APPLICATION OF ISOZYMES AND SSR MARKERS FOR THE ANALYSIS OF THE GENETIC BACKGROUND OF SOME ROOTSTOCKS DERIVED FROM TELEKI'S SEEDLINGS (TELEKI 5C, KOBER 5BB, SO4)

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The aim of this work was to determine the SSR profile of 20 Vitis rootstocks at 15 SSR loci and 3 isozyme systems to find genetic relatedness between them. Based on the isozyme results, it can be established, that all of the three enzymes (acid phosphatase, catechol-oxidase, glutamate-oxalacetate transaminase) showed polymorphism for the assessment of relatedness. Teleki's hybrids (Teleki 5C, Kober 5BB, SO4) show high similarity. Based on the dendogram of isozyme data these items also show close similarity to the '*Vitis berlandieri* Rességuier' (V._berl._R107, V._berl._R1) accessions. The examined *Vitis riparia* and *Vitis rupestris* items also show relatedness, but for the Teleki's seedlings the similarity is lower, which can be traced back to the complex hybrid origin of the seedlings. Based on the SSR results the Teleki hybrids (Teleki 5C, Kober 5BB, SO4) show the highest similarity with V. riparia derivates. In the combined isozymes-microsatellite dendogram Teleki hybrids show close relatedness with the 'V. berlandieri Rességuier' (R107, R1) accessions.
Keywords: Vitis, grapevine, rootstocks, molecular markers, microsatellite

Anwendung von Isoenzymen und SSR-Markern für die genetische Analyse der Unterlagsrebsorten, die von Teleki-Sämlingen abstammen (Teleki 5C, Kober 5BB, SO4): Ziel dieser Arbeit war es, an 20 Unterlagsrebsorten mit Hilfe von SSR-Profilen an 15 SSR-Loci und dreier Isoenzym-Systeme die genetische Verwandtschaft zu analysieren. Basierend auf den Ergebnissen der Isoenzym-Analysen kann festgestellt werden, dass alle drei Enzyme (Saure Phosphatase, Polyphenoloxidase, Glutamat-Oxalacetat-Transaminase) einen ausreichenden Polymorphismus für die Abschätzung des Verwandtschaftsgrades aufwiesen. Die Analyse der Teleki-Hybriden Teleki 5C, Kober 5BB sowie SO4 ergab eine nahe Verwandtschaft. Auf der Grundlage der Isoenzym-Analysen erstellte Dendogramme zeigen, dass die drei Unterlagsrebsorten auch Ähnlichkeit mit '*Vitis berlandieri* Rességuier' (V._berl._R107, V._berl._R1)-Typen haben. Die untersuchten *Vitis riparia*- und *Vitis rupestris*-Typen zeigten ebenfalls Verwandtschaft zu den drei Unterlagsrebsorten. Allerdings ergaben die Analysen in letzterem Fall einen geringeren Grad an Verwandtschaft, eine Tatsache, die auf die komplexe Hybrid-Herkunft der Sämlinge zurückzuführen ist. Basierend auf der SSR-Analyse

ergeben sich folgende mögliche Eltern-Nachkommen-Kombinationen: Teleki 5C – 'Riparia Gloire de Montpellier'. Die Teleki-Hybriden (SO4, Teleki 5C, Kober 5BB) zeigen die größte Ähnlichkeit mit *V. riparia*-Abkömmlingen. Im kombinierten Isozyme-SSR-Dendogramm zeigen die Teleki-Hybriden enge Verwandtschaft mit den 'Vitis berlandieri Rességuier' (R107, R1)-Akzessionen.
Schlagwörter: Vitis, Rebe, Unterlagen, Molekularmarker, Mikrosatelliten

Grape rootstocks became important at the end of the 19th century, after the appearance of the phylloxera epidemic in Europe at the end of the 1860s, beginning of 1870s. Planting with grafts became common in winegrowing after the destruction caused by the phylloxera in the historical wine districts of Hungary, amounting to about 20 to 25 % of the vineyards (Csepregi and Zilai, 1973). The rootstock variety became the basis for production in viticulture, determining the phylloxera resistance (Boubals, 1966), nutrient uptake (Erdei et al., 1985; Ruhl, 1989; Kocsis and Lehoczky, 2000; Csikászné Krizsics, 2008), life span, soil requirements, drought tolerance, salt tolerance (Downtown, 1977) and lime tolerance of the grafts (Kocsis, 1998), the primary growth and the time of the commencement of the production of stocks (Rives, 1971; Mannini et al., 1990). It also influences the quality and quantity of crop, and the economy of grape production (Lefort and Legisle, 1977; Howell, 1987).

The clonal selection and cross breeding of grape rootstocks dates back to more than one hundred years. In 1896 Sigmund Teleki, the well-known rootstock breeder aimed at breeding rootstocks with high lime tolerance (Németh, 1975; Bakonyi and Kocsis, 2004). He bought grape seeds from a famous French *Vitis berlandieri* breeder, Euryale Rességuier.

The pedigree of these 40.000 seedlings has not been clarified to the present day (Poczai et al., 2013). So it is unknown which species the world's most widespread rootstocks Teleki 5C, Kober 5BB and SO4 originated from (Schmid et al., 2005; Goldammer, 2013).

Teleki sorted his seedlings to 10 groups. The groups 1, 2 and 3 contained *vinifera* type individuals, which were not further propagated. He classified the *riparia* phenotype individuals with naked internode to the group 4, 5 and 6, and to the *berlandieri*-like, downy-internoded ones to the groups 7, 8 and 9. To the group no. 10 the *rupestris*-like individuals were sorted. Among these features, the flower types were recorded, which made the characterisation of varieties more systematic (Table 1).

The characterisation of grapevine by isoenzymes began in the seventies, using starch gel electrophoresis (Wolfe, 1976; Schwennesen et al., 1982; Arulsekar and Parfitt, 1986), followed by the use of polyacrilamide gel electrophoresis (Sánchez-Escribano et al., 1998) and isoelectric focusing (Bachmann and Blaich, 1988; Royo et al., 1989). Later an evidence was given, that the environment has no influence on the isoenzyme patterns of some enzymes (peroxidase, catechol-oxidase etc.) of the grape, if the samples are taken from the woody stems in dormancy (Kozma et al., 1990; Royo et al., 1997).

