

GenRes Bridge



Genetic resources for food-secure
and forested Europe



GenRes Bridge - Joining forces for genetic resources and biodiversity management

Workshop on Phytosanitary barriers for genetic resources

24 February 2021, 9:30 – 12:00 and 14:00 to 16:00

online meeting on MS Teams

Developing a proposal for phytosanitary regulation for import
of *Vitis* germplasm to the EU

Oswaldo Failla – University of Milan

Full professor in Arboriculture and Fruit tree growing



The Faculty of Agriculture of Milano was founded as Royal Higher School of Agriculture in 1870

It is the second oldest Italian higher educational institution in the agricultural field

A long tradition in the germplasm conservation and evaluation

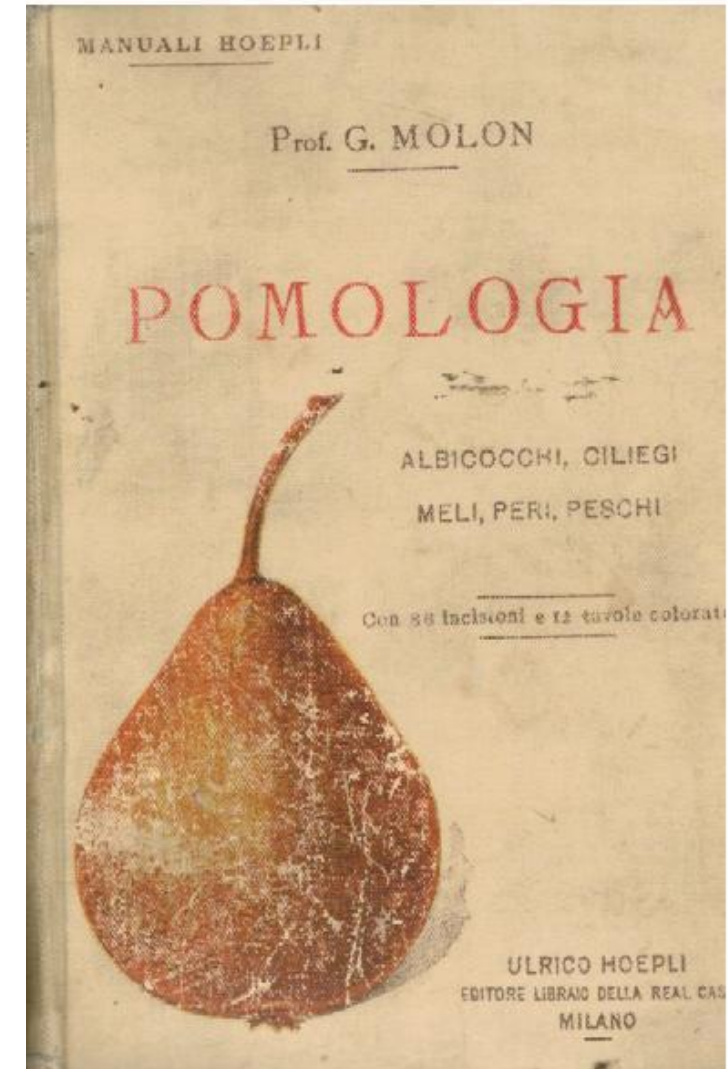


Girolamo Molon (1860 – 1937)

Professor of Horticulture (1897 – 1935)



Molon G. 1906 Ampelografia. Descrizione delle migliori varietà di viti per uve da vino, uve da tavola, portinnesti e produttori diretti. Milano, Ed. Hoepli, voll. 2, pp. XLIV-1243



Molon G. 1901 Pomologia. Descrizioni delle migliori varietà di albicocchi, ciliegi, meli, peri, peschi. Milano, Ed. Hoepli, pp. XXIX-717.



Vitis **54** (Special Issue), 271–272 (2015)

Phytosanitary rules for grapevine (*Vitis vinifera* L.) propagation material introduction into EU for germplasm conservation and scientific purposes

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The main purpose of the present proposal is to simplify and make more consistent the regulation for grapevine (*Vitis vinifera* L.) propagation material introduction into EU for germplasm conservation, scientific purposes and breeding.



East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding - GRAPENET

FA1003

Start date: 04/11/2010

End date: 03/11/2014

Osvaldo FAILLA

Chair

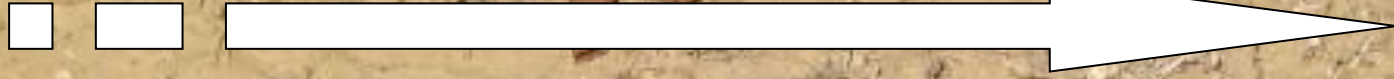
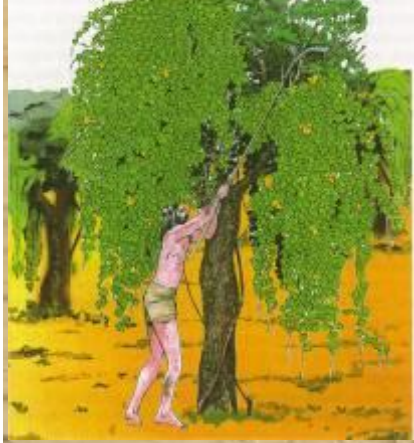
University of Milano / Italy



Why east to west collaboration?

- due to evolution of the vinicultural system, nowadays viticulture is based on a limited number of grapevine varieties;
- most of the grapevines biodiversity, built along the history of viticulture, is only maintained in the germplasm collections
- in east Europe this trend is particularly remarkable: hundreds of grapevine cultivars are only maintained in germplasm collections;
- the research facilities and capabilities on east Europe have to be aided by western European research institutions by developing collaborative research projects aimed to germplasm exploitation and sustainable conservation.

