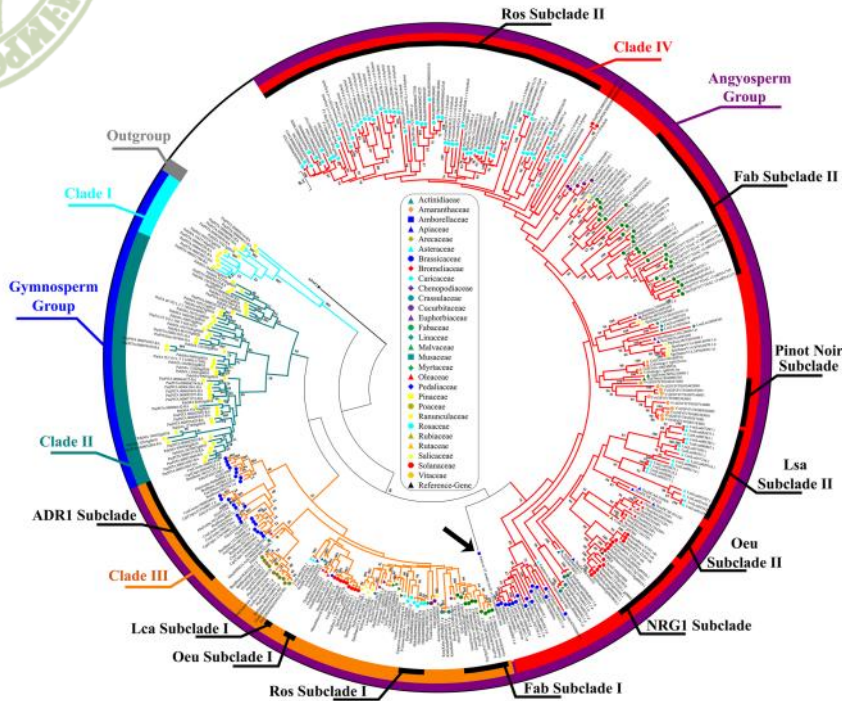




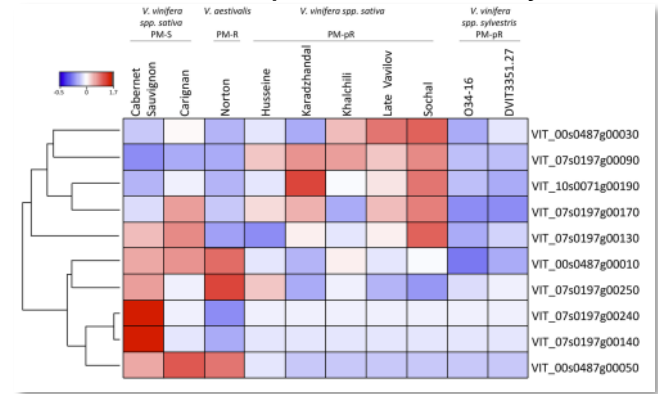
Immunity genes identified at DA



Phylogenetic analysis



Transcriptional variability



Planta (2020) 251:32
<https://doi.org/10.1007/s00425-019-03324-x>

ORIGINAL ARTICLE



Inferring RPW8-NLRs's evolution patterns in seed plants: case study in *Vitis vinifera*

Giuseppe Andolfo¹ · Clizia Villano¹ · Angela Errico¹ · Luigi Frusciantè¹ · Domenico Carputo¹ · Riccardo Aversano¹ · Maria R. Ercolano¹