There are only limited analyses of the isozymes' variability of grapevine rootstocks at that time. Twenty-seven varieties and feral accessions from four *Vitis* species were examined by Subden et al. (1987) for 12 isozyme systems exhibiting polymorphism. Using extracts from woody tissue and a protocol to avoid isozyme inactivation by polyphenolics and other materials, 27 of 29 strains exhibited unique isozyme banding patterns for glucose-6-phosphate isomerase, peptidase, and acid phosphatase. Implications for genetic homogeneity screening of nursery stock or identifying unknown samples are discussed.

Starch gel electrophoresis was used by Walters et al. (1989) for the analysis of *Vitis vinifera L.* varieties, interspecific *Vitis* hybrids and wild individuals of *Vitis riparia* Michx. They suggest a simple and inexpensive procedure for the extraction of active enzymes from grape, which is rapid and efficient. Starch gel electrophoresis with different optimized gel electrode buffer systems is used for 40 different isoenzymes, 14 of which were consistently resolvable and showed variation among different varieties. Isozyme analysis by starch gel electrophoresis for glucose phosphate isomerase (GPI) from roots and woody cane extracts showed that this material can be used for the identification of rootstocks (Boursiquot and Parra, 1992). Concerning the roots the best results were

obtained at the first stage of growth (during spring) when the plants were in good physiological state but this material is rather delicate. Authors suggested working with extracts of scrapings from woody canes which give good results and fair resolutions. Nine phenotypes have been found with the thirty rootstocks tested in the GPI-2 system. It was possible to identify Fercal, 41 B, 333E.M., 161-49 C, and Vialla. The other rootstocks could be sorted in four groups.

Starch gel electrophoresis was used to produce isozyme banding patterns for the 60 grape rootstocks available from the University of California, Davis grape collections. Strips of tissue from under the bark consisting of young phloem, young xylem, and vascular cambium provided the sample material for protein extraction. Isozyme patterns were produced with the following enzyme systems: glucose phosphate isomerase (GPI), aspartate aminotransferase (AAT), phosphogluconate dehydrogenase (6-PGD), phosphoglucomutase (PGM), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), leucine amino-peptidase (LAP). The resulting patterns were consolidated and arranged in a grid designed to function as a taxonomic key. A unique isozyme profile was obtained for each rootstock. Ten unknown rootstocks were tested and their identities were determined using this system (Walker and Liu, 1995).

Rosbarceló et al. (1996) studied the gene expression of isozymes of peroxidase in downy mildew resistant (*Vitis vinifera* x *Vitis rupestris*) x *Vitis riparia* hybrids and in the susceptible *Vitis vinifera* parent. The peroxidase isoenzyme type B3 (PI = 8,9) expressed in the phloem of resistant hybrids, was completely absent in the susceptible parent.

Tests were carried out on different types of calli and somatic embryos of *V. rupestris* using 2-D electrophoresis. The investigation carried out by Martinelli et al. (1993) was focused on the isozyme patterns of AcP (acid phosphatase), ADH (alcohol dehydrogenase), EST (esterase), G6PDH (gluconate-6-phosphate dehydrogenase) and PGM (phosphoglucomutase). A typical variation of isozyme pattern could be observed during the different steps of somatic embryogenesis. Dedifferentiated callus showed other types of isoenzyme patterns compared to those obtained during the development of somatic embryos.

The new knowledge obtained by the development and application of DNA markers provides several further possibilities in grape genetics researches. The DNA markers make it possible to characterise and identify the different varieties, so the origin and relationships of the varieties can be discovered. It opens the possibility for the verification of former systems and classifications.

The microsatellite markers (also called SSR) belong to the most effective types of DNA markers. Environmental conditions, diseases, growing conditions, time of sampling do not influence the results. The microsatellites are highly polymorphic DNA markers comprised of mononucleotides, dinucleotides, trinucleotides or tetranucleotides that are repeated in tandem arrays and distributed throughout the genome. As they are regularly shorter than 100 bp. and are bordered by special sequences, they can be amplified by polymerase chain reaction (PCR). The polymorphism of microsatellites originates from the number of repeats. This polymorphism is stable enough, to make the marker useable in genetic analysis (Hearne et al., 1992; Thomas et al., 1994).

Microsatellite analyses were frequently used for rootstock analyses in the last decades. The DNA extracted from the cambium tissues of grape (*Vitis spp*., *Muscadinia rotundifolia* Small) rootstocks was found to be suitable for molecular analysis. Its quality was equivalent to that of DNA extracted from leaf tissues, although the yield was higher from leaves. The use of cambium tissue allows DNA extractions during dormancy or from grafted rootstocks where leaves are not available (Lin and Walker, 1997).

The first genetic linkage map of grape derived from rootstock parents was constructed using 188 progeny from a cross of Ramsey (*Vitis champinii*) × *Riparia Gloire* (*V. riparia*) (Lowe and Walker, 2006). Eleven microsatellites isolated from grapevine (*Vitis vinifera*) were used to study the degree of conservation of these sequences across different Vitis species. The results demonstrate the possibility of extending the use of microsatellite markers on wild germplasm and interspecific hybrids.

A representative group of rootstock accessions and varieties of the Vitis species commonly used in rootstock breeding (*V. vinifera, V. berlandieri, V. riparia, and V. rupestris*) and conserved in the largest European germplasm banks of Vitis were analyzed using sequence tagged microsatellite sites (STMS) and amplified fragment length polymorphism (AFLP) markers. The STMS ana-

Table 1: The origin and classification of Teleki's varieties based on morphological characters (according to BAKONYI and KOCSIS, 2004)

Group no.	Type	Internode	Colour of tip	Flower type	Variety name
1*, 2*, 3*	Vinifera	-	-	-	-
4A	-	naked	bronze	hermaphrodite male	Binova SO4
5A	Riparia	naked	bronze	female male	5BB G.K.67
			green	female male	G.K.62 5C
6A	-	naked	green	female	G.K.1
7B	-	downy	bronze	male female	125 A, 127 125 AA
8B	Berlandieri	downy	bronze	female male	Cosmo 2, 10, G.K.10 Durlach 50, 52, G.K.9
9B	-	downy	green	female	Barr 503, 513
10A	Rupestris	naked	bronze	male	T.10A