Origin and development of biodiversity



Wild grapevines

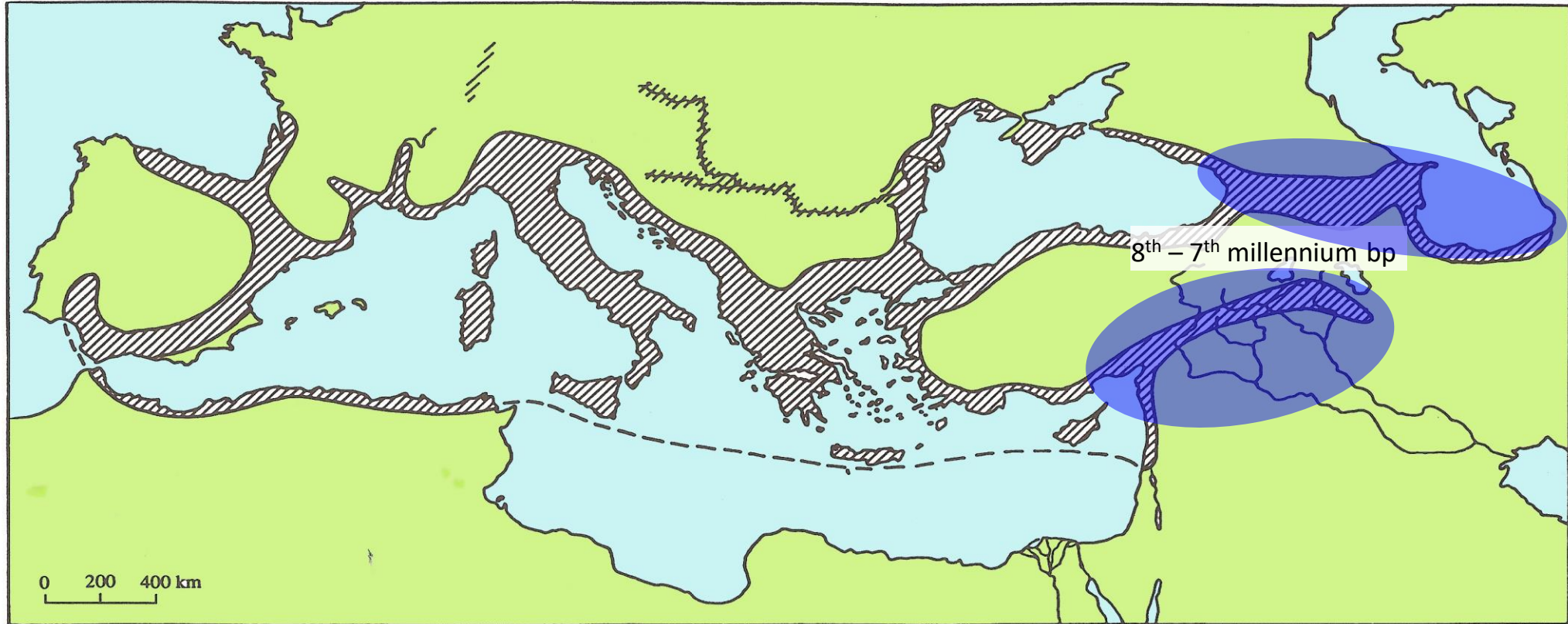
Vitis vinifera subsp. *silvestris* (Gmel.) Hegi



Domestic grapevines

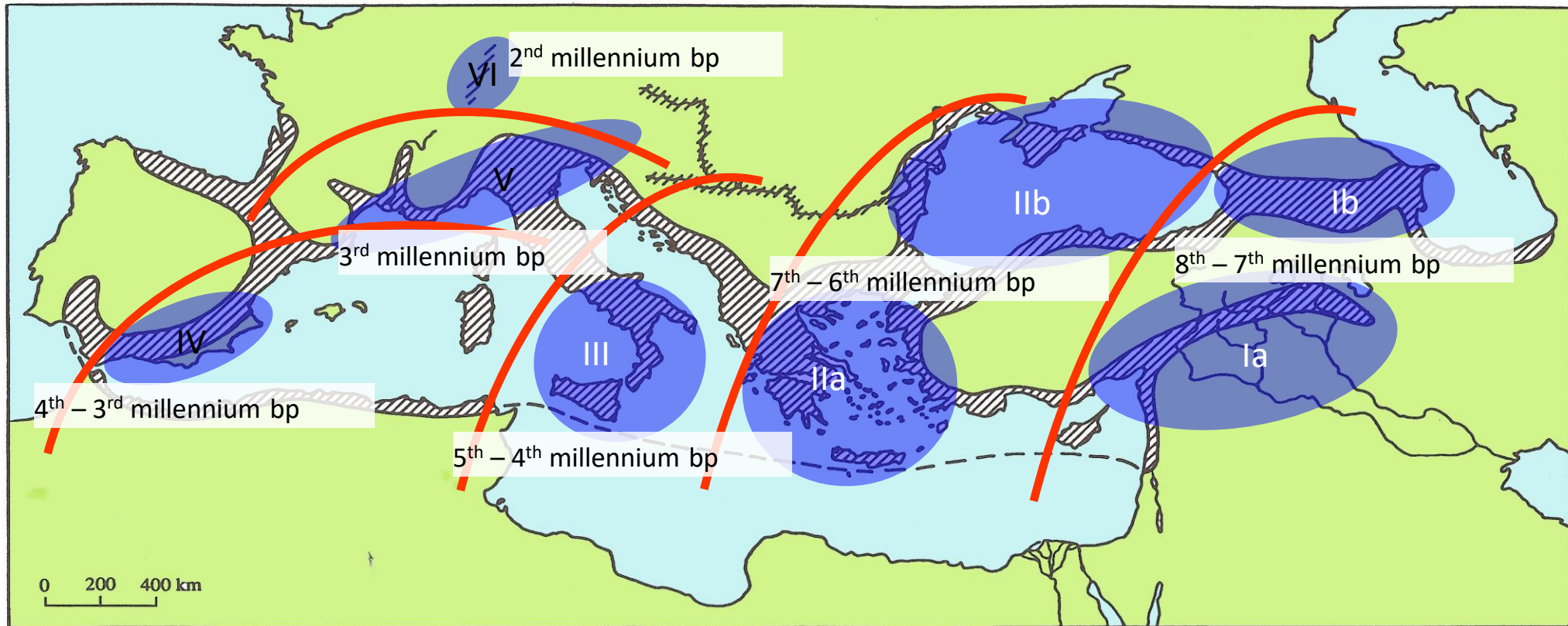
Vitis vinifera L. subsp. *vinifera*

Origin and development of biodiversity



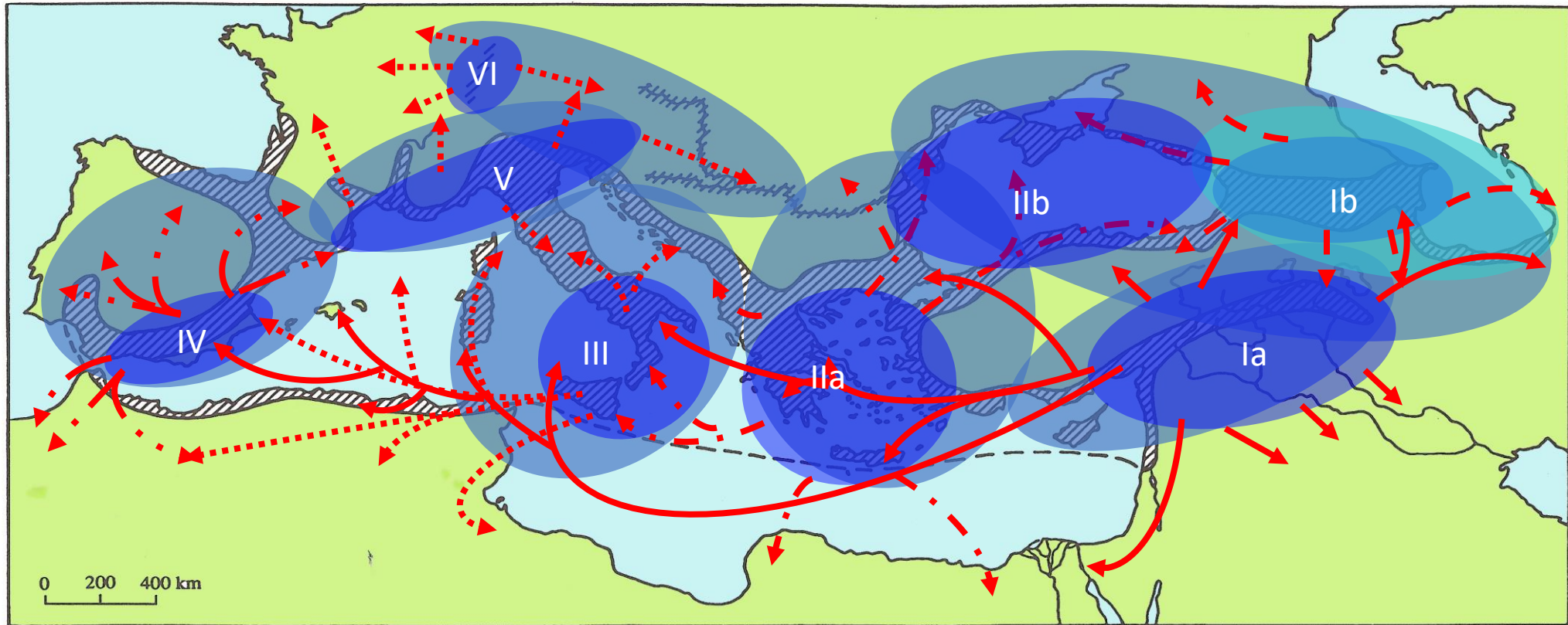
The primary centers of domestication of *Vitis vinifera* L.

Origin and development of biodiversity



The possible secondary centers of domestication (*Vitis vinifera* L.)