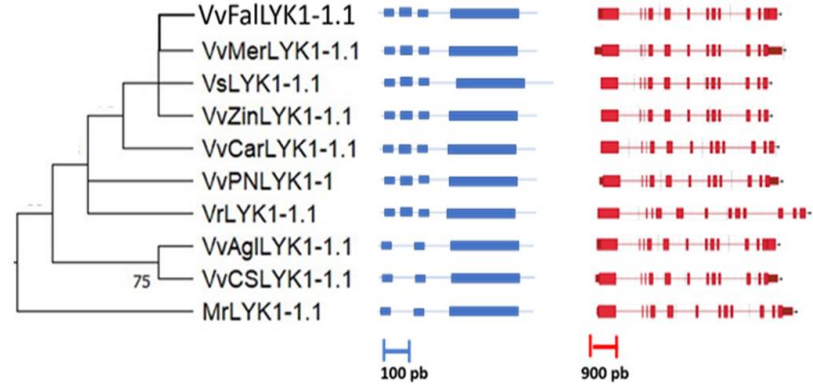
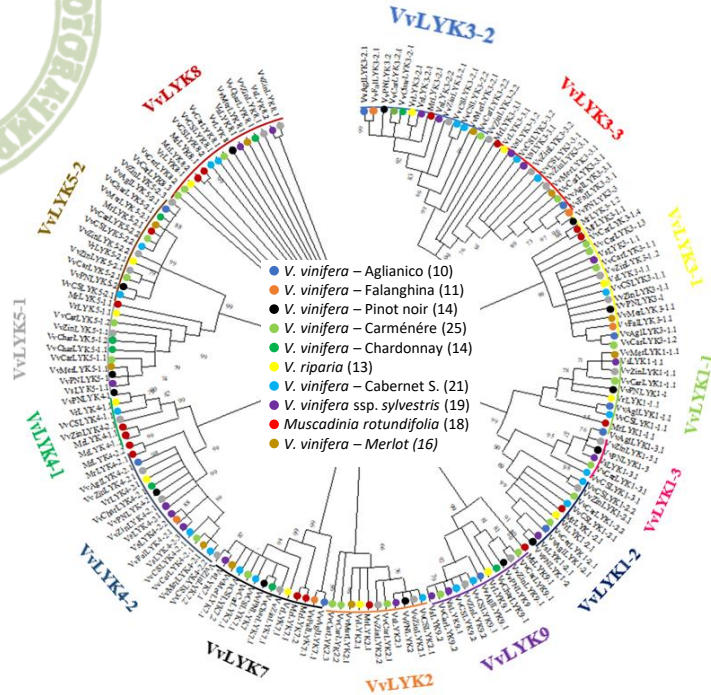
Received: 14 August 2019 / Accepted: 3 December 2019 / Published online: 10 December 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019



Immunity genes identified at DA

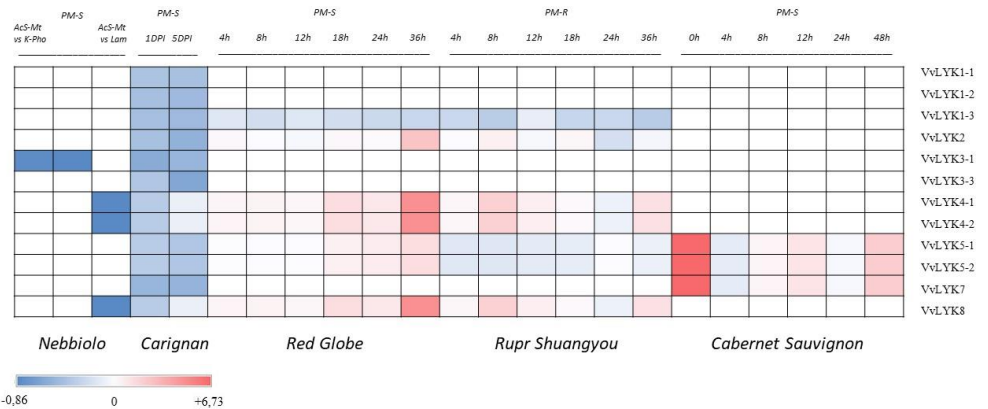


VvLYK1-1 shows differences in the genetic and proteic structure and...