*Not propagated because of low vigour

Table 2: List of the analyzed Vitis accessions

No.	ID	Accession name	Genetic origin	Origin of the accession
1	V._berl._R1	Resseguier N1	V. berlandieri	INRA, Domaine de Vassal, France
2	V._rup._FW3	Fort Worth N3	V. rupestris	
3	V._rup._T	Taylor	V. rupestris	
4	V._cord.	8029 Mtp2	V. cordifolia	
5	V._rip._GdM	Gloire de Montpellier	V. riparia	
6	Aramon_rup_G1	Aramon Ganzin N1	V. vinifera x V. rupestris	
7	V._vip._Ggb	Riparia Grand glabre	V. riparia	
8	V._rup._FW1	Fort Worth N1	V. rupestris	
9	Jacquez	Jaquez	V. Bourquina (Vinifera x Aestivalis)	
10	Vialla	Vialla	V. labrusca x V. riparia	
11	V._cin._Arnold	Cinerea Arnold	V. cinerea	
12	V._aest._S.	Sauvage	V. aestivalis	
13	V._sol.	Solonis	V. solonis	
14	V._rup._FW2	Fort Worth N2	V. rupestris	
15	V._berl._R107	Resseguier N107	V. berlandieri	
16	Aramon_rup_G2	Aramon Ganzin N2	V. vinifera x V. rupestris	
17	N._Mex.	V. Novo Mexicana	V. riparia x V. candicans	
18	T5C	Teleki 5C E20	V. berlandieri x V. riparia	Cserszegtomaj, Hungary
19	SO4	SO4 (133)	V. berlandieri x V. riparia	
20	5BB	Kober 5BB	V. berlandieri x V. riparia	

lysis allowed assigning a microsatellite genotype to most rootstock varieties, although it revealed numerous misclassified accessions in the studied collections. Genetic similarity among the different genotypes was analysed using AFLP, which provided information on the genetic relationships within and between hybrid groups (DEANDRÉS et al., 2007).

The aim of this study was to analyse the similarity and genetic relationship among three rootstocks (Teleki 5C, Kober 5BB, SO4) and their possible relatives.

In this study both molecular and biochemical markers were used: the SSR profile in 15 loci and the isoenzyme patterns were determined in three systems.

MATERIAL AND METHODS

PLANT MATERIAL

The plant material (dormant canes) of 20 *Vitis* accessions (Table 2) for enzyme and DNA extraction originated from INRA, Domaine de Vassal, France (1 to 17) and from the collection of the University of Pannonia in Cserszegtomaj (Hungary). Enzymes and DNA were extracted from the materials originating from France, the remaining were propagated for conservation in Cserszegtomaj, Hungary. Repeated samples were collected three times in the dormant period of 2010 in Cserszegtomaj to ensure reproducibility.

ENZYME EXTRACTION

The canes of the samples were peeled and the phloem was removed. 150 mg polyvinylpolypyrrolidone (Sigma-Aldrich Steinheim, Germany) and 1 ml of cold (4 °C) extraction buffer (Arulsekar and Parfitt (1986) were added to 300 mg of plant material, and homogenized in a mortar. After centrifugation at 4 °C, 20 ml of clear supernatant were applied for isoenzyme analyses. The extracts for further examination were stored at -75 °C.

ISOENZYME ANALYSIS

Vertical polyacrylamide gel electrophoresis was used for the separation of isoenzymes of catechol-oxidase (CO), peroxidase (PER), glutamate-oxalacetate-transaminase (GOT) and acid phosphatase (AcP) as described by Royo et al. (1997). Bromophenol blue was added for every sample as tracking dye.
After electrophoresis the gels were stained for AcP, GOT and PER with the staining solutions as described by Arulsekar and Parfitt (1986), or for CO as described by Sánchez-Yélamo (1992). The electrophoresis for all of the enzyme systems was repeated three times with every sample to ensure reproducibility.
The isoenzyme patterns were evaluated visually. Rf (relative mobility) values were calculated for every single band as follows: the distance migrated by a band divided by the distance migrated by the dye (bromophenol blue) front.

DNA EXTRACTION

DNA was extracted from the phloem of the dormant canes with DNA Plant Mini Kit (Quiagen Hilden, Germany), following the instructions of the producer. Both the quantity and quality of DNA were determined spectrophotometrically. The amount of DNA was determined by measuring the 260 nm absorbance and calculating as follows: concentration of DNA (μg/ ml) = A260 x 50 x dilution ratio, the quality was estimated by measuring the 260 to 280 nm absorbance ratio (A260/A280). The DNA was diluted to a concentration of 10 ng/ml.

SSR ANALYSIS

Microsatellite (SSR) analysis was performed in 15 loci (Table 3). The primers had been chosen from different chromosomes (Costantini et al., 2007) to give well-defined heterozygosis.
Polymerase chain reactions were carried out in a total volume of 25 μl containing 12.5 μl of Hot Start Master Mix (Quiagen), 0.2 μM of each primer, and 50 ng of template DNA, using the following thermal profile: (1) 94 °C for 45 min; (2) 94°C for 1 min, at the annealing temperature (Table 3) for 1 min, 73 °C for 1 min per 35 cycles; (3) 73 °C for 7 min.
One primer of each primer pair was fluorescently labelled with FAM (6FAM) on the 5' end of the DNA chain. PCR products were run on a PE-Applied Biosystem 3100 Automated Capillary DNA Sequencer, the length of the products was determined using GeneScan 2.0 software (Applied Biosystem, Foster City, USA).

DATA ANALYSIS

The isoenzyme bands were scored as present (1) or absent (0) across all genotypes. Estimates of genetic similarity between pairs were calculated by the Jaccard index (Jaccard, 1908). In the Jaccard index joint absences are excluded from consideration, equal weight is given to matches and non-matches. For the generation of distance matrices and UPGMA dendograms a demo version of MolMarker – a platform-independent software for the analyses of molecular marker data – was used (Györffyné Jahnke and Smidla, 2014).