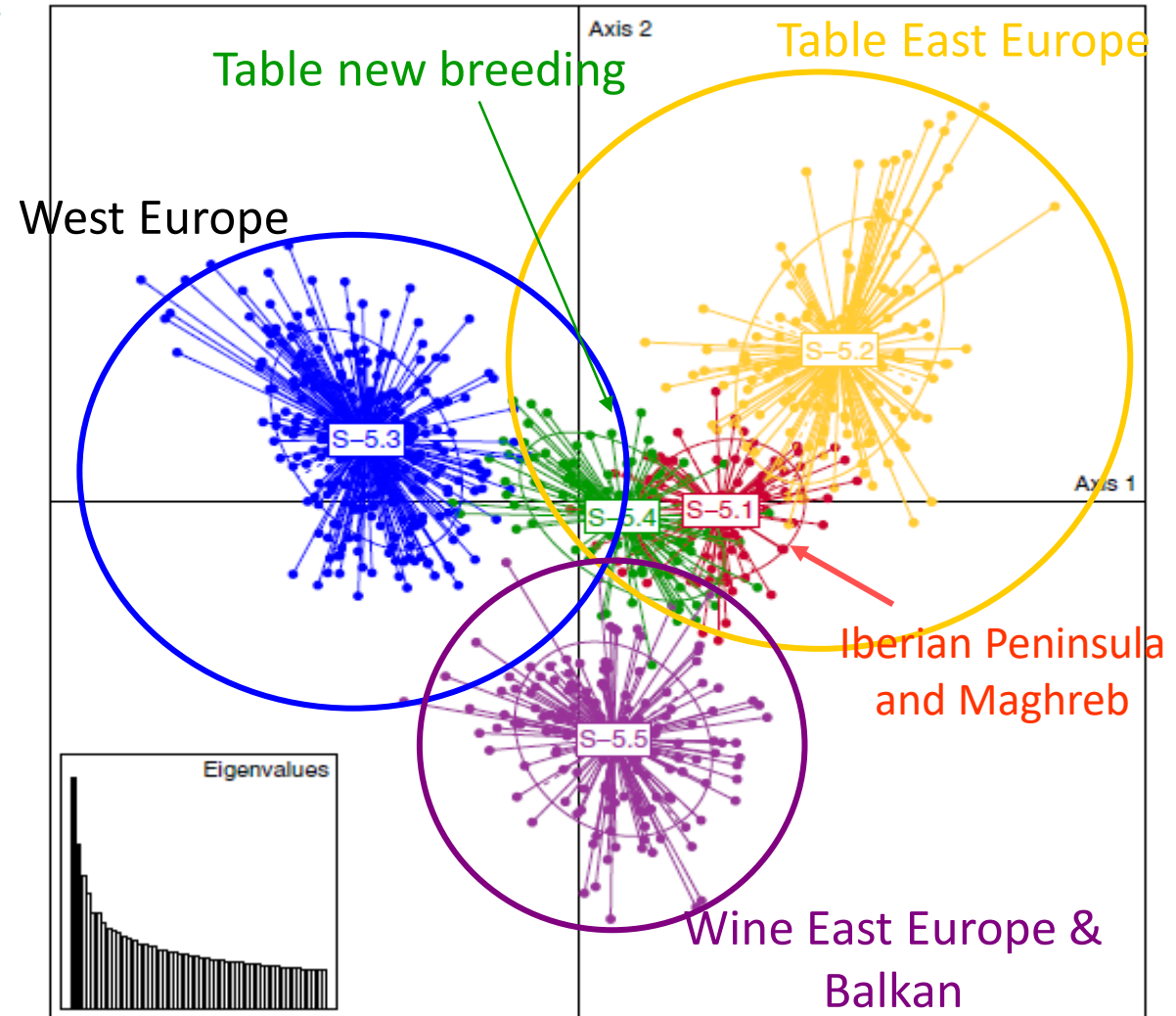
Origin and development of biodiversity



The secondary routes of cultivar dissemination: an historic stratification

Genetic structure in cultivated grapevines is linked to geography and human selection

Roberto Bacilieri^{1*}, Thierry Lacombe^{1,2}, Loïc Le Cunff³, Manuel Di Vecchi-Staraz¹, Valérie Laucou¹, Blaise Genna², Jean-Pierre Péros¹, Patrice This¹ and Jean-Michel Boursiquot⁴



Biodiversity of *V. vinifera* germplasm

Tree Genetics & Genomes
DOI 10.1007/s11295-013-0597-9

OPINION PAPER

From the cradle of grapevine domestication: molecular overview and description of Georgian grapevine (*Vitis vinifera* L.) germplasm

Serena Imazio · David Maghradze · Gabriella De Lorenzis · Roberto Bacilieri · Valérie Laucou · Patrice This · Attilio Scienza · Osvaldo Failla

De Lorenzis et al. *BMC Plant Biology* (2019) 19:7
<https://doi.org/10.1186/s12870-018-1576-y>

RESEARCH ARTICLE

Open Access

SNP genotyping elucidates the genetic diversity of *Magna Graecia* grapevine germplasm and its historical origin and dissemination

Gabriella De Lorenzis¹, Francesco Mercati², Carlo Bergamini³, Maria Francesca Cardone¹, Antonio Lupini⁴, Antonio Mauceri⁴, Angelo Raffaele Caputo¹, Loredana Abbate², Maria Gabriella Barbagallo³, Donato Antonacci⁵, Ersimur Gencar⁶ and Loris Brancaccio⁷

Riaz et al. *BMC Plant Biology* (2018) 18:137
<https://doi.org/10.1186/s12870-018-1351-0>