...in the transcriptional behaviour.

The LYK gene family is highly variable at inter- and intra-specific level



Plant Biotechnology Journal (2019) 17, pp. 812–825

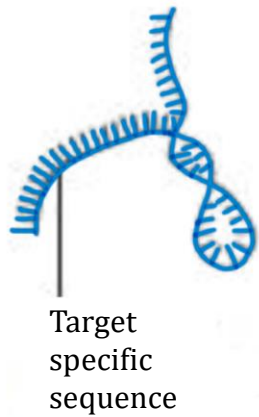
doi: 10.1111/pbi.13017

The grapevine (*Vitis vinifera*) LysM receptor kinases VvLYK1-1 and VvLYK1-2 mediate chitoooligosaccharide-triggered immunity

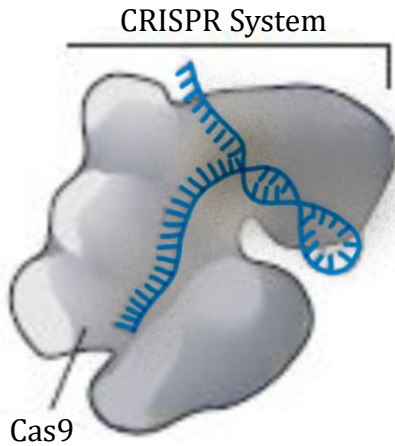
Daphnée Brulé^{1,*}, Clizia Villano^{2,*}, Laura J. Davies³, Lucie Trdá¹, Justine Claverie¹, Marie-Claire Héloir¹, Annick Chiltz¹, Marielle Adrian¹, Benoit Darblade⁴, Pablo Tornero⁵, Lena Stransfeld⁶, Freddy Boutrot⁶, Cyril Zipfel⁶, Ian B. Dry^{3,*} and Benoit Poinsot^{1,*}