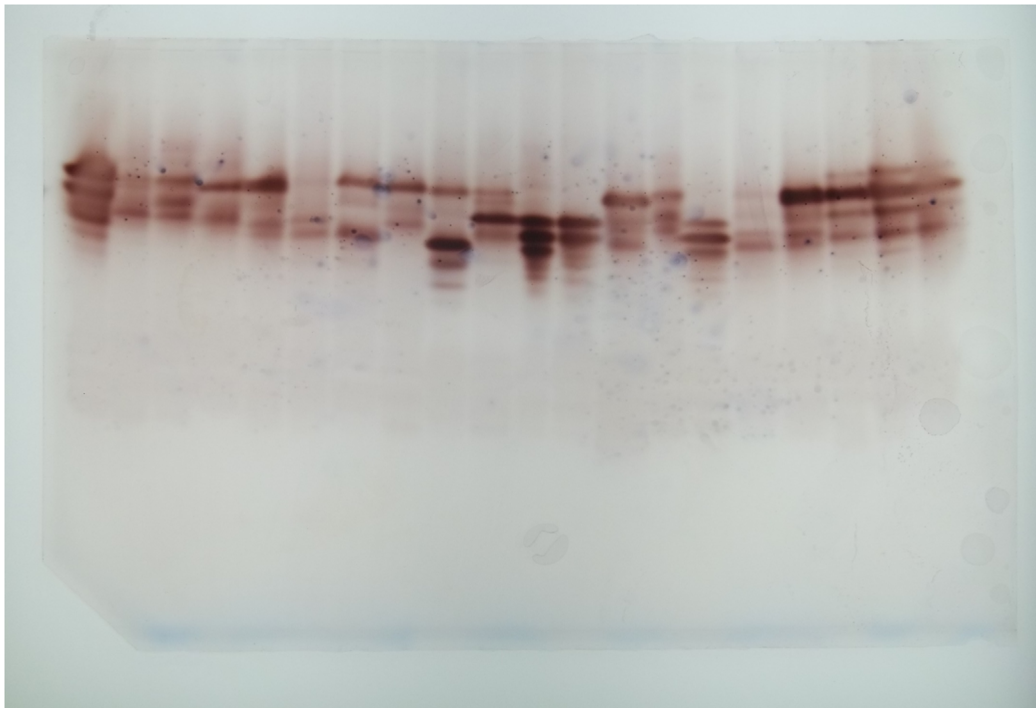

Fig. 1: Catechol-oxidase banding patterns (order of accessions as in Table 4)

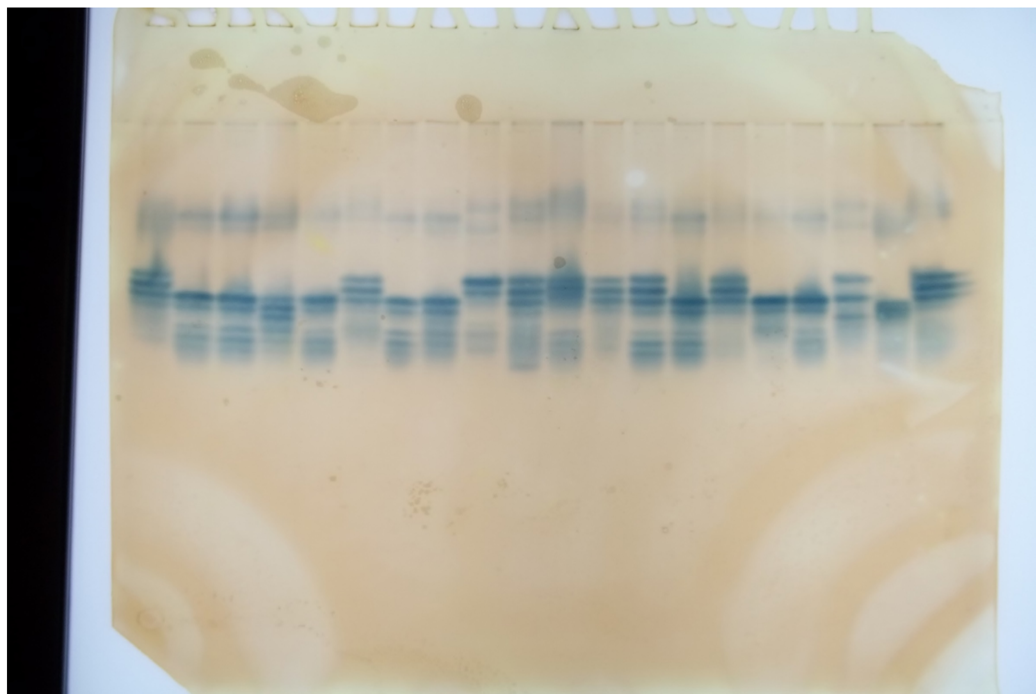

Fig. 2: Glutamate-oxalacetate-transaminase banding patterns (order of accessions as in Table 5)

Table 3: List of the analyzed SSR loci

Linkage group[a]	SSR locus name	Annealing temperature
1	VMC8A7	64 °C
2	VMC7G3	60 °C
3	VVMD28	62 °C
4	VrZag21	62 °C
5	VrZag79	60 °C
8	VMC1F10	57 °C
9	VMC1C10	60 °C
10	VrZag25	67 °C
13	VMC3D12	57 °C
12	VMC2H4	57 °C
15	VMC5G8	58 °C
14	VMCNG1E1	58 °C
17	Scu06vv	60 °C
18	VVIM10	57 °C
19	VMC5E9	58 °C

[a]Linkage groups are numbered according to ADAM-BLONDON et al. (2004)

RESULTS AND DISCUSSIONS

ISOENZYME ANALYSIS

In case of catechol-oxidase (CO), glutamate-oxalacetate transaminase (GOT) and acid phosphatase (AcP) enzymes the results were reproducible and the zymograms of the woody stem extracts were independent of the time of sampling in the dormant period of the grape. Similar conclusions were made by ROYO et al. (1997). They found, that during the resting period (fall/winter) a good resolution of reproducible bands were obtained, while during the growing season, a variable number of erratic bands were often present. WALKER and BOURSIYUOT (1992) also stated that cambial scrapings from dormant canes produced reliable and consistent isozyme profiles with aspartate aminotransferase (same as GOT).

Similarly to the results of ROYO et al. (1997) and ORITZ et al. (2004) the catechol-oxidase (CO) was the most polymorphic system: 14 bands and 18 patterns were observed. Based on these patterns 16 of the 20 analysed

Table 4: The banding patterns by Rf (relative mobility) values for catechol-oxidase