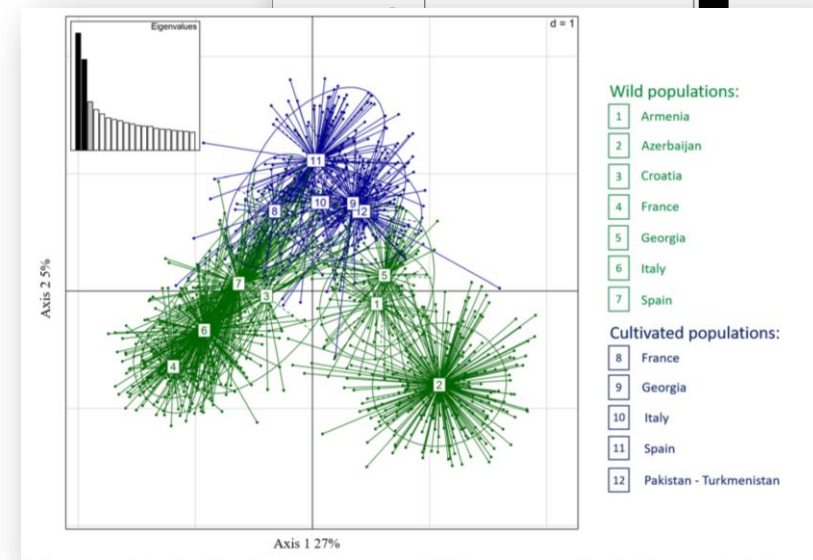
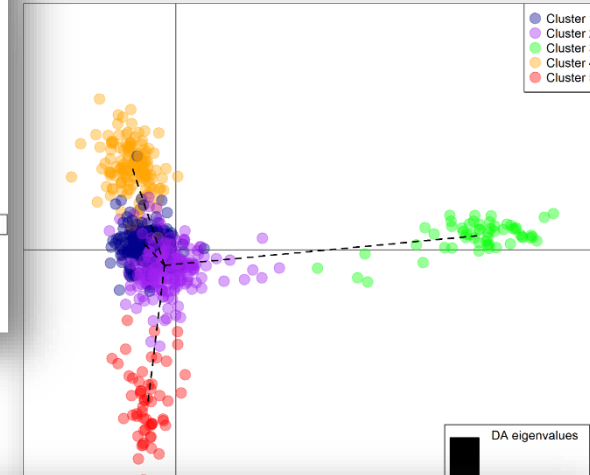
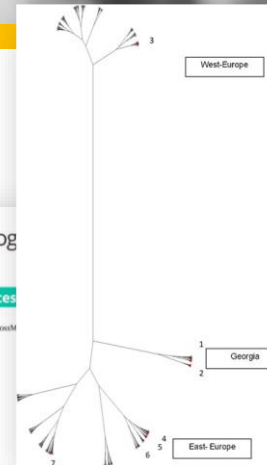
BMC Plant Biology

RESEARCH ARTICLE

Open Access

Genetic diversity analysis of cultivated and wild grapevine (*Vitis vinifera* L.) accessions around the Mediterranean basin and Central Asia

Summaira Riaz^{1*}, Gabriella De Lorenzis^{2*}, Dianne Velasco³, Anne Koehmstedt⁴, David Maghradze⁵, Zviad Bobokashvili⁶, Mirza Musayev⁶, Goran Zdrunic⁷, Valerie Laucou⁸, M. Andrew Walker⁹, Osvaldo Failla², John E. Preece⁸, Mallikarjuna Aradhya¹⁰ and Rosa Arroyo-Garcia¹¹



The Caucasian germplasm showed the highest genetic diversity

Action Parties



Participating countries (35)

Cost (25)

AT, BA, BE, BG, CH, CY, CZ, DE, EL,
ES, FR, HR, HU, IL, IT, LU, LV, MK,
PL, PT, RO, SI, SK, SE, SR

No Cost (10)

AL, AM, AZ, GE, MA, ME, MD,
NZ, RU, UA

+ New Zealand

Action objectives

- Sharing experiences, responsibilities, information and materials for the development and improvements of genotyping and phenotyping methods for association genetics studies.
- **The main object of the action is to define a core collection able to represent and conserve the highest genetic diversity with the lowest number of plant accessions.**
- To improve the impact of the research conducted by each partner and to introduce innovative areas of research at the European level, creating:
 - knowledge;
 - long-term conservation and
 - a qualitative and sustainable grape production in Europe.

Grapenet germplasm collection network

24 institutions in 18 countries



Identification and characterization of grapevine genetic resources maintained in Eastern European Collections

E. MAUL¹⁾, R. TÖPFER¹⁾, F. CARKA²⁾, V. CORNEA³⁾, M. CRESPIAN⁴⁾, M. DALLAKYAN⁵⁾, T. DE ANDRÉS DOMÍNGUEZ⁶⁾, G. DE LORENZIS⁷⁾, L. DEJEU⁸⁾, S. GORYSLAVETS⁹⁾, S. GRANDO¹⁰⁾, N. HOVANNISYAN⁵⁾, M. HUDCOVICOVA¹¹⁾, T. HVARLEVA¹²⁾, J. IBÁÑEZ¹³⁾, E. KISS¹⁴⁾, L. KOCIS¹⁵⁾, T. LACOMBE¹⁶⁾, V. LAUCOU¹⁶⁾, D. MAGHRADZE¹⁷⁾, E. MALETIĆ¹⁸⁾, G. MELYAN¹⁹⁾, M. Z. MIHALJEVIĆ¹⁸⁾, G. MUÑOZ-ORGANERO⁶⁾, M. MUSAYEV²⁰⁾, A. NEBISH²¹⁾, C. F. POPESCU⁸⁾, F. REGNER²²⁾, V. RISOVANNA⁹⁾, S. RUISA²³⁾, V. SALIMOV²⁴⁾, G. SAVIN³⁾, A. SCHNEIDER²⁵⁾, N. STAJNER²⁶⁾, L. UJMAJURIDZE²⁷⁾ and O. FAILLA⁷⁾

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³⁾Research and Practical Institute for Horticulture and Food Technologies, Chisinau, Republic of Moldova

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⁷⁾Università degli studi di Milano, Dipartimento di Scienze Agrarie ed Ambientali, Milano, Italy

⁸⁾University of Agronomic Sciences and Veterinary Medicine, Faculty of Horticulture, Bucharest, Romania

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¹⁴⁾Szent István University, Institute of Genetics and Biotechnology, Gödöllő, Hungary

¹⁵⁾University of Pannonia, Georgikon Faculty, Department of Horticulture, Keszthely, Hungary

¹⁶⁾INRA – SupAgro Montpellier, UMR AGAP, Équipe Diversité, Adaptation et Amélioration de la Vigne, Montpellier, France

¹⁷⁾Institute of Horticulture, Viticulture and Oenology, Agrarian University of Georgia, University Campus at Digomi, Tbilisi, Georgia

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¹⁹⁾Armenian Academy of Viticulture and Wine-making, Yerevan, Armenia

