

Corvinus University of Budapest



**INVESTIGATION OF *VITIS SYLVESTRIS* C.C. GMEL. (WILD GRAPE)
AND OTHER *VITIS* TAXA WITH MORPHOLOGICAL CHARACTERS AND
MOLECULAR MARKERS**

PhD. thesis

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1. RESEARCH HISTORY

Molecular biological methods are used in almost all fields of agricultural and botanical research. In most cases, the aim of these works is to understand the genetic resource of the useful properties of taxa, and to follow them in the offspring generations. Another important goal is to investigate, and detect the pedigree of hybrid grapevine cultivars. With the application of the molecular methods the diversity of the natural populations can be estimated, too. Methods, such as DNA-based marker-techniques, recombinant DNA techniques, genome sequencing, genetic transformation, and genome-mapping now form an integral part of the agricultural activities and determine the guidelines for the scientific development of botany. Morphological research is also accompanied by molecular tests and these are used together in researching the evolution of taxa.

Grapevine has one of the biggest fruit growing surfaces in the world, therefore the molecular genetic methods were adapted quickly in its research. The complete mapping of the genome sequence of the plant offers further possibilities. The new techniques make the investigation, identification, and separation of *Vitis* taxa, *conculata* members and grapevine cultivars possible, as well as the proof/refutation of the origin of their hybrids. In addition, the search for characteristics as seedlessness, pathogen and pest resistance, or genome mapping became everyday practises. With the help of these methods, the molecular marker based selection (MAS) of the hybrid populations became also possible.

The origin of the vine is a long-discussed topic of researchers, grapevine growers and winemakers. The exact time and place of domestication is unclear, but it was proved that the origin of the taxa is related to the wild grape (*Vitis sylvestris* C.C. Gmel.). The knowledge of this Europe-wide protected wildlife species, the exploration and protection of its habitats is a significant botanical issue for conservation of biological diversity.

Since the wild grape could still possess the adaptive properties which are lost in the cultivated grape taxa, therefore the species could be also important as a gene source in the future. The wild forms can bear resistance resources and adaptive traits which can be used in the breeding processes. In addition, the wild grape is a symbol of tradition and culture for the wine-growing industry.

One of the most serious causes of the degradation of the habitats is the spread of alien north-American *Vitis* species (*Vitis riparia* Michx, *Vitis rupestris* Schee.). These taxa easily escape from plantations and displace the natural plants in its habitats. In order to

protect the *Vitis sylvestris* C.C. Gmel. its morphological and molecular identification is absolutely necessary in the natural habitats.

In sub-levels of the species, the identification and protection of the grown and collected cultivars is an essential step. Nowadays, with molecular techniques genetic fingerprints are possible to be made. It is important in the notification of the new varieties and in the differentiation of the varieties from each other at the same time, especially when the varieties can be hardly distinguished from each other.

The *conculta*, also known as group of cultivars, is a taxonomic item over the “taxon” in which the individuals are colour variants of a common ancestor meaning that the only difference among them are the colours of the berries and the autumn foliage. Between *conculta* members (like 'Pinot noir', 'Pinot gris' and 'Pinot blanc') even SSR methods may not be effective for the separation. The difference between the white and red berried cultivars is that the white cultivars are not capable of anthocyanin production. The reason for this is that one transcription factor gene (*VvMybA1*) of biosynthetic pathway is not expressed while a Ty3–gypsy-type retro-transposon named *Gret1* blocks the promoter region and the gene function. This retroelement can be found in all grape cultivars at least in one copy, however, in the white varieties the *VvMybA1* is contained in both alleles, this is the reason for, no anthocyanin production. In contrast, the one dominant allele of the red berried cultivars, without the *Gret1* is capable of pigment production. According to this conclusion white and red berried cultivars can be distinguished according to the presence or the absence of the allele with *Gret1*. If there is any allele without the *Gret1*, it is a red berried cultivar.