No.	ID	Rf value														Banding pattern type
		0,170	0,200	0,225	0,235	0,250	0,255	0,260	0,265	0,270	0,275	0,280	0,290	0,310	0,350	
1	V._berl._R1	0	1	0	1	0	0	0	0	0	0	0	1	1	0	G
2	V._rup._FW3	0	1	0	0	0	0	0	0	0	1	0	0	0	0	K
3	V._rup._T	0	1	0	1	0	0	0	0	0	1	0	0	0	0	F
4	V._cord.	0	1	0	0	0	0	0	0	0	1	0	0	0	0	K
5	V._rip._GdM	0	1	0	0	0	0	0	0	0	1	0	1	0	0	J
6	Aramon_rup_G1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	R
7	V._vip._Ggb	0	1	0	0	0	0	0	0	0	0	0	1	0	0	M
8	V._rup._FW1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	H
9	Jacquez	0	1	0	0	0	0	0	0	0	0	0	1	1	1	L
10	Vialla	0	1	0	1	0	0	0	1	0	0	0	0	0	0	E
11	V._cin._Arnold	0	0	0	1	0	0	0	1	0	0	0	1	0	0	O
12	V._aest._S.	0	0	0	1	0	0	0	1	0	0	0	0	0	0	P
13	V._sol.	0	1	0	0	0	0	0	0	1	0	0	0	0	0	I
14	V._rup._FW2	0	1	1	0	1	0	0	0	0	0	0	0	0	0	C
15	V._berl._R107	0	0	0	1	0	0	1	0	0	0	1	0	0	0	N
16	Aramon_rup_G2	0	0	0	0	0	1	0	1	0	0	0	0	0	0	Q
17	N._Mex.	0	1	0	1	0	1	0	1	0	0	0	0	0	0	D
18	T5C	1	1	0	1	0	0	0	1	0	0	0	0	0	0	B
19	SO4	1	1	0	1	0	0	0	1	0	0	0	0	1	0	A
20	5BB	1	1	0	1	0	0	0	1	0	0	0	0	0	0	B

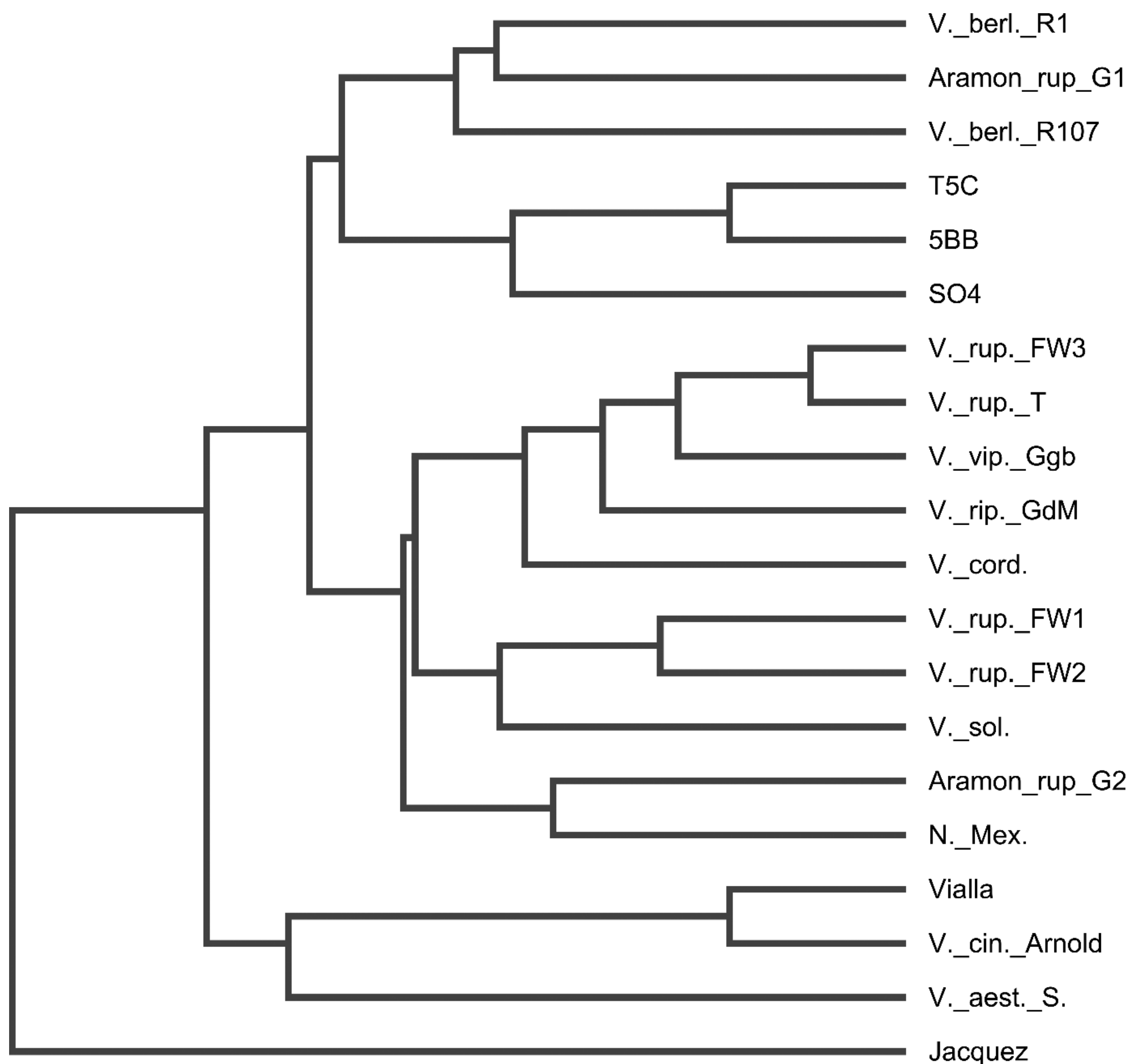

Fig. 3: Dendogram based on isoenzyme results

accessions were identifiable. The banding patterns by Rf (relative mobility) values are presented in Table 4. The catechol-oxidase banding patterns are shown in Figure 1. In the case of glutamate-oxalacetate-transaminase (GOT) 9 bands and 13 patterns were observed. Based on these patterns 9 of the analysed accessions can be identified. The banding patterns by Rf (relative mobility) values are presented in Table 5. The glutamate-oxalacetate-transaminase banding patterns are shown in Figure 2. Royo et al. (1997) and Oritz et al. (2004) also found the GOT isozyme system highly polymorphic.