Genome editing

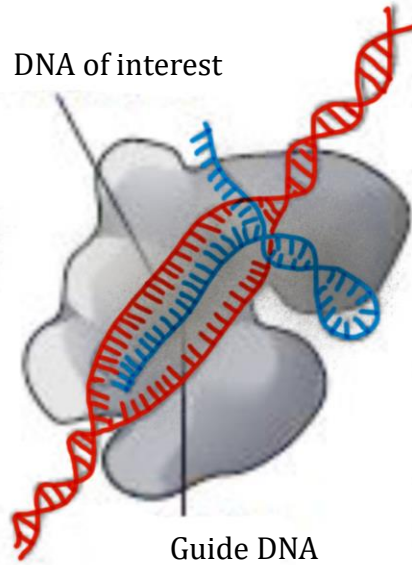


Guide RNA specific for target DNA

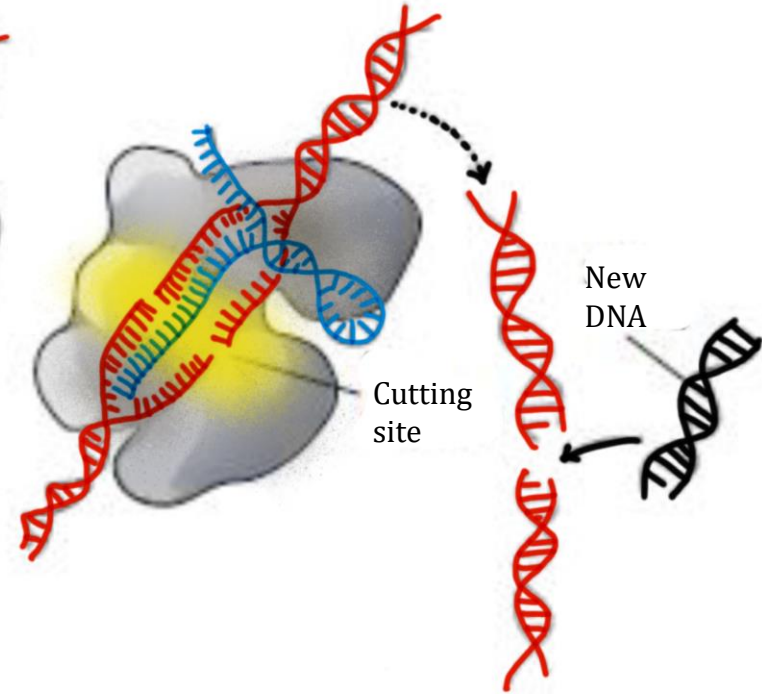


CRISPR system made by the Guide RNA and the enzyme able to cut the target DNA, named Cas9

DNA of interest



CRISPR-Cas9 complex recognizing the target DNA



Cas9 cutting the DNA target in the chosen site. It can be changed, deleted or substituted.



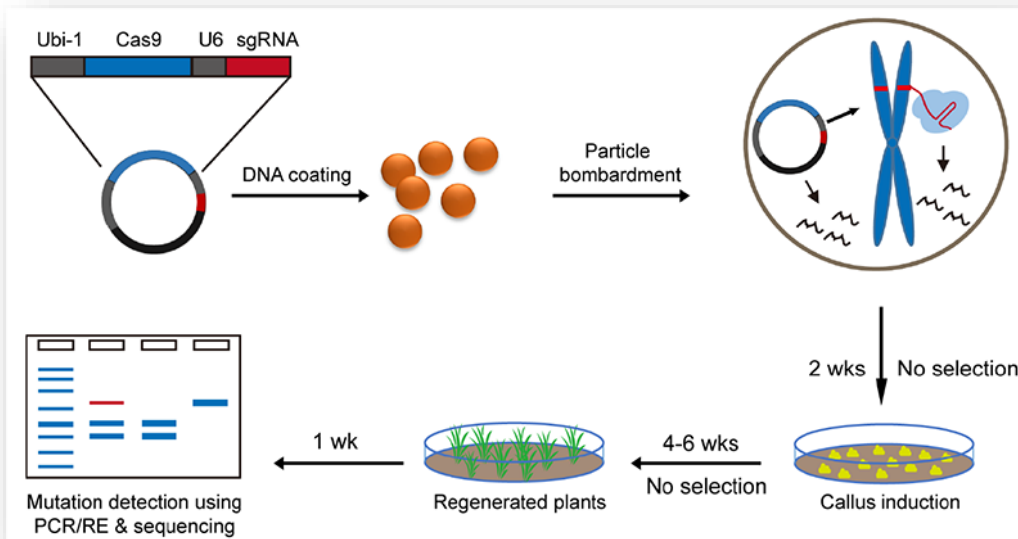
Using the intervarietal diversity



Genome editing

VvAg1LYK1-1	MKQKVLGPFVLLSVFCAVDSQC <small>SRGCDL</small> LALGSYYVWQGSNLT <small>FISG</small> .PQTTISEILSYN	60
VvFal1LYK1-1	MKQKVLGPFVLLSVFCAVDSQC <small>SRGCDL</small> LALGSYYVWQGSNLT <small>FISG</small> .PQTTISEILSYN	60
VvPNLYK1-1	MKQKVLGPFVLLSVFCAVDSQC <small>SRGCDL</small> LALGSYYVWQGSNLT <small>FISG</small> .PQTTISEILSYN	60
VrLYK1-1	MKQKVLGPFVLLSVFCAVDSQC <small>SGGCDL</small> LALGSYYVWQGSNLT <small>FISG</small> .PQTTISEILSYN	60
VrLYK1-1.2	MKQKVLGPFVLLSVFCAVDSQC <small>SGGCDL</small> LALGSYYVWQGSNLT <small>FISG</small> .PQTTISEILSYN	60