²⁰⁾Genetic Resources Institute of the Azerbaijan National Academy of Sciences (AGRI), Baku, Azerbaijan

²¹⁾Department of Genetics, Yerevan State University, Armenia

²²⁾HBLAuBA Klosterneuburg, Klosterneuburg, Austria

²³⁾Latvia State Institute of Fruit-Growing, Dobeles, Latvia

²⁴⁾Azerbaijani Scientific Research Institute of Viticulture and Winemaking, Baku, Azerbaijan

²⁵⁾Consiglio Nazionale delle Ricerche, Istituto di Virologia Vegetale, Grugliasco, Torino, Italy

²⁶⁾University of Ljubljana, Ljubljana, Slovenia

²⁷⁾AGRO - National Center for Grapevine and Fruit Tree Planting Material Propagation, Mtskheta, Georgia

Table 1

Summary of the analyzed accessions: Albania, Armenia, Austria, Azerbaijan, Bulgaria, Croatia, Georgia, Hungary, Latvia, Moldova, Romania, Slovakia, Slovenia and Ukraine

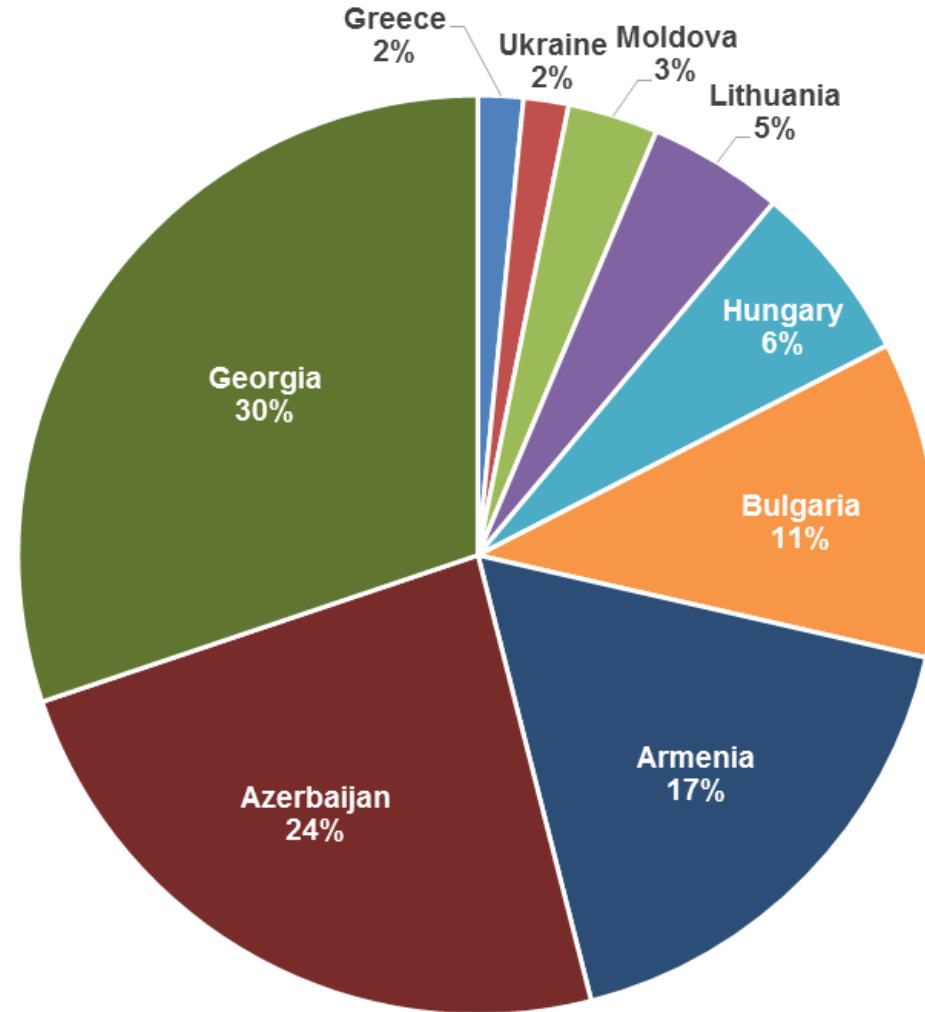
Country	Institute code	No. of accessions	No. of accessions unique or repeated 2 to 9 times									No. of unique profiles	Unique genotypes not repeated in Western Europe	
			unique	2	3	4	5	6	7	8	9		No.	%
1. Albania	ALB017	13	11	1								12	6	50
2. Armenia	ARM011	95	50	9	3	1		1		1		65	49	75
3. Austria	AUT024*	10	10									10	3	30
4. Azerbaijan	AZE007, AZE015	108	45	13	3	7						68	49	72
5. Bulgaria	BGR013*	189	173	8								181	63	35
6. Croatia	HRV014*	21	17	2								19	9	47
7. Georgia	GEO014, GEO015, GEO036, GEO037	370	129	34	22	5	5	3	5		1	204	106	52
8. Hungaria	HUN 08, HUN005*, HUN007, HUN045	47	41	3								44	28	64
9. Moldavia	MDA004	41	31	2	2							35	17	49
10. Romania	ROM 06/ROM045	57	47	3		1						51	3	6
11. Ukraine	UKR050	57	50	2	1							53	19	36
12. Slovenia	SVN018*	29	24	1	1							26	7	27
		1037	628									768	359	