The acid phosphatase (AcP) enzyme provided only 7 bands and 4 patterns, so by this enzyme only two of the 20 accessions were identifiable. The banding patterns

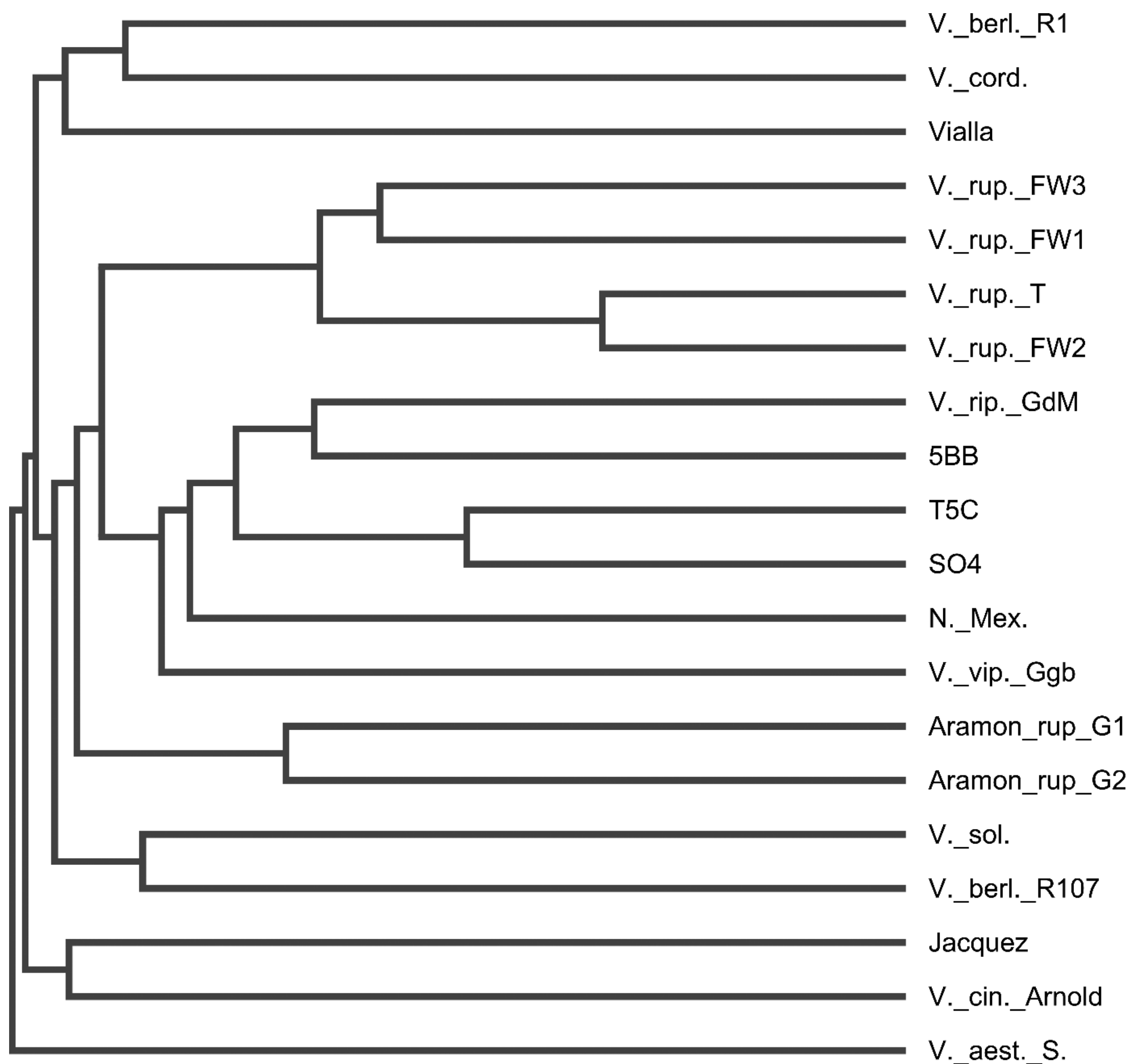

Fig. 4: Dendogram based on microsatellite results

by Rf (relative mobility) values are presented in Table 6. The acid phosphatase banding patterns are shown in Figure 3. In our former studies a special acid phosphatase isozyme pattern (with an additional band) was found (Jahnke et al., 2009), which was completely absent in the varieties involved in this study.

Based on the totted up isoenzyme results a dendogram was constructed (Fig. 3). The Teleki 5C, Kober 5BB and SO4 rootstocks show close relatedness. Based on this dendogram these items also show close similarity with the '*Vitis berlandieri* Rességuier' (V._berl._R107, V._berl._R1) accessions which confirm the information

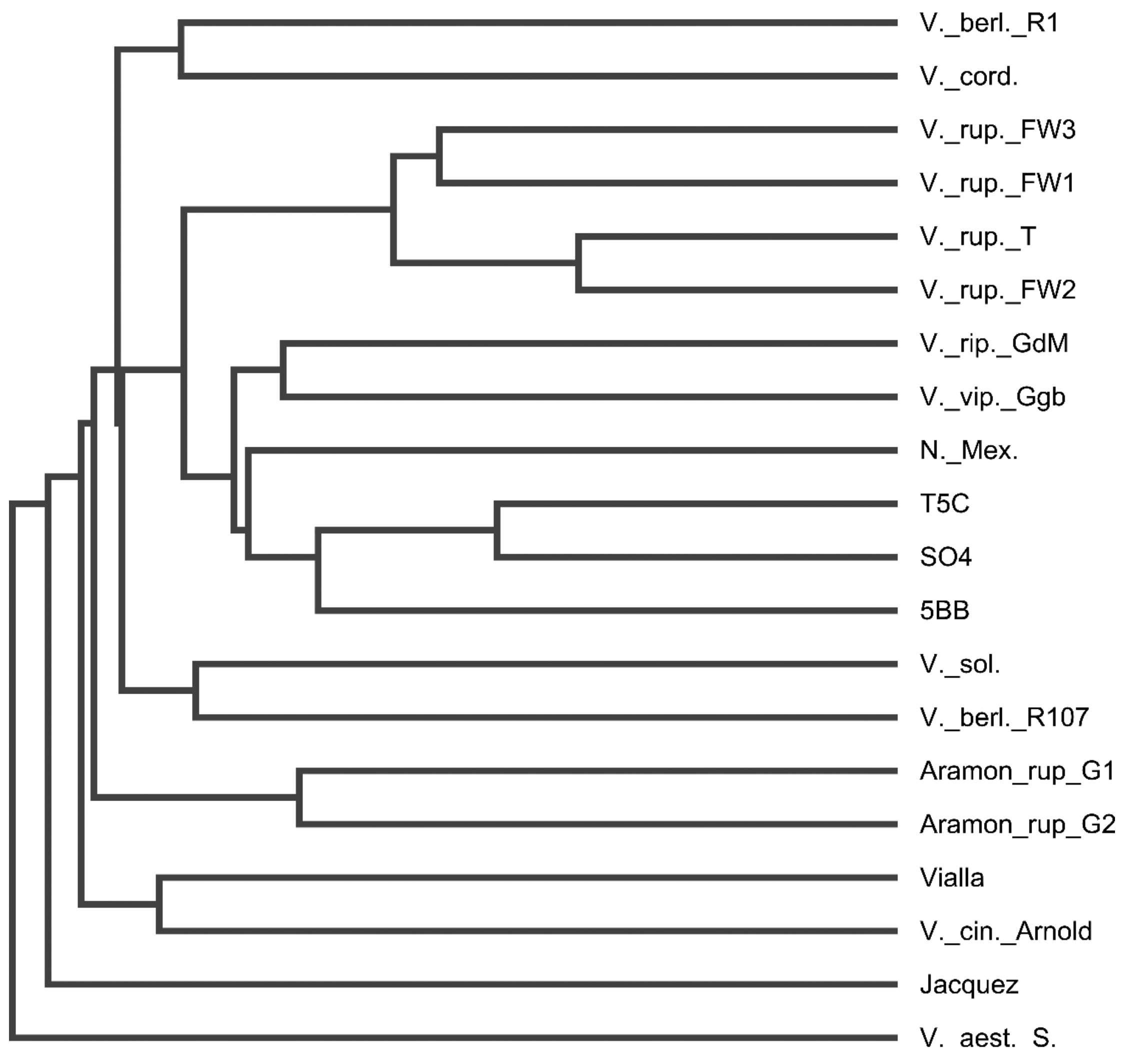

Fig. 5: Combined isozymes-microsatellite dendogram

according to Németh (1975) that Zsigmond Teleki had purchased the seeds for selection from Euryale Rességuier (France).

The examined *Vitis riparia* and *Vitis rupestris* items also show relatedness, but for Teleki's seedlings the relatedness is higher for *Vitis ripara.*

MICROSATELLITE ANALYSIS

The detailed SSR data are presented in Table 7. Based on the results a distance matrix (not presented) and a UPGMA dendogram (Fig. 5) were created.

The possible parent-offspring combination was assumed, where two genotypes shared at least one allele per each locus, or being homozygous (supposing a shared null allele (Dakin and Avise, 2004). A possible parent

Table 5: The banding patterns by Rf (relative mobility) values for glutamate-oxalacetate-transaminase

No.	ID	Rf value									Banding pattern type
		0,255	0,270	0,290	0,310	0,330	0,350	0,370	0,390	0,420	
1	V._berl._R1	1	1	1	1	0	0	0	0	0	B
2	V._rup._FW3	0	0	1	1	0	1	1	0	0	I
3	V._rup._T	0	0	1	1	0	1	1	0	0	I
4	V._cord.	0	0	1	1	1	0	0	0	0	G
5	V._rip._GdM	0	0	1	0	0	0	1	0	0	L
6	Aramon_rup_G1	1	1	1	0	0	0	0	0	0	D
7	V._vip._Ggb	0	0	1	1	0	1	1	0	0	I
8	V._rup._FW1	0	0	1	1	0	1	1	1	0	H
9	Jacquez	1	1	0	0	0	1	1	1	0	E
10	Vialla	1	1	1	0	0	1	1	0	1	C
11	V._cin._Arnold	1	1	1	0	0	1	1	0	1	C
12	V._aest._S.	1	1	1	0	0	0	0	0	0	D
13	V._sol.	1	1	1	1	0	0	1	1	0	A
14	V._rup._FW2	0	0	1	1	0	1	1	1	0	H
15	V._berl._R107	1	1	1	0	0	0	0	0	0	D
16	Aramon_rup_G2	0	0	1	1	0	1	0	0	0	J
17	N._Mex.	0	0	1	1	0	0	1	0	0	K
18	T5C	1	0	1	0	1	0	0	0	0	F
19	SO4	0	0	0	1	1	0	0	0	0	M
20	5BB	1	1	1	0	0	0	0	0	0	D

Table 6: The banding patterns by Rf (relative mobility) values for acid-phosphatase

No.	ID	Rf value							Banding pattern type
		0,475	0,490	0,530	0,535	0,575	0,590	0,635	
1	V._berl._R1	1	1	1	1	0	1	0	A
2	V._rup._FW3	1	1	1	1	0	1	0	A
3	V._rup._T	1	1	1	1	0	1	0	A
4	V._cord.	1	1	1	1	0	1	0	A
5	V._rip._GdM	1	1	1	1	0	1	0	A
6	Aramon_rup_G1	1	1	1	1	0	1	0	A
7	V._vip._Ggb	1	1	1	1	0	1	0	A
8	V._rup._FW1	1	1	1	1	0	1	0	A
9	Jacquez	1	0	1	0	1	0	1	B
10	Vialla	0	1	1	0	0	1	0	C
11	V._cin._Arnold	0	1	1	0	0	1	0	C
12	V._aest._S.	0	1	0	1	0	0	0	D
13	V._sol.	1	1	1	1	0	1	0	A
14	V._rup._FW2	1	1	1	1	0	1	0	A
15	V._berl._R107	1	1	1	1	0	1	0	A
16	Aramon_rup_G2	1	1	1	1	0	1	0	A
17	N._Mex.	1	1	1	1	0	1	0	A
18	T5C	1	1	1	1	0	1	0	A
19	SO4	1	1	1	1	0	1	0	A
20	5BB	1	1	1	1	0	1	0	A

Table 7: Results of microsatellite (SSR) analysis in 15 loci (part 1 of 3)

ID	VMC7G3		VMC3D12		VMC1F10		VVMD28		VMC8A7	
V._berl._R1	114	-	-	-	-	-	-	-	162	-
V._rup._FW3	118	-	198	222	194	196	-	-	158	172
V._rup._T	122	134	198	220	194	202	-	-	158	172
V._cord.	116	128	200	220	190	-	235	-	154	164
V._rip._GdM	120	126	198	204	196	-	237	261	160	162
Aramon_rup_G1	118	-	198	212	192	198	233	249	150	160
V._vip._Ggb	122	126	186	198	196	-	233	249	160	-
V._rup._FW1	118	134	186	198	194	196	237	-	164	172
Jacquez	116	126	206	208	196	200	231	237	152	156
Vialla	114	124	214	216	200	230	227	237	158	162
V._cin._Arnold	114	-	202	208	208	-	261	283	148	158
V._aest._S.	140	144	204	-	204	-	247	-	160	164
V._sol.	122	-	198	218	196	224	249	251	160	172
V._rup._FW2	122	134	198	222	194	202	237	247	158	172
V._berl._R107	114	-	210	218	194	194	243	-	160	-
Aramon_rup_G2	116	122	198	212	192	198	-	-	160	-
N._Mex.	122	130	198	-	194	200	247	-	160	-
T5C	114	126	198	218	196	-	-	-	160	166
SO4	114	126	198	218	196	-	-	-	160	166
5BB	120	126	-	-	196	-	217	253	162	-

The minus sign (-) indicates no amplification/homozygous genotype for a null allele, or that the variety might be either homozygous or heterozygous with a null allele

Table 7: Results of microsatellite (SSR) analysis in 15 loci (part 2 of 3)

ID	VMCNG1E1		Scu06vv		VMC1C10		VVIM10		VMC2H4	
V._berl._R1	96	-	178	190	132	140	362	-	208	-
V._rup._FW3	90	-	184	-	142	-	364	-	204	228
V._rup._T	80	90	184	-	142	-	362	-	196	204
V._cord.	94	96	184	-	136	-	362	366	204	212
V._rip._GdM	84	88	170	204	142	-	364	-	204	212
Aramon_rup_G1	80	92	172	178	156	168	364	-	210	-
V._vip._Ggb	80	88	170	188	142	-	364	-	202	-
V._rup._FW1	84	90	184	192	142	-	364	-	166	228
Jacquez	92	-	168	174	140	164	374	-	208	220
Vialla	80	-	178	-	140	144	364	-	200	-
V._cin._Arnold	94	-	180	182	140	-	-	-	208	-
V._aest._S.	78	-	170	-	140	-	-	-	206	214
V._sol.	90	-	188	196	150	-	-	-	-	-
V._rup._FW2	80	90	184	-	142	142	362	-	196	204
V._berl._R107	92	-	172	178	150	-	-	-	208	-
Aramon_rup_G2	94	96	-	-	156	168	364	366	206	210
N._Mex.	84	90	180	188	142	150	364	-	208	-
T5C	84	-	170	180	142	150	364	-	208	212
SO4	84	-	-	-	142	150	364	-	208	-
5BB	84	-	170	-	142	150	364	-	208	-

The minus sign (-) indicates no amplification/homozygous genotype for a null allele, or that the variety might be either homozygous or heterozygous with a null allele

Table 7: Results of microsatellite (SSR) analysis in 15 loci (part 3 of 3)

ID	VrZag21		VrZag25		VMC5G8		VMC5E9		VrZag79	
V._berl._R1	203	205	239	261	304	306	196	204	240	242
V._rup._FW3	203	207	217	241	302	304	198	214	252	254
V._rup._T	203	207	187	259	302	304	194	196	252	260
V._cord.	203	205	239	247	300	304	194	196	250	260
V._rip._GdM	203	209	241	251	286	304	202	-	252	256
Aramon_rup_G1	195	215	253	259	302	314	194	196	240	252
V._vip._Ggb	205	211	253	259	292	300	202	-	252	-
V._rup._FW1	203	207	-	-	304	-	194	214	254	260
Jacquez	193	201	243	-	302	318	194	210	246	248
Vialla	201	209	241	-	300	304	196	198	-	-
V._cin._Arnold	203	217	239	-	302	322	208	214	248	252
V._aest._S.	189	197	237	243	286	298	200	208	250	-
V._sol.	197	205	247	251	300	-	204	208	254	258
V._rup._FW2	203	207	259	-	302	304	198	214	252	260
V._berl._R107	205	209	239	-	300	-	198	200	254	256
Aramon_rup_G2	199	215	241	251	302	314	194	208	252	254
N._Mex.	203	209	251	251	300	304	202	-	250	260
T5C	195	209	241	253	286	294	202	210	252	-
SO4	195	209	-	-	284	294	-	-	248	-
5BB	201	209	241	-	302	304	200	202	248	256

The minus sign (-) indicates no amplification/homozygous genotype for a null allele, or that the variety might be either homozygous or heterozygous with a null allele

for the Teleki 5C, is the rootstock Riparia Gloire de Montpellier (also suggested previously by Jahnke et al. (2011)). Considering the possibility of null allele in the ISV4 locus the same can be supposed based on the results of Crespan et al. (2009). The rootstock Riparia Gloire de Montpellier was selected in 1880 in Montpellier (France) by L. Vialla and R. Michel. This rootstock is male-flowered and was one of the first to be used after the phylloxera crisis in Europe, but it does not provide enough iron to its scions in limestone-based soils (Németh, 1975).

A possible parent-offspring relation can also be supposed between the Vitis rupestris accessions: Fort Worth N1 and Fort Worth N3. The first accession (N1) was selected by Charles de Grasset, as the second (N3) by Franz Richter from *Vitis rupestris* populations of Fort Worth (Texas, USA). Both of the accessions have female flowers, so a parent and offspring relation is possible. These data do not provide enough evidence for parentage (Galet, 1968 and 1979).

Based on the results of the present study and the results of Crespan et al. (2009), a possible parent-offspring relation between Teleki 5C and SO4 can be supposed, but cannot be confirmed by our former results (Jahnke et al., 2011), where these accessions share one allele in 15 loci, show one allele in two loci (can share null alleles), but show different profiles in two loci (VrZag21 and Scu06vv) out of 19 loci. On the other hand the close relation of these rootstocks is clear from these results, and from the history of these accessions (Németh, 1975; Bakonyi and Kocsis, 2004).

The analysed species (*V. rupestris*, *V. berlandieri*, *V aestivalis, V. cordifolia*) form well-defined groups in the dendogram. The analysed Teleki seedlings (Teleki 5C, Kober 5BB, SO4) show the closest similarity with *V. riparia* derivates.

To show true-to-typeness of Teleki 5C, Kober 5BB and SO4 with comparison of already published SSR results is hard because of the few matching loci and the lack of control varieties. Barankova (2014) compared her SSR results to others (Lin and Walker, 1998; Thomas et al., 1994; This et al., 2004; Sefc et al., 2000; Crespan et al., 2004) and to our former results (Jahnke et al., 2011). This comparison confirmed the true-to-typeness of our material (The same accessions were used in this study).

COMBINED ISOZYMES MICROSATELLITE RESULTS

The combined isozymes-microsatellite dendogram is shown in Figure 5.
In this dendogram the close relation of *Vitis rupestris* accessions Fort Worth N1 and Fort Worth N3 (possible parent-offspring by SSR data - see above) is clearly visible, as they form a distinct group.
Similarly to the isozyme (Fig. 3) and SSR (Fig. 4) dendograms, the so-called Teleki hybrids (Teleki 5C, Kober 5BB, SO4) show close relatedness to each other (Fig. 5). In comparison to other results we can conclude that the Teleki 5C, Kober 5BB and SO4 rootstocks are closely related, but all of them have several clones, which makes the correct identification difficult. This enhances the importance of a precise register of maintained rootstock varieties. Further analyses with other molecular tools are needed.

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