VvAg1LYK1-1	RLVGTFGYMP <small>EG</small> FAQYGDVSPKVDVYAFGVVLYELISAKEAVVKENG <small>SVAESKGLVALFE</small>	537
VvFal1LYK1-1	RLVGTFGYMP <small>EE</small> FAQYGDVSPKVDVYAFGVVLYELISAKEAVVKENG <small>SVAESKGLVALFE</small>	540
VvPNLYK1-1	RLVGTFGYMP <small>EE</small> FAQYGDVSPKVDVYAFGVVLYELISAKEAVVKENG <small>SVAESKGLVALFE</small>	529
VrLYK1-1	RLVGTFGYMP <small>EE</small> FAQYGDVSPKVDVYAFGVVLYELISAKEAVVKENG <small>SVAESKGLVALFE</small>	539
VrLYK1-1.2	RLVGTFGYMP <small>EE</small> FAQYGDVSPKVDVYAFGVVLYELISAKEAVVKENG <small>SVAESKGLVALFE</small>	500





Perspectives

- Investigate grapevine germplasm with NGS-based molecular markers
- Classical and innovative molecular breeding
- Identify new candidates: in terms of genes and varieties

We need to preserve traditional varieties using innovative approaches!



References

- Wolkovich, E. M., de Cortázar-Atauri, I. G., Morales-Castilla, I., Nicholas, K. A., & Lacombe, T. (2018). From Pinot to Xinomavro in the world's future wine-growing regions. *Nature Climate Change*, 8(1), 29-37.
- Villano, C., Carputo, D., Frusciante, L., Santoro, X., & Aversano, R. (2014). Use of SSR and retrotransposon-based markers to interpret the population structure of native grapevines from southern Italy. *Molecular biotechnology*, 56(11), 1011-1020.
- Aversano, R., Basile, B., Buonincontri, M. P., Carucci, F., Carputo, D., Frusciante, L., & Di Pasquale, G. (2017). Dating the beginning of the Roman viticultural model in the Western Mediterranean: The case study of Chianti (Central Italy). *PloS one*, 12(11), e0186298.
- Villano, C., Lisanti, M. T., Gambuti, A., Vecchio, R., Moio, L., Frusciante, L., ... & Carputo, D. (2017). Wine varietal authentication based on phenolics, volatiles and DNA markers: State of the art, perspectives and drawbacks. *Food Control*, 80, 1-10.
- Brulé, D., Villano, C., Davies, L. J., Trdá, L., Claverie, J., Héloir, M. C., ... & Poinssot, B. (2019). The grapevine (*Vitis vinifera*) LysM receptor kinases Vv LYK 1-1 and Vv LYK 1-2 mediate chitooligosaccharide-triggered immunity. *Plant biotechnology journal*, 17(4), 812-825.
- Andolfo, G., Villano, C., Errico, A., Frusciante, L., Carputo, D., Aversano, R., & Ercolano, M. R. (2020). Inferring RPW8-NLRs's evolution patterns in seed plants: case study in *Vitis vinifera*. *Planta*, 251(1), 1-13.
- Qiu, W., Feechan, A., & Dry, I. (2015). Current understanding of grapevine defense mechanisms against the biotrophic fungus (*Erysiphe necator*), the causal agent of powdery mildew disease. *Horticulture research*, 2(1), 1-9.
- Marks, R. A., Hotaling, S., Frandsen, P. B., & VanBuren, R. (2021). Lessons from 20 years of plant genome sequencing: an unprecedented resource in need of more diverse representation. *bioRxiv*.
- Visciano, P., & Schirone, M. (2021). Food frauds: Global incidents and misleading situations. *Trends in Food Science & Technology*.
- Holmberg, L. (2010). Wine fraud. *International Journal of Wine Research*, 2, 105-113.



13 Ottobre 2020

FELICIA MASUCCI

Feed for Food
come l'alimentazione animale
influenza quel che mangiamo e tanto altro