Core collection

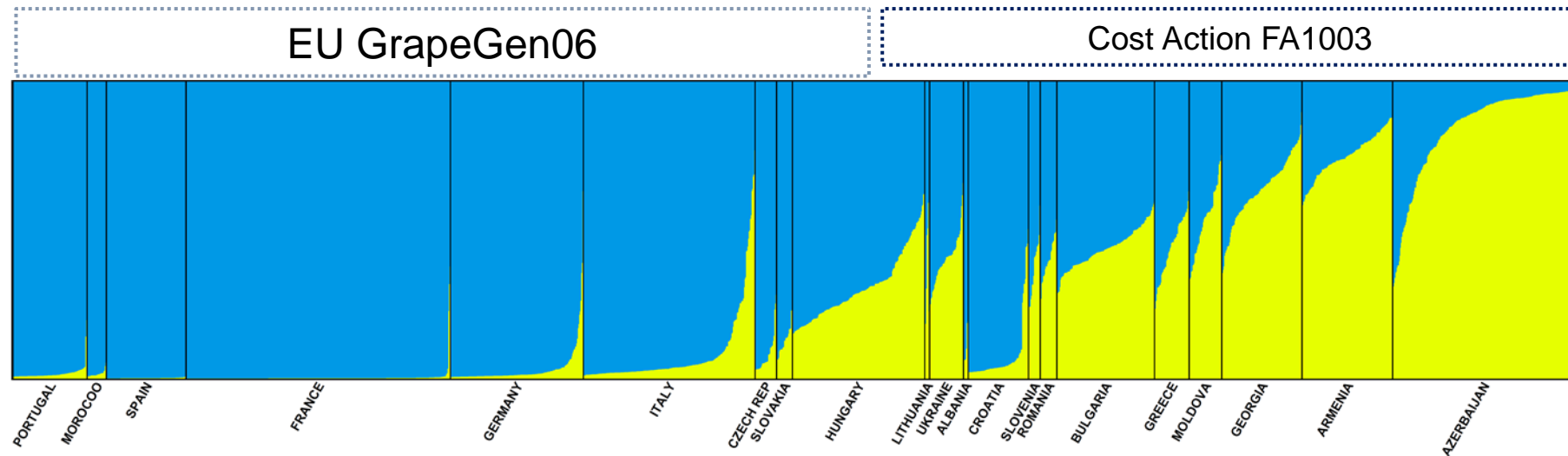
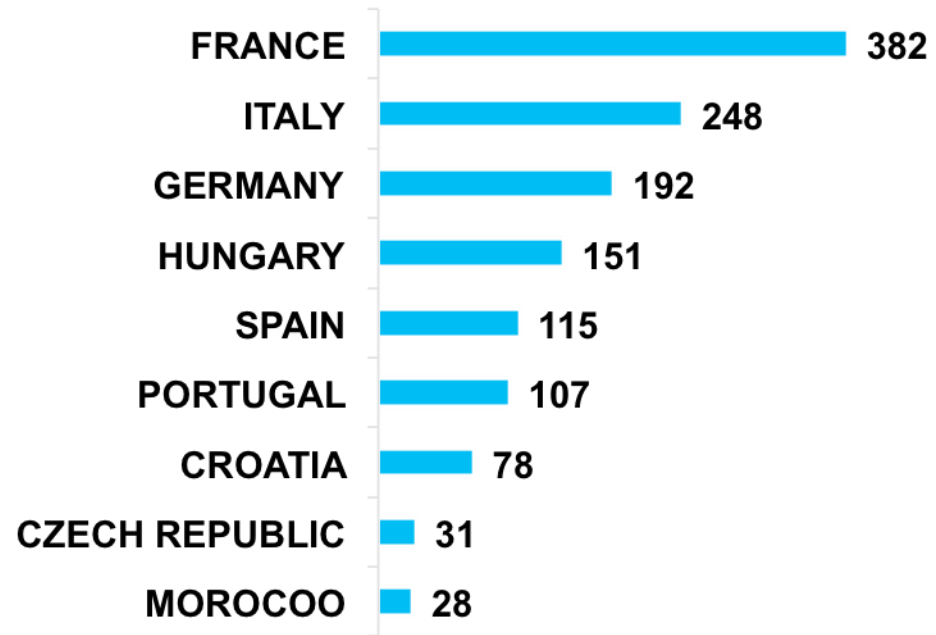
To develop a set of accessions with reduced allele redundancy, and thus with enhanced richness of potentially useful alleles in the entire sample, the M (maximization) strategy in the PowerCore software was applied.

It allowed to extract the accessions with higher diversity representing the entire coverage of variables and gave a 100% reproducibility.

Sample	N	A	9 SSRs		
			Alleles retained	H _E	H _O
GRAPENET	775	178	100%	0.867	0.789
Core collection	63	178	100%	0.916	0.771



*Lukasz Grzeskowiak, M. Stella Grando
Lisbon 2014*



PHYTOSANITARY RULES FOR GRAPEVINE (*Vitis vinifera* L.)
PROPAGATION MATERIAL INTRODUCTION INTO EU FOR GERMPLASM
CONSERVATION AND SCIENTIFIC PURPOSES

1. Purpose of the document proposal
2. Current regulations
3. Principles of the transfer material proposal
4. Procedure of the plant material introduction into EU
5. Specific procedures for dormant cuttings introduction
6. List of quarantine organisms
7. List of other important pathogens

Purpose of the document proposal

1. to simplify, unify and harmonize the regulation for grapevine (*Vitis vinifera* L.) propagation material introduction into EU **for germplasm conservation, scientific purposes and breeding**
2. to **improve efficiency** of the plant material transfer into EU

Current regulations

1. **FAO/IBPGR Technical Guidelines for the Safe Movement of Grapevine Germplasm (1991)**

taking however into account that the guideline does not include the most recent knowledge and methods currently available and used. Moreover, it should be noted that:

- the Technical guidelines are very complicated, **not applicable** for practical and fast plant material introduction into EU because the evaluation and therapy of infected material are rather difficult, time consuming and expensive; moreover, the effect of the thermotherapy may be critical on the phenotypic trueness to type and stability;
- more than one pesticides recommended by the Technical guidelines is not yet allowed;
- the therapy procedures should be used just in case of a unique material

Current (2014) regulations

2. EU regulations

- transfer of plant material into EU is regulated by **Directive 2000/29/EC**;
- **introduction** of the grapevine plant material into EU is **prohibited** with exception of the material transfer for scientific purposes and breeding;
- the conditions under which plants, and plant products may be introduced into or moved within the Community **for trial or scientific purposes and for work on varietal selections** is regulated by Commission **Directive 2008/61/EC** of 17 June 2008

REGULATION (EU) 2016/2031

COMMISSION DELEGATED REGULATION (EU) 2019/829

Principles of the transfer material proposal

Protocol for safe introduction into EU of grapevine propagation material for germplasm conservation, scientific purposes and breeding

1. the present protocol refers only to **dormant woody cuttings**
2. the plant **material** transferred into EU **should be free from EU quarantine organisms**
3. mother plants **evaluation in the country of origin** have to be based on:
 - **visual** assessment of disease symptoms;
 - **ELISA** specific tests;
 - **PCR-based diagnostic tools** – EU approved and/or available methods for pathogen detection and identification.
4. based on the **negative test results** in detection of quarantine organisms, the plant material will be subjected to the procedure for the **introduction of plant material into the EU**, following in force regulations

Procedure of the plant material introduction into EU

Prerequisites before application for material introduction approval

approved quarantine conditions in relation to **qualifications of the personnel, quarantine containment conditions** of the location and facilities

Procedure of the plant material introduction into EU

Progressive steps of the approval of plant material introduction – duties for National Plant Protection Organization of Importer Member State (IMS) or Exporter Third Country (ETC)

- **Application for introduction** of plant material (IMS);
- **Approval of the planned activities concerned** (IMS). The nature and objectives of the activities for which the material is to be introduced or moved shall have been examined by the responsible official body of importing Country and found to comply with the concept of trial or scientific purposes and or work on varietal selections provided for under Directive 2008/61/EC.
- **Approval of post-entry quarantine station** (IMS). Quarantine site and personal should be approved by official body.
- **Field and laboratory controls** on propagating materials and related mother plants (ETC);

Procedure of the plant material introduction into EU

Progressive steps of the approval of plant material introduction – duties for National Plant Protection Organization of Importer Member State (IMS) or Exporter Third Country (ETC)

- **Approval letter of authority** for introduction of plants for trial or scientific purposes and for work on varietal selections (IMS); the responsible official body shall limit the quantity of material to an amount that is adequate for the approved activities and in any case the amount shall not exceed quantities which have been determined having regard to available quarantine containment facilities; the import permit shall be released on the basis of the approval of the activities, and on the assurance that quarantine containment conditions shall be applied during movement and detection of the material
- Export **phytosanitary certificate** (PC) issue (ETC). The PC shall indicate an additional declaration stating that *“the consignment complies with Directive 2008/61/EC”*
- **Phytosanitary inspection** at the Border Inspection Point and introduction in UE, to post-entry quarantine approved station (IMS)

Specific procedures for dormant cuttings introduction

A. Pre-shipping controls

- **field controls on mother plants at the place of origin**, in appropriate timing
- **selection** (exclusion of all symptomatic vines)
- **ELISA/PCR tests** for grapevine known harmful pathogens; analysis results should be indicated in additional statement in phytosanitary certificate
- **collecting woody canes**
- dipping in appropriate **insecticide and fungicide**
- deliver of appropriate **Phytosanitary Certificate** (specific additional declaration) for each consignment

B. Entry controls

- entry controls performed in approved **Border Inspection Point (BIP)**

Specific procedures for dormant cuttings introduction

C. Post-entry controls

- **arrival in approved** “Post Entry Quarantine Station”, under official control by National Plant Protection Organization (NPPO)
- further accurate **Visual Testing** (insects, mites, epiphytic bacteria and fungi)
- detecting, isolation and identification of **epiphytic bacteria and fungi** (e.g. Biolog)
- ELISA or RT-PCR (for relevant grapevine **harmful viruses and phytoplasmas**)
- **grafting** on appropriate rootstock, if needed
- production of rooted **plants**, if needed
- field **planting in guarded conditions** including evaluation of vector’s presence
- **monitoring** during experimental or conservation activity

Specific procedures for dormant cuttings introduction

D. Additional remarks

Release of any imported propagating material **for circulation and trade purposes** should provide further and closer investigation about sanitary status of the materials that have to include:

- **Next generation sequencing** (NGS) to detect any known harmful organism (HOs);
- **Pest Risk Analysis** for EU, per each HO; only for potentially HOs for EU should be provide adequate restrictions or phytosanitary measures

List of quarantine organisms

1. Blueberry leaf mottle virus
2. Grapevine Flavescence dorée MLO and other grapevine yellows
3. Peach rosette mosaic virus
4. Tobacco ringspot virus
5. Tomato ringspot virus (strain 'yellow vein' and other strains)
6. *Xylella fastidiosa* (Well & Raju)
7. *Xylophilus ampelinus* (Panagopoulos) Willems et al.
8. Ajinashika disease (for ETC where the disease is known to be present)
9. Grapevine stunt (for ETC where the disease is known to be present)
10. Summer mottle (for ETC where the disease is known to be present)

List of other important pathogens

1. Grapevine fanleaf virus (GFLV)
2. Arabic mosaic virus (ArMV)
3. Grapevine leafroll associated virus 1 (GLRaV1)
4. Grapevine leafroll associated virus 3 (GRLaV3)
5. Grapevine fleck virus (GFkV)