2. AIMS

The experiments were designed to answer the following questions:

- Is there still some *Vitis sylvestris* C.C. Gmel. (wild grape) habitat in Hungary? To answer this question I've planned to re-visit the habitats which were described and characterized by András Terpó. Beside this my goal was to collect and preserve the plants deserving protection in gene banks.
- Is the characterization and diversity assessment of the Hungarian population of *Vitis sylvestris* C.C. Gmel. and the collected herbarium specimens made by András Terpó possible with morphological and molecular methods?
- I investigated *Vitis sylvestris* C.C. Gmel. items, wildly North American *Vitis* taxa which are still used as rootstocks (*Vitis riparia* Michx., *Vitis vulpina* L., *Vitis rupestris* Schee.), and *Vitis vinifera* L. cultivars with SSR markers to answer the following questions:
 - Is it possible to group the Hungarian and Turkish *Vitis sylvestris* C.C. Gmel. items and to compare the genetic variability of the samples?
 - Can the wild grape taxa in the Hungarian habitats be separated and identified?
 - Are the parent-offspring connections of the cultivars investigated true?
 - Is it possible to characterize the connection between *Vitis riparia* Michx. and *Vitis vulpina* L.
- Are the SSR and *Gret1* based marker techniques suitable for the separation of the *conculata* members (colour variants)?

3. MATERIALS AND METHODS

I visited several previously described *Vitis sylvestris* C.C. Gmel. habitats, to ascertain their status, to get a clear picture about the area of wild grape today, to collect plant material for my morphological and molecular investigations and to create a germplasm collection. During my work two main methods were used: the different *Vitis* taxa were compared with the help of morphological patterns and molecular markers.

The morphological comparison was made according to OIV and NÉMETH (1966). In this part of my work I compared the following samples according to 20 morphological patterns: 11 *Vitis sylvestris* C.C. Gmel. samples from different Hungarian habitats, 14 herbarium specimens collected and made by Terpó András, 3 *Vitis vinifera* L. cultivars ('Chasselas', 'Kadarka', 'Pinot noir') and 1 *Vitis riparia* Michx. and 1 *Vitis rupestris* Schee. items. The applied patterns were transferred to a numerical system and evaluated with NMDS (*Non-metric multidimensional scaling*), and cluster analysis methods, and depicted on a dendrogram.

During the molecular investigations 8 variable SSR markers were used (VvS1, VvS2, VvMD5, VvMD7, VvMD27, VrZag62, VrZag79) to estimate the genetic variability and to characterise 10 *Vitis sylvestris* C.C. Gmel. items, 1 herbarium specimens made András Terpó, and 6 *Vitis sylvestris* C.C. Gmel. samples from Turkey, 1 *Vitis riparia* Michx., 1 *Vitis labrusca* L., and 1 *Vitis vulpina* L. samples. The results were evaluated with NMDS, and cluster analysis as well, and depicted on a dendrogram.

Additional molecular investigations were made with the above mentioned SSR markers for the characterization and separation of 42 grapevine cultivars containing several *conculta* member items. For the separation of the *conculta* members 3 earlier published (THIS et al. 2007) and 1 own designed oligonucleotide primers were used to detect the *Gret1* retroelement.

4. RESULTS

It was found that the wild grape is still present in the habitats characterized by András Terpó. The conditions of the habitats are deteriorating similarly to the other European regions. The size and the quality of the area is reduced due to human intervention. The North American *Vitis* taxa, especially the *Vitis riparia* Michx. escapes the vineyards and threatens the habitat of the *Vitis sylvestris* C.C. Gmel. This invasive species adapts well to the environment, and supplants the wild grape from the habitats. In order to separate the taxon from each other, both morphological and molecular methods were tried to discover the suitable characteristics and markers.. It was found that both methods are suitable for the separation of the taxon. In the case of the morphological method, the erected hairs on the lower side of the leafs and the hair on the petiole proved to separate the different species. Beside this, the molecular SSR method is also effective to differentiate between the invasive taxon and the native wild grape populations (*Figure 1*).

