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Absence of an acid phosphatase isozyme locus as a marker candidate for true to typeness in woodland grape (*Vitis vinifera* L. ssp. *sylvestris* Gmelin)

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Abstract

The quest and conservation of existing populations of woodland grape (*Vitis vinifera* L. ssp. *sylvestris* Gmelin), the supposed progenitor of the European grapevine (*Vitis vinifera* L. ssp. *sativa*) and a significant actor in the evolution of grapevine, has great importance in preserving biodiversity. The proof of true-to-typeness is highly important in ex-situ conservation, because the contamination risk of the woodland grape populations is very high. Some characteristic "*sylvestris*" simple sequence repeats (SSR) alleles were identified, but they are only characteristic in a specific population. In our recent study, the SSR profiles of 32 woodland grapes were compared to those of 16 European grapevine varieties and 20 rootstocks. Morphology and SSR analyses suggested that the analysed *Vitis vinifera* ssp. *sylvestris* Gmelin accessions were true-to-type. In this report, the results of the acid phosphatase isoenzyme analyses of the same woodland grape accessions are presented and a new marker for true-to-typeness is suggested.

Keywords : woodland grape, *Vitis sylvestris*, wild grapevine, true-to-type, isozyme, acid phosphatase, Hungary

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Table 1. List of the analysed *Vitis* accessions

No	ID	Accession Name	Genetic Origin	Origin of the Accession
1	Sziren	Szirén	*Vitis vinifera* ssp. *sativa*	Kecskemet, Hungary
2	Trilla	Trilla		
3	Gesztus	Gesztus		
4	Heureka	Heuréka		
5	Generosa	Generosa		
6	Kecskemet_7	Kecskemét 7		
7	Cserszegi_fuszeres	Cserszegi fűszeres		
8	Irsai_Oliver	Irsai Olivér		
9	Kovidinka	Kövidinka		
10	Pinot_gris	Pinot gris		
11	Ezerjo	Ezerjó		
12	Pozsonyi_feher	Pozsonyi fehér		
13	Kadarka	Kadarka		
14	Muscat_Lunel	Muscat Lunel		
15	Muscat_ottonel	Muscat ottonel		
16	Piros_tramini	Piros tramini		
17	S1	Sylvestris S1	*Vitis vinifera* ssp. *sylvestris*	Badacsony, Hungary (ex-situ collection from Szigetköz, Hungary)
18	S4_1	Sylvestris S4/1		
19	S4_2	Sylvestris S4/2		
20	S4_3	Sylvestris S4/3		
21	S6_1	Sylvestris S6/1		
22	S6_2	Sylvestris S6/2		
23	S6_4	Sylvestris S6/4		
24	S7	Sylvestris S7		
25	B1	Sylvestris B1		
26	B2	Sylvestris B2		
27	B5	Sylvestris B5		
28	B10	Sylvestris B10		
29	B12	Sylvestris B12		
30	B13	Sylvestris B13		
31	B16	Sylvestris B16		
32	B19	Sylvestris B19		
33	B21	Sylvestris B21		
34	B24	Sylvestris B24		
35	B26	Sylvestris B26		
36	B27	Sylvestris B27		
37	B30	Sylvestris B30		
38	B31	Sylvestris B31		
39	B33	Sylvestris B33		
40	B34	Sylvestris B34		
41	B36	Sylvestris B36		
42	B37	Sylvestris B37		
43	B41	Sylvestris B41		
44	B47	Sylvestris B47		
45	B48	Sylvestris B48		
46	B49	Sylvestris B49		
47	B50	Sylvestris B50		
48	B51	Sylvestris B51		
49	V._berl._R1	Resseguier N1	*V. berlandieri*	INRA, Domaine de Vassal, France
50	V._rup._FW3	Fort Worth N3	*V. rupestris*	
51	V._rup._T	Taylor	*V. rupestris*	
52	V._cord.	8029 Mtp2	*V. cordifolia*	
53	V._rip._GdM	Gloire de Montpellier	*V. riparia*	
54	Aramon_rup_G1	Aramon Ganzin N1	*V. vinifera x V. rupestris*	
55	V._vip._Ggb	Riparia Grand glabre	*V. riparia*	
56	V._rup._FW1	Fort Worth N1	*V. rupestris*	
57	Jacquez	Jaquez	*V. Bourquina (Vinifera x Aestivalis)*	
58	Vialla	Vialla	*V. labrusca x V. riparia*	
59	V._cin._Arnold	Cinerea Arnold	*V. cinerea*	
60	V._aest._S.	Sauvage	*V. aestivalis*	
61	V._sol.	Solonis	*V. solonis*	
62	V._rup._FW2	Fort Worth N2	*V. rupestris*	
63	V._berl._R107	Resseguier N107	*V. berlandieri*	
64	Aramon_rup_G2	Aramon Ganzin N2	*V. vinifera x V. rupestris*	
65	N._Mex.	V. Novo Mexicana	*V. riparia x V. candicans*	
66	T5C	Teleki 5C E20	*V. berlandieri x V. riparia*	Kecskemet, Hungary
67	SO4	Teleki-Fuhr SO4 (133)	*V. berlandieri x V. riparia*	Cserszegtomaj, Hungary
68	5BB	Teleki-Kober 5BB	*V. berlandieri x V. riparia*	

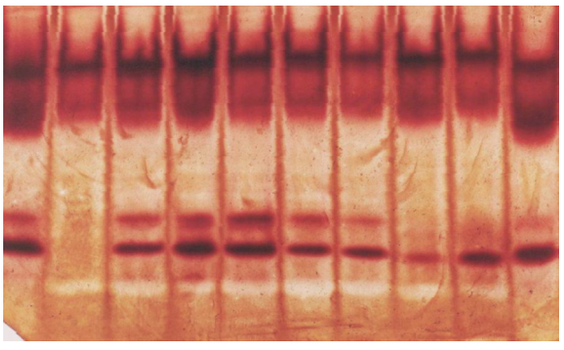

Figure 1. Gel photo of *Vitis vinifera* ssp. *sylvestris* accessions (from left to right: sylvestris S4/3, B10, B12, B13, B33, B37, B41, B35, B49, and S6/1).

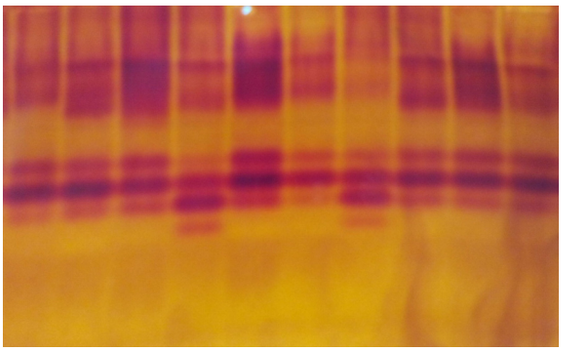

Figure 2. Gel photo of *Vitis vinifera* ssp. *sativa* accessions (from left to right: Pinot gris, Irsai Olivér, Szirén, Pozsonyi fehér, Muscat ottonel, Piros tramini, Kövidinka, Szirén, Heuréka, and Generosa).

Introduction

The woodland grape (*Vitis vinifera* L. ssp. *sylvestris* Gmelin) is supposed to be the progenitor of the European grapevine (*Vitis vinifera* L. ssp. *sativa*) (Arroyo-García *et al.,* 2006). This wild subspecies is endangered, as its populations are destroyed by phylloxera (*Daktulosphaira vitifoliae* Fitch), fungal diseases (eg. downy mildew, powdery mildew), contamination and human activity (Ocete *et al.,* 2015).

The woodland grape is protected in Hungary (Farkas, 1999). The quest and conservation of its existing populations has great importance in preserving biodiversity, also because of its role in the evolution of grapevine.

The proof of true-to-typeness has high importance in ex-situ conservation (This *et al.*, 2006). The contamination risk of woodland grape populations is very high, as *Vitis vinifera* L. ssp. *sylvestris* Gmelin can easily be crossed with other *Vitis* species, such as the invasive *Vitis riparