

Genetic resources of wild grapevine (*Vitis vinifera* subsp. *sylvestris*) in Croatia

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Outline

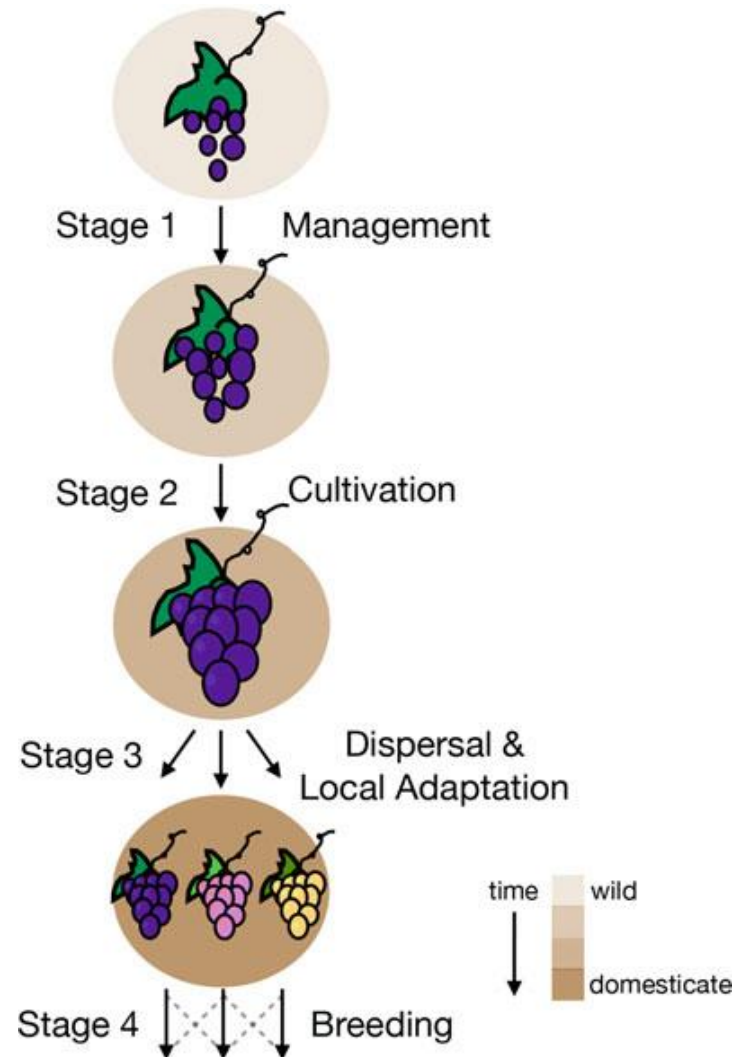
1. What is wild grapevine?
2. Why is important to consider genetic resources of wild grape for viticulture?
3. Challenges in conservation and introduction of new genes into cultivated grapevine
4. Case study of Croatian wild grapevine – WI.GRA.GENE project
5. Conclusions

Eurasian grapevine (*Vitis vinifera* L.)

- Grapes are most widely cultivated and highest value horticultural crops
- 7.1 million hectares producing 77.4 million tons of fruit globally in 2016 (OIV, 2015)
- Grown from hot dry desert to tropical climates, to very cold areas
- Cultivated (domesticated) grapes are cultivars of *Vitis vinifera* L. subsp. *vinifera* considered to have the highest fruit quality; the fruit is used for wine, table grape and raisin production
- *Vitis vinifera* L. subsp. *vinifera* has thousands of cultivars; mostly with hermaphroditic type of flowers
- European forms of wild *Vitis vinifera* L. are within subspecies *sylvestris*
- The key defining characteristics of wild grape (*V. vinifera* subsp. *sylvestris*) are dioecious with male or female flowered vines

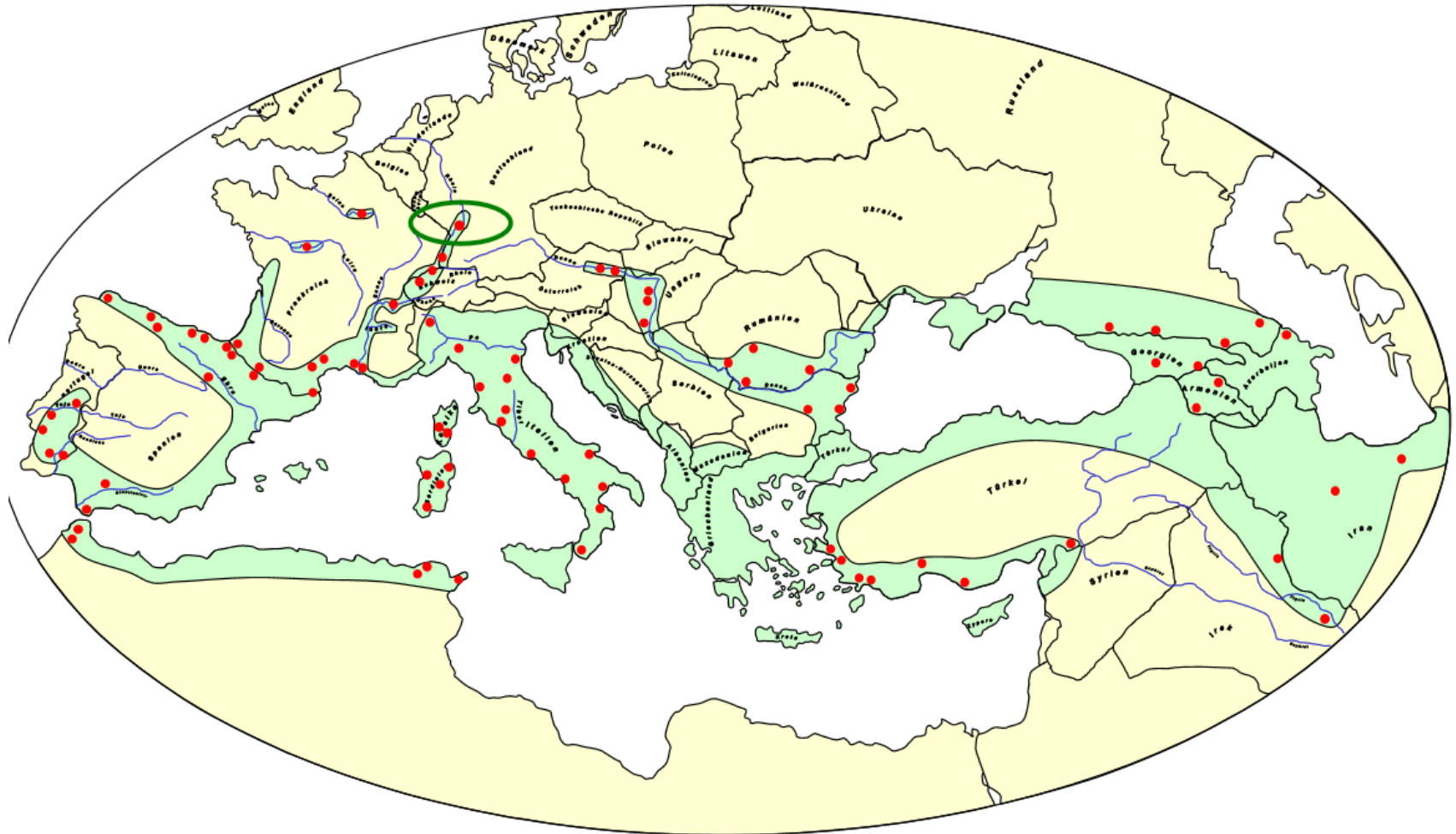
Domestication process in grapes

- Grapes among the first cultivated perennial crops
- Earliest evidence of wine production dated to the 8000–7800 ya from Kvemo province of modern-day Georgia (McGovern et al. 2017)
- Transcaucasia – primary center of domestication
- Chloroplast DNA (cpDNA) suggests multiple origins (Arroyo-Garcia et al., 2006)
- Genetic diversity decreased during domestication
- Wild grape retain some unique alleles and traits



Zhou et al. (2019)

Distribution of European wild grape *Vitis vinifera* subsp. *sylvestris* (Portugal to Afghanistan)



- Distribution of *Vitis vinifera* L. subsp. *sylvestris*
- Habitats published between 1998 and 2010

First written records in Croatia since 1879

Vinarsko zelce, Vinarsko žleže, *Lysimachia Nummul. L. (Frey.)*.

Vinena tikva, *Cucurbita Lagenaria L. (Nov.)*.

Vinerski zelce (*Bl.*), v. Vinarsko zelce.

Viničevina (*Zag.*), Viničina (*Kalnik*), v. Vinika.

Vinik (= phoenix, φοινίξ), palma (*Stulli*), *Phoenix dactylifera L.*

Vinika. *Vitis vinifera silv. Gmel. (Vis. Freyer, Jambr.)*, v. Vinjaga.

Vinoboja, *Phytolacca decandra L. (Panč.)*.

Vinoloza (*Vukas.*), Vinova loza, *rus. виноградъ, polj. winogrosł, češ. vinový kmen, Vitis vinifera L. (Vuk, Panč.)*.

(Voda od vinove loze dobra je ženskinju za kosu, raste od toga. *Milić.*)

(Sadila Mara vinograd

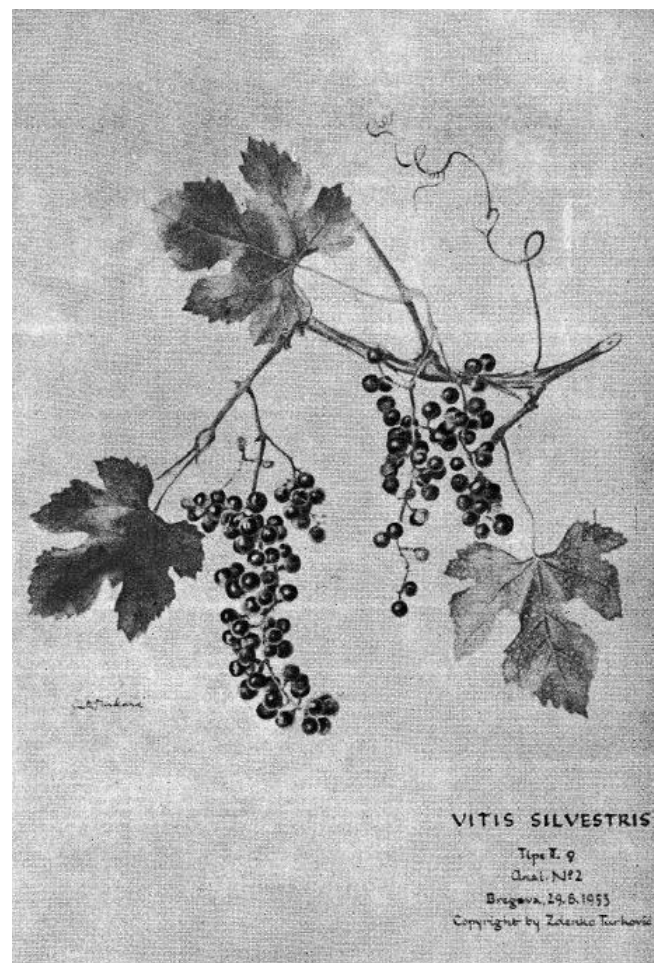
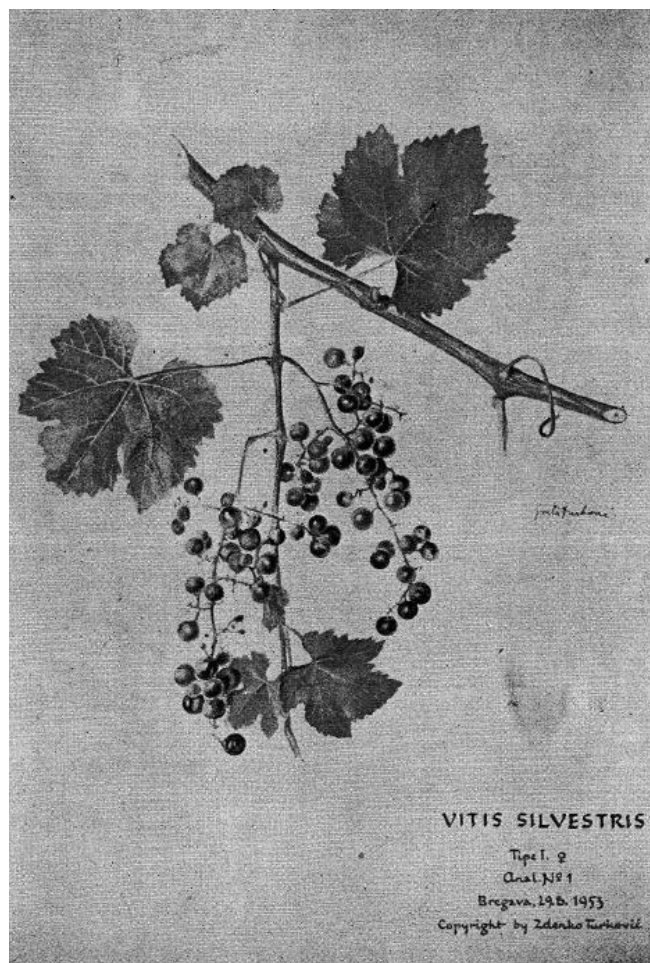
i bielu lozu vinovu. *Nar. pj. Vuk.*)

(Zagonetke: Lipa krava lipova, otelila liepa sina a manita unuka. *Vuk.*

Otac kotac, mati dropljuša, sitna djeca, al obiesna. *Slav.*)

Šulek (1879.), Croatian names: „*vinika*” or „*vinjaga*”

Description of wild grape from Herzegovina



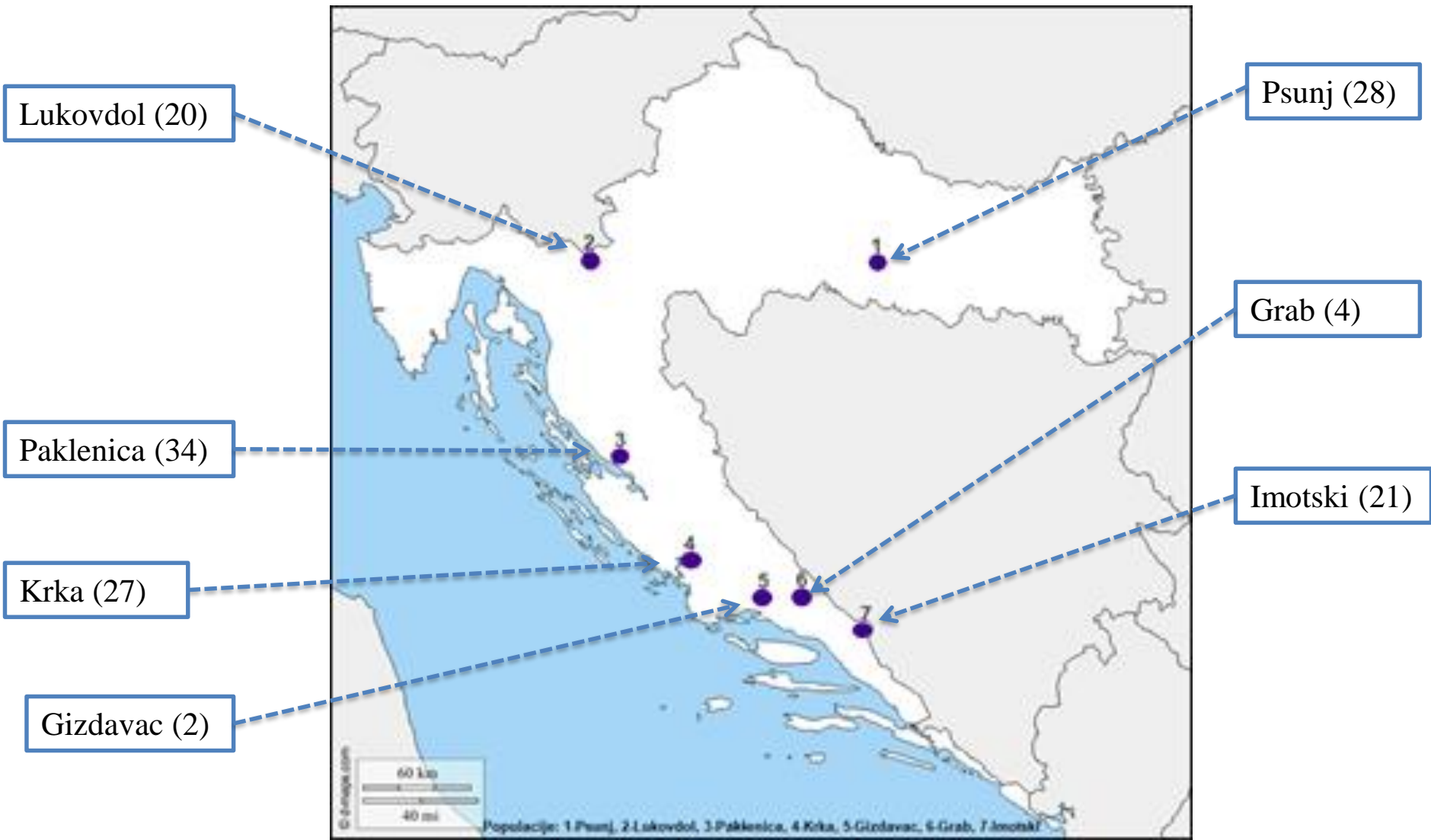
Turković and Sučević – Šafar (1953)

Why is important to consider genetic resources of wild grape for viticulture?

WI.GRA.GENE project supported by Croatian Science Foundation

- HRZZ - Installation Research Project (UIP-2014-09-9737)
- 01 July 2015 – 30 June 2018
- The main objectives: 1) assess the overall genetic and phenotypic diversity of wild grape in this region and their relatedness to local cultivated grapevine 2) investigate morphological and genetic characteristics of special interest to breeding
- Doctoral thesis Dr. Katarina Lukšić
- Scientific trainings for 7 researchers at European research institutions (Germany, Italy, Slovenia, Austria, United Kingdom)
- New collaboration established with numerous researchers
- InWiGrape project with 16 partners from 11 EU countries founded by ECPGR
- 10 WOSCC papers published

Distribution of wild grape in Croatia



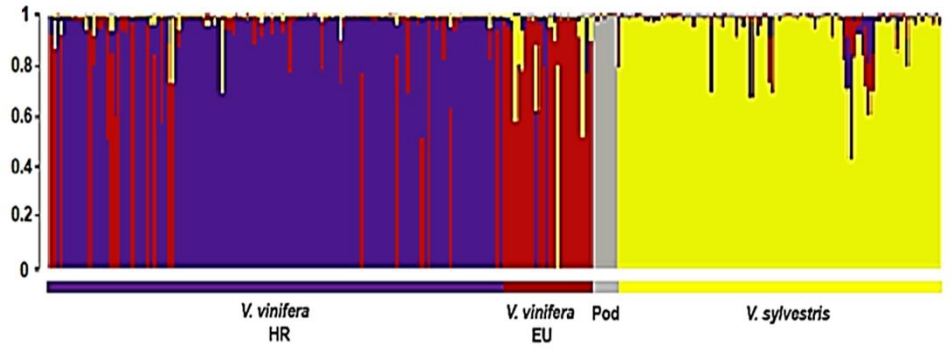
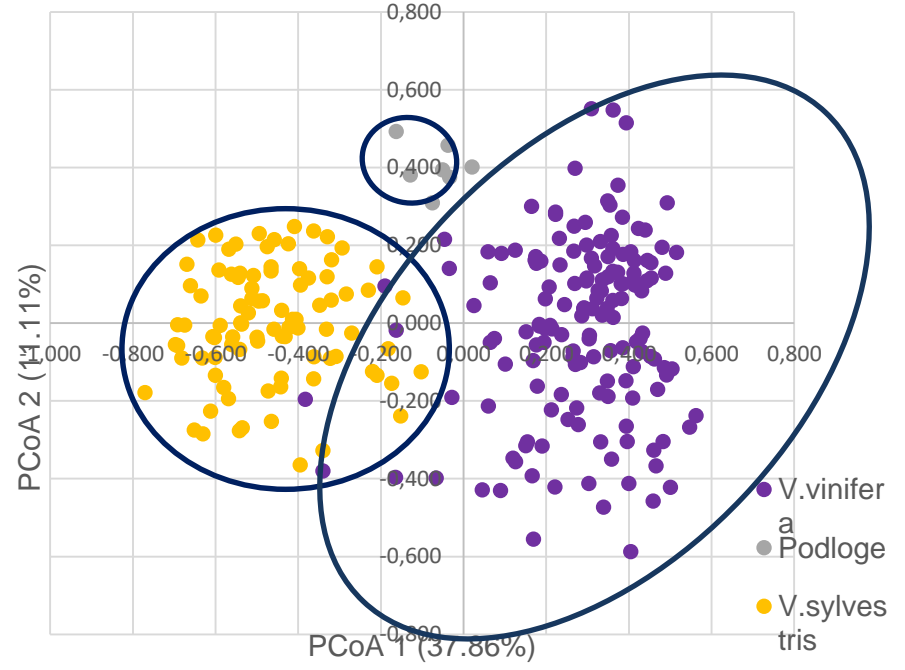
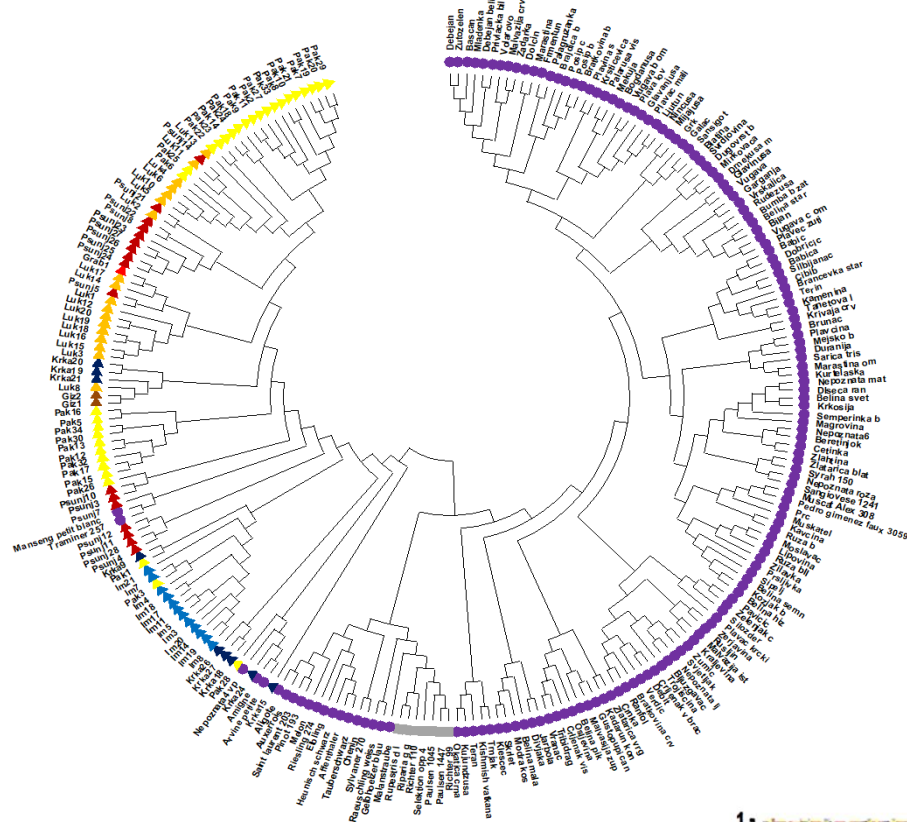
Karstic area



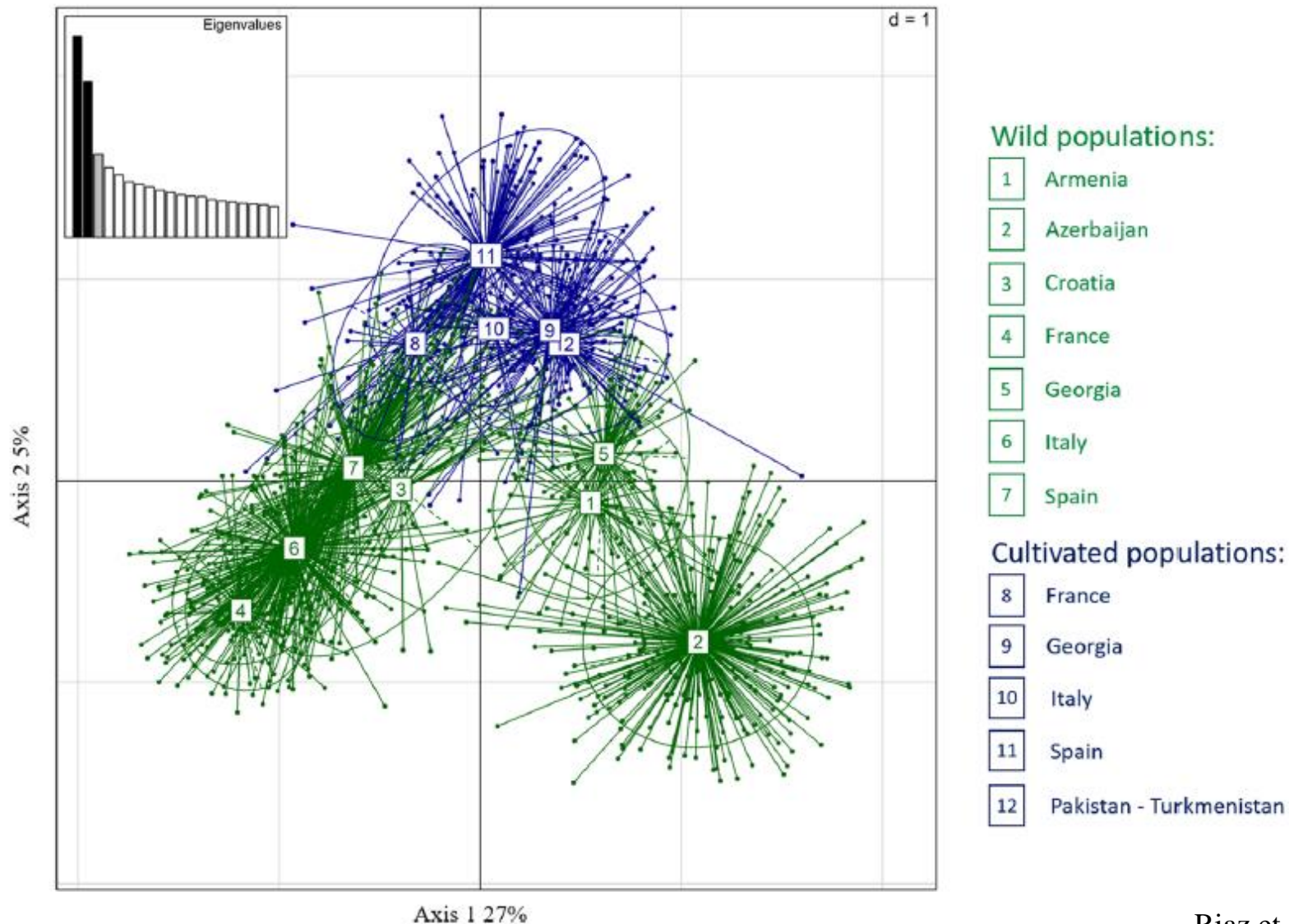
Psunj – deep wood



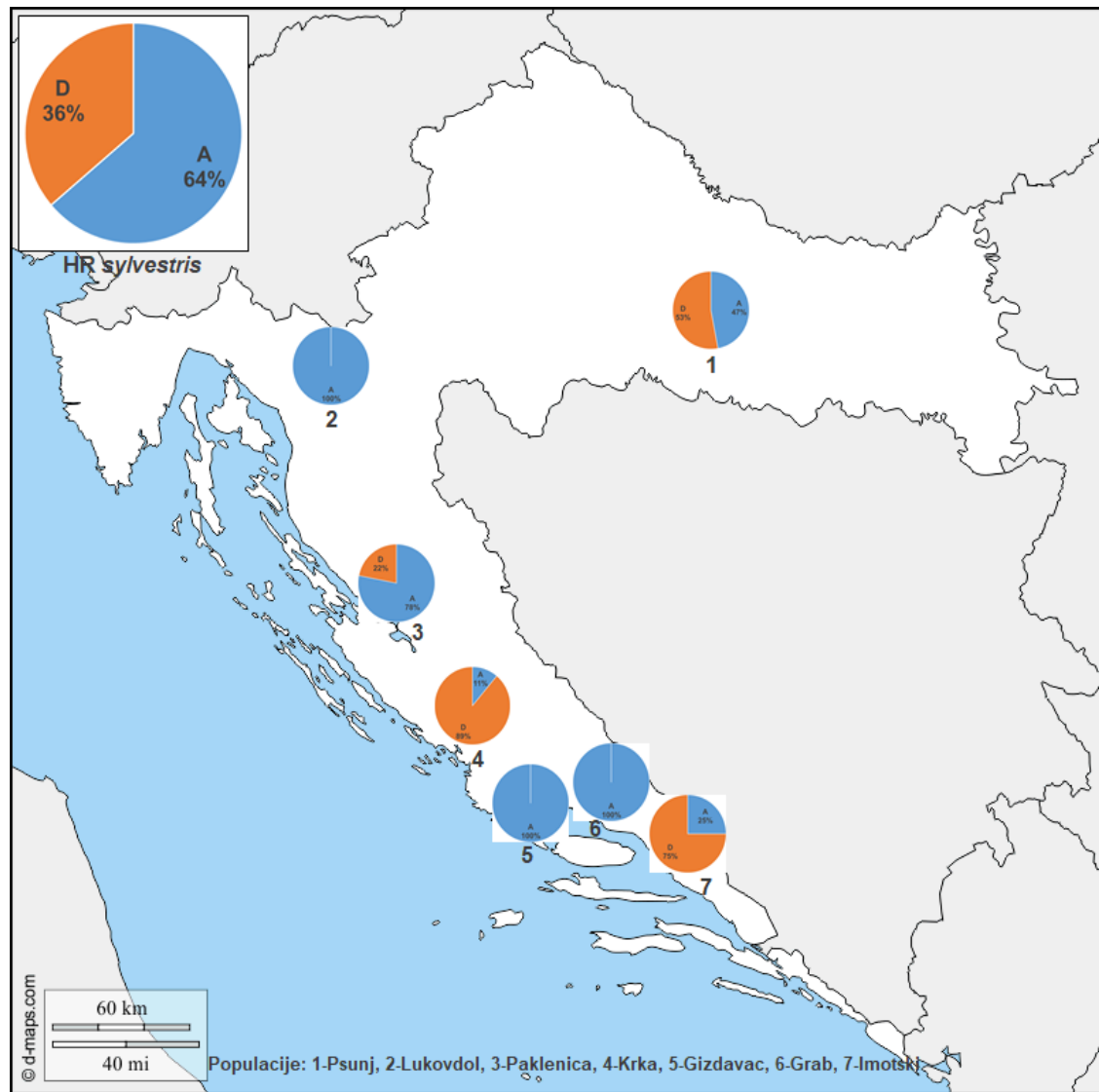
Genetic relationship between sylvestris and cultivated genotypes



Comparison Croatian *syvestris* with other European populations

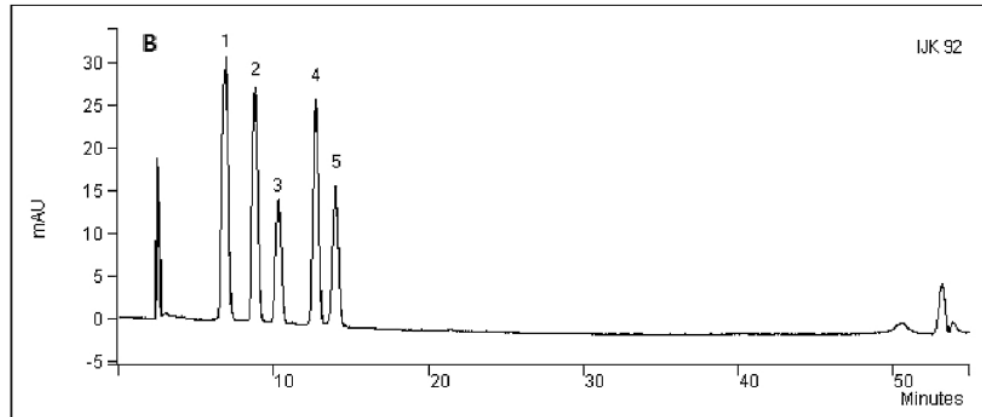
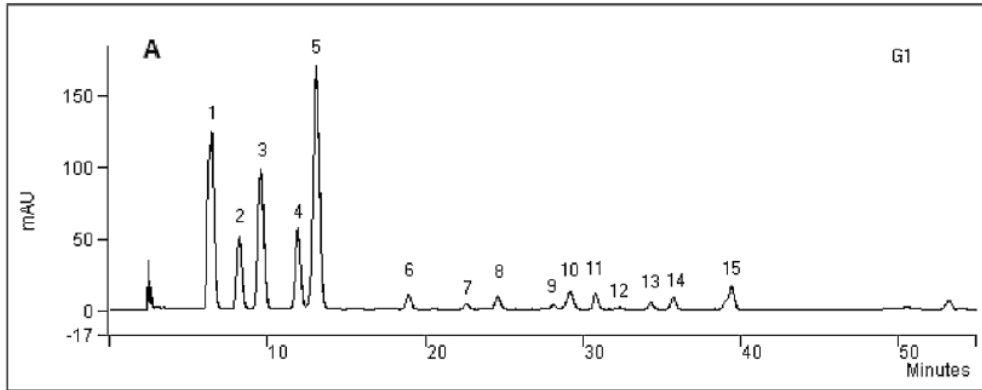


Chlorotype diversity of *syvestris*; overall: A = 64%, D = 36%



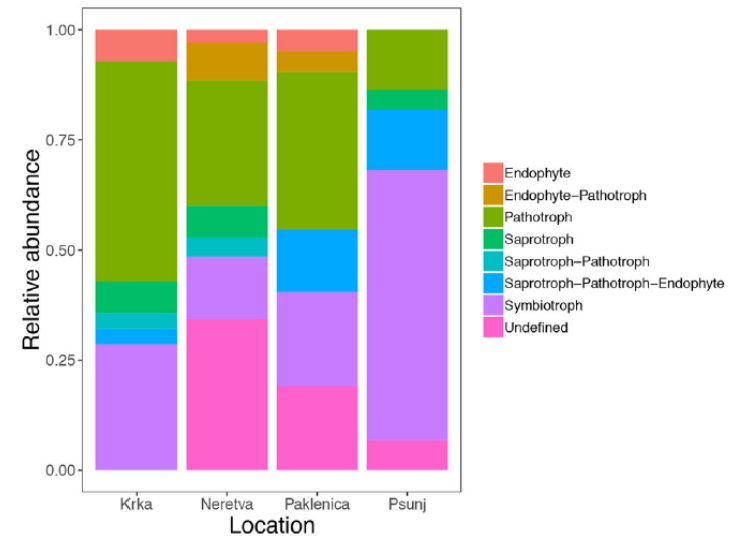
Beneficial traits in wild grape

- Two wild genotypes lacked acylated forms of anthocyanins



Budić-Leto et al (2018)

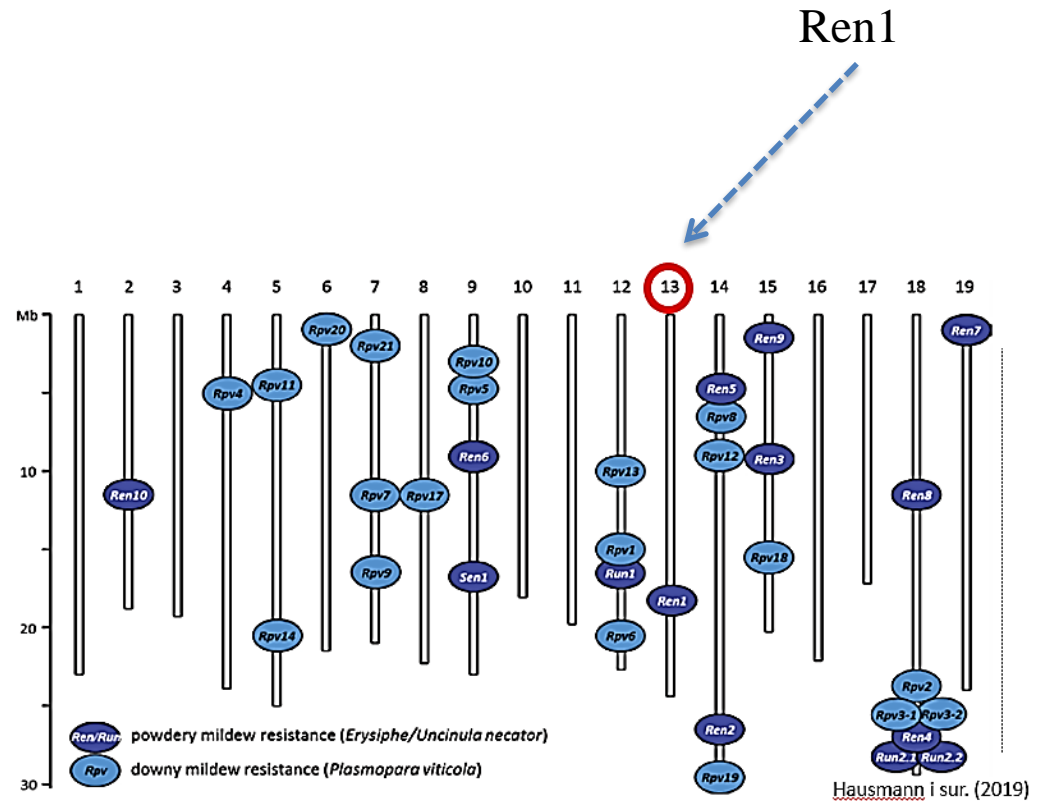
- High taxonomic diversity Root-associated fungal communities



Radić et al (2021)

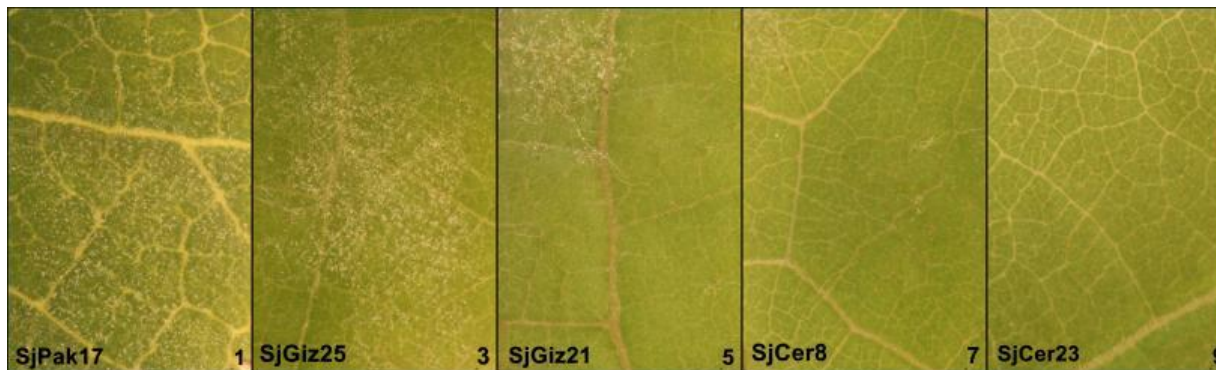
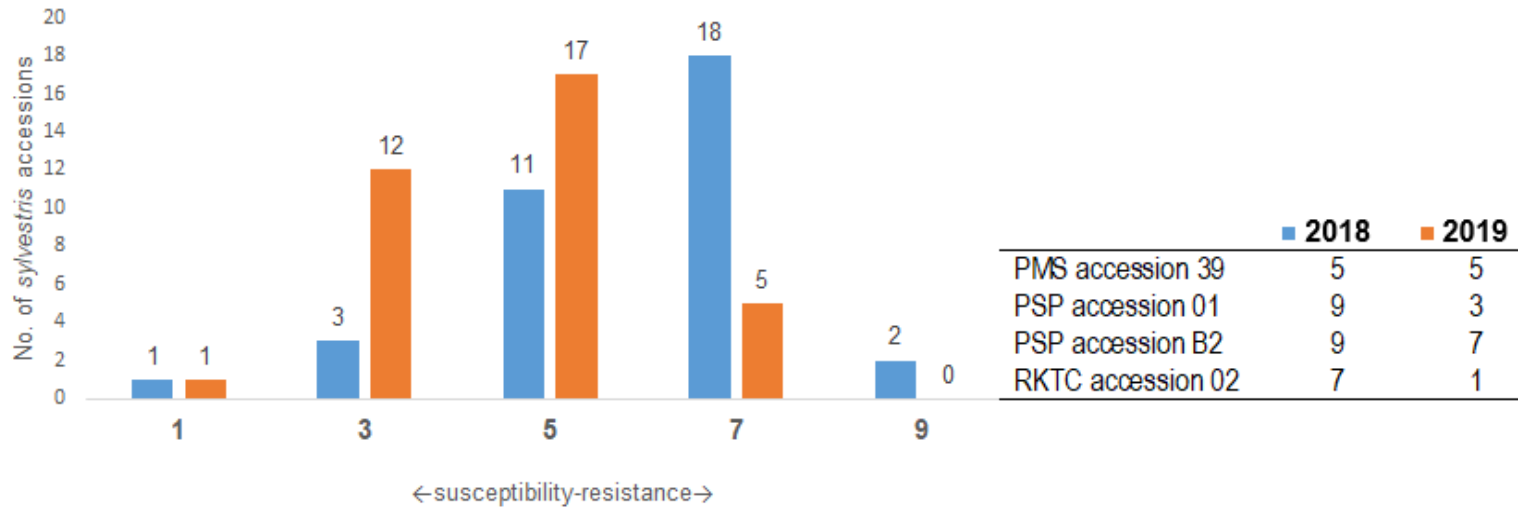
Resistance to *Erysiphe necator*

- *Muscadinia rotundifolia*: **Run1**
(Resistance to *Uncinula necator*),
Rpv1 (Resistance to *Plasmopara viticola*) (Merdinoglu i sur., 2003)
- *Vitis vinifera* subsp. *vinifera*: **Ren1**
(Resistance to *Erysiphe necator*),
cv. Kishmish vatkana (Hoffmann et al., 2008)
- *Vitis vinifera* subcp. *sylvestris* **Ren1**
SSR loci: VMCNg4e10.1, Sc47–18, SC8–0071–14, UDV124, VMC3d1 (Riaz et al., 2020)



Resistance to Erysiphe necator

At SSR locus SC47-18 presence of resistance alleles 239 and 246 within Croatian sylvestris



Non-*sylvestris* individuals within population

- Rootstocks, cultivars, hybrids
- Registered within each population observed



1. Distinctive morphology

2. SSR unspecific alleles, cluster

analysis, parent-offspring test



♂ ♀



♀



♂



Flower type

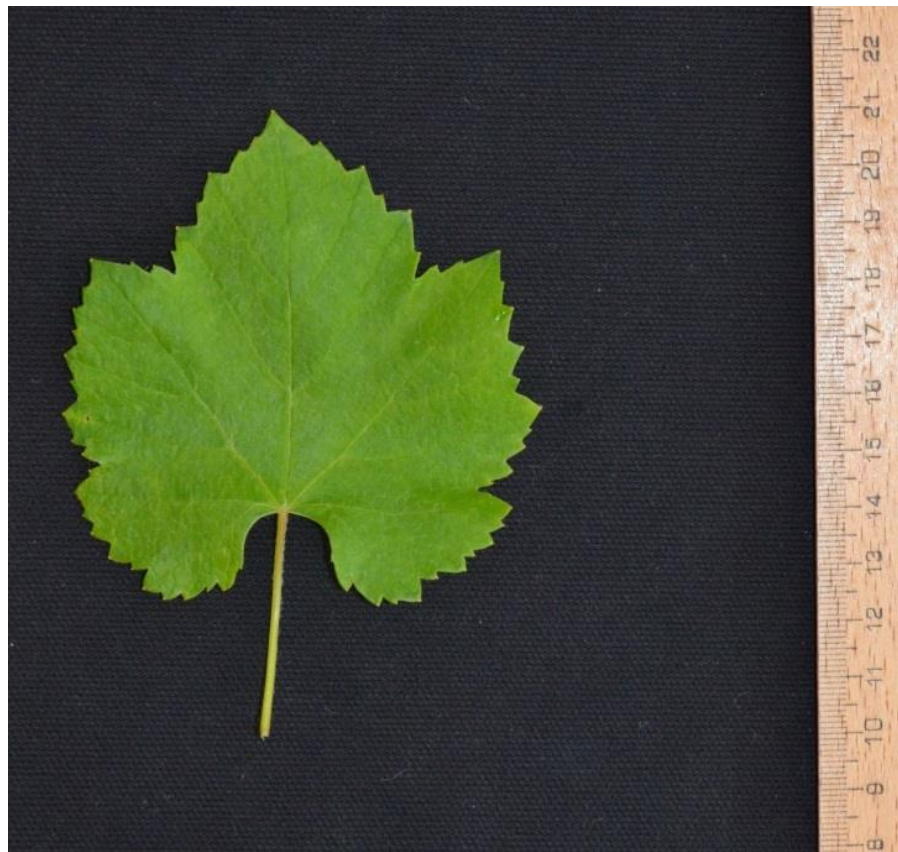
♂ Male



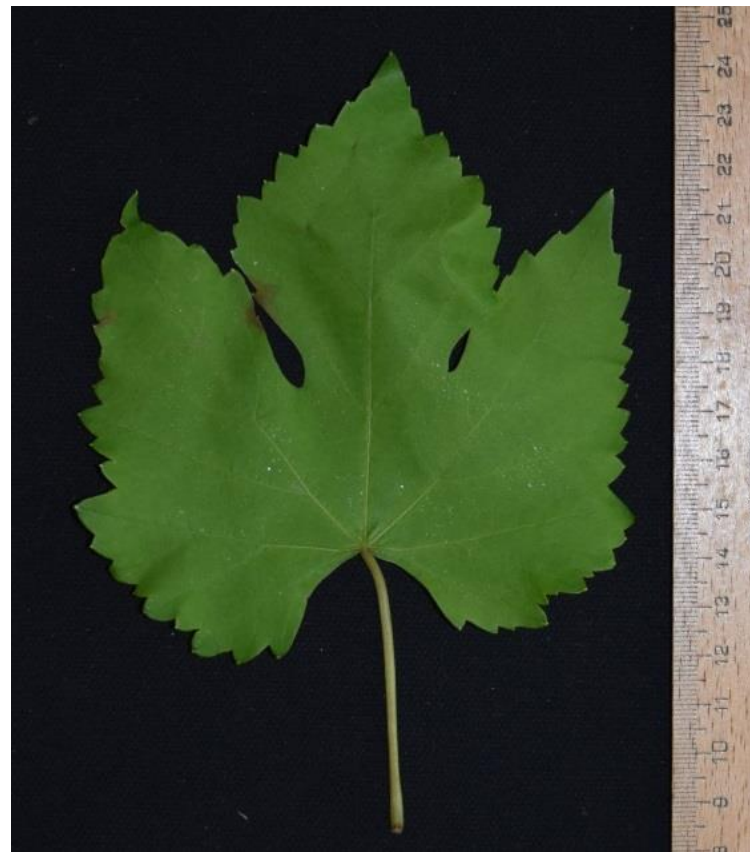
♀ Female



Leaf morphology



Gizdavac G1



Paklenica PK1

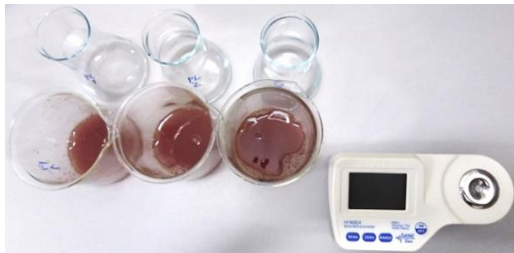
Bunch dimensions

	cultivars (n=400)				<i>syvestris</i> (n=385)			
	Mean	Min	Max	StDev	Mean	Min	Max	StDev
Cluster length (cm)	20.6	8.2	38.9	5.0	9.3	3.4	17.5	2.1
Cluster width (cm)	12.7	5.4	27.7	3.5	5.4	2.9	11.1	1.6
Cluster weight (g)	307.4	26.8	955.7	168.6	14.1	1.0	43.7	7.3
Rachis fresh weight (g)	15.6	0.1	182.3	15.6	1.2	0.3	3.5	0.6
Berries weight (g)	258.4	23.5	923.9	141.7	12.9	0.4	42.7	6.9
Number of berries	167.2	9.8	1000.0	108.3	34.1	7.0	83.0	14.5



Sugar, acidity and pH

Sample	Year	Sugar (°Oe)	Total acidity (g/l)	pH
Krka20	2017	100	10.9	2.99
Im3		100	3.2	3.29
Im11		107	3.0	4.07
Im14		98	12.2	2.96
Im17		97	10.4	3.08
Giz1	2018	77	24.6	2.86
Im3		94	8.4	3.26
Im11		106	8.3	3.22
Im17		75	20.9	2.99



Conclusions on phenotyping and conservation:

- Clear distinction between *sylvestris* and *cultivars* based on leaf and cluster morphology
- Non-*sylvestris* individuals (rootstocks, cultivars, hybrids) observed in each population; 19% tested non-*sylvestris*
- Morphology seems powerful tool for discrimination between two subspecies (OIV151 Flower type, OIV079 Petiole sinus, OIV082 Lateral sinus, OIV076 Shape of teeth = diagnostic descriptors, *in situ*)
- Conservation and protection of biodiversity – highly needed – *ex situ* germplasm collection

Conclusions on genetic diversity and benefits for breeding:

- 20 SSR successfully identified wild grape genotypes
- Distance and model-based cluster analysis differentiated among genotypes of wild and cultivated grape
- Low genetic diversity of wild grape in comparison to cultivated grape
- Structure separated genotypes into 4 groups: sylvestris, rootstocks, western cultivars and Croatian cultivars
- cpDNA analysis revealed 2 chlorotypes: A (64%) and D (36%)
- Powdery mildew resistance in sylvestris: R-alleles (239, 246 at SC47-18)
- Bunch architecture, high acidity, could be of interest for breeding

Wi.GRA.GENE project: The wild grape (*Vitis vinifera* subsp. *sylvestris*): a valuable source of genes for grape breeding



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Thank you for your attentions



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