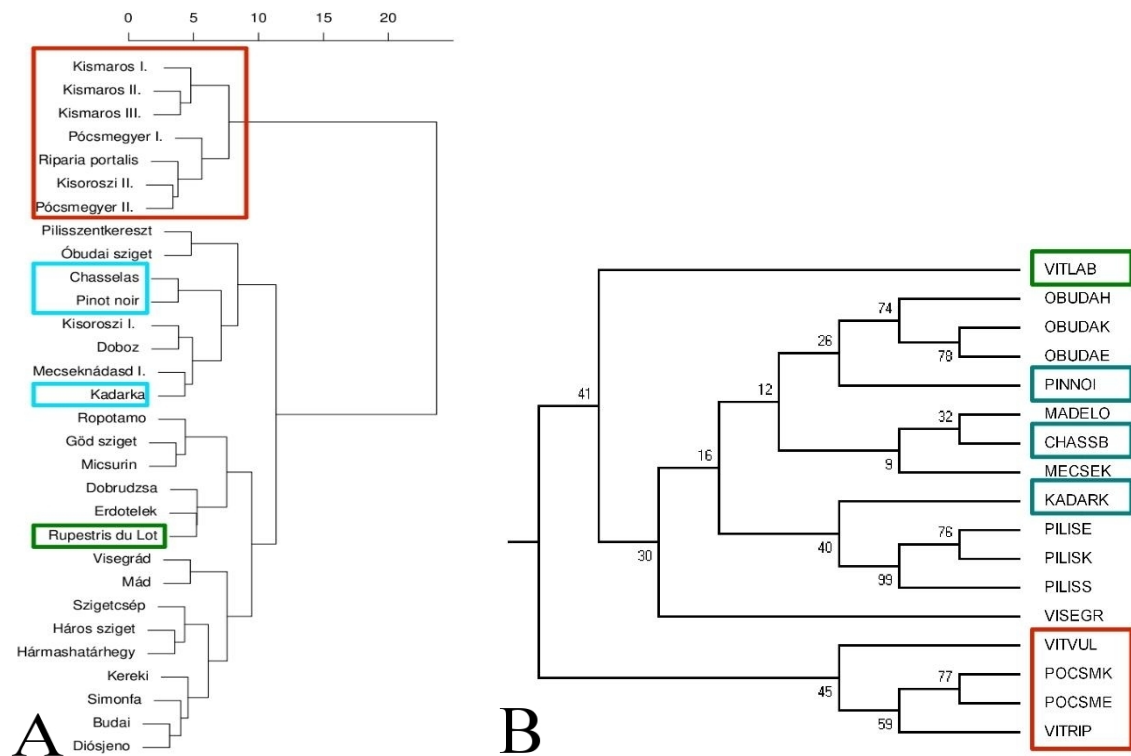


Figure 1.: The separation of the examined *Vitis* taxon with the help of morphological (A) and molecular (B) methods. Frame-codes: RED: *Vitis riparia* Michx. and hybrids, BLUE: *Vitis vinifera* L., GREEN: a *Vitis rupestris* Schee. and *Vitis labrusca* L., NO FRAMES: *Vitis sylvestris* C.C. Gmel.

The examination of the *Vitis vinifera* L. showed that not all of the colour variants can be distinguished according to the presence or absence of the *Gret1* element (Table 1.), as some white berried cultivars showed red berried genotypes. The earlier published general theory about the *VvMybA1* locus and the berry colour has to be reviewed.

Table 1.: The separation of the *conculta* members. The places of sample collection: Pécs-PTE Institute of Viticulture and Enology; Nagyréde-Szőlőskert Zrt.; Helvécia-MgSzH Helvéciai Research Farm; Eger-KRF Research Institute of Viticulture and Enology.

Sample	Natural classification/Pedigree	Berry colour	Methods for differentiation	
			SSR	Gret1
Bajor blackwooden	<i>convar. pontica</i>	red	no	no
Bajor blue	<i>convar. pontica</i>	red	yes	no
Bajor grey	<i>convar. pontica</i>	grey	no	no
Chasselas rouge	<i>convar. orientalis</i>	pink	no	no
Chasselas blanc	<i>convar. orientalis</i>	white	no	no
Csaba gyöngye	Madeleine Angevine × Muscat Fleur d'Oranger	white	no	no
Csaba gyöngye red	Csaba gyöngye × Mathiász Jánosné muskotály	pink	no	no
Delaware white	hibrid	white	yes	yes
Delaware red	hibrid	pink	yes	yes
Gohér red	<i>convar. pontica</i>	pink	no	no
Gohér white	<i>convar. pontica</i>	white	no	no
Gohér altered	<i>convar. pontica</i>	white	yes	no
Járdovány black	<i>convar. pontica</i>	red	yes	n.a.
Járdovány white	<i>convar. pontica</i>	white	yes	n.a.
Merlot gris	<i>convar. occidentalis</i>	grey	no	no
Merlot	<i>convar. occidentalis</i>	red	no	no
Mustos	<i>convar. pontica</i>	white	yes	n.a.
Mustos white	<i>convar. pontica</i>	white	yes	n.a.
Pinot noir	<i>convar. occidentalis</i>	red	no	yes
Pinot gris	<i>convar. occidentalis</i>	grey	no	no
Pinot blanc	<i>convar. occidentalis</i>	white	no	yes
Sárfehér	<i>convar. pontica</i>	white	yes	n.a.
Sárpiros	<i>convar. pontica</i>	red	yes	n.a.
Tramini	<i>convar. occidentalis</i>	white	no	no
Tramini red	<i>convar. occidentalis</i>	pink	no	no

5. NEW SCIENTIFIC RESULTS

1. **According to the field works and the morphological and molecular investigations it was proved that the *Vitis sylvestris* C.C. Gmel. is still present in the habitats earlier characterized by András Terpó.**

In all visited habitats except for Pócsmegyer, even if in a small number but the wild grape was present. The area of the species decreased to very small territory, due to the spread of the invasive North American species and the degradation of the area.

2. **A germplasm collection was established to keep the genetic variability, using the collected and the seeding plant material from natural habitats.**

The collected seeds and plant material were grown and planted in a plant collection. The seedlings and cuttings are signed with the name of the habitat so that in the future morphological characters will be possible to be examined.

3. **With the help of the morphological and molecular examinations, *Vitis sylvestris* C.C. Gmel., *Vitis riparia* Michx., hybrid plants and populations were separated and identified.**

Appropriate morphological characters and molecular markers were found which allow the separation of the wild grape individuals from the rootstock cultivars bred from the North American *Vitis* taxon, which most commonly escapes from the vineyards.

4. **Results show that the best differential characteristics to separate the *Vitis sylvestris* C.C. Gmel. from the *Vitis riparia* Michx. are the patterns of erected hairs on the lower side of the leaf and petioles on the latter taxon.**

According to the 20 morphological characters it can be concluded that although the *Vitis sylvestris* C.C. Gmel. has variable type of hairs on the organs, but no erected hairs on the leaf or petioles. This character is suitable for the separation of taxon during field works.

- 5. Based on the plant material from the University of Pécs's Institute of Viticulture and Enology, this research was the first to prove the long-debated theory: *Vitis riparia* Michx. and *Vitis vulpina* L. are the same taxon. Probably the latter is the synonym of the *Vitis riparia* Michx.**

The connection between the two species was not clarified without doubt until now. Some researchers considered them as separate species, according to others *Vitis vulpina* L. and *Vitis riparia* Michx. are synonyms.

- 6. Neither the examined morphological characters, nor the molecular genetic SSR markers used in this study are suitable for the separation of the *Vitis sylvestris* C.C. Gmel and the *Vitis vinifera* L. taxon.**

The botanical connection between the two species is a current issue nowadays as well. According to some opinions the wild grape is not an individual species, but the subspecies of the *Vitis vinifera* L., others accept it as separate species. It is proved the grapevine evolves from the wildgrape with the modification of some characters like dioecious flowers to monoecious, or berry and cluster increase. Neither the morphological characters, nor the molecular markers are capable of separating the two taxon.

- 7. The theory of the 'Delaware white' and 'Delaware red' having a parent-offspring connection was confuted with the help of molecular markers. The connection between the 'Budai gohér' and 'Török gohér' cultivars was clarified as well as the theory that the 'Fekete járdovány' and 'Fehér járdovány' do not form a *conculata*, the two names are just similar to each other.**

This is the first report about the connection between the 'Delaware white' and 'Delaware red'. According to an earlier hypothesis the 'Delaware white' is the seedling of 'Delaware red', this examination rejected this statement.

8. Results proved that not all colour variants can be separated according to the presence of the *Gret1* retroelement in the promoter region of the *VvMybA1* transcription factor gene.

According to earlier investigations the dominant factor in the colour of the *Vitis vinifera* L. cultivars is the presence of the *Gret1* retrotransposon in the promoter region of the *VvMybA1* transcription factor gene, which blocks the expression of the gene. Consequently the anthocyanin biosynthetic pathway is stuck, and the plant is not able to produce anthocyanin. Results show that not in all cases is the *Gret1* responsible for the colour-loss mutation. According to this, the general theory was refuted, namely, that the white and red berried *Vitis vinifera* L. cultivars can be distinguished in all cases according to the presence of the retroelement.

6. PUBLICATIONS

Scientific publications in English with impact factors

Bodor P., Höhn, M., Pedryc A., Deák T., Dücső I., Uzun, I., Böhm É., Cseke K., Bisztray Gy. D. (2009): Conservation value of the native Hungarian wild grape (*Vitis sylvestris* Gmel.) evaluated by microsatellite markers. *Vitis*. 49 (1) 23-27. (If:0,798)

Refereed scientific publications in English without impact factors

Bodor P., Varga Zs., Deák T., Pedryc A., Bisztray Gy. D. (2008): Old Hungarian grapevine cultivars and their relations characterized with microsatellite markers. *Int. J. Hort. Sci.* 14, (4): 27-32.

Varga Zs., Bényei F., **Bodor P. Jr.**, Fazekas I. (2007): Investigation of the cultivation value of old grapevine cultivars in Tokaj in 2004-2006. *Int. J. Hort. Sci.* 13, (2): 23-27.

Bodor P., Deák T., Bényei F., Varga Zs., Bisztray Gy. D. (2007): A mikroszatelliteken alapuló molekuláris markerezés előnyei és hátrányai szőlő (*Vitis vinifera* L.) esetében. *Kertgazdaság*. 39, (2): 57-62.

Refereed scientific publications in Hungarian without impact factor

Bodor P., Varga Zs. (2009): Szülő-utód kapcsolatok szőlőfajtáink között. *Gyakorlati agrofórum*. 2009. Extra 30. 12-13.

Ifj. Bodor P., Varga Zs., Lőrincz A., Bisztray Gy. D. (2008): Hibrid szőlőfajták eredetének nyomon követése morfológiai és molekuláris szinten. *Borászati Füzetek*. 18, (2) Kutatás: 1-4.

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Proceedings and oral presentations

Bodor, P., Dücső, I., Deák, T., Pedryc, A., Bisztray, Gy.D., Böhm, É., Höhn, M. (2009) A ligeti szőlő (*Vitis sylvestris* Gmel.) diverzitásvizsgálata molekuláris markerek segítségével. *Lippay J.- Ormos I.- Vas K. Tudományos Ülésszak, Szőlészettudományi Szekció*, 2009. október 28-30. Budapest. Összefoglalók. 258-259 p.

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Bodor P. – Bisztray Gy. D. – Pedryc A. (2008) The separation of grapevine (*Vitis vinifera* L.) conculta members with molecular markers. *MendelNet Agro '08. International Ph.D. Students Conference*. 2008.november 26. Brno (CD rom) ISBN: 978-80-7375-239-2

Bodor, P., Bisztray, Gy. D., Deák, T., Dücső, I., Pedryc A., Höhn, M. (2009) Diversity and conservation value of native *Vitis sylvestris* Gmel. stands from Hungary, evaluated by morphological and molecular markers. *2nd European Congress of Conservation Biology*. Prague, Czech Republic, 01-05 September, 2009. Book of Abstract 57. p.

Bodor P. Jr. (2007): Old Hungarian grapevine (*Vitis vinifera* L.) cultivars invetigated by microsatellite markers. *Proceedings of the International Life Sciences Students` Conference*. 2007. November 7.-11. Ljubljana, Slovenia p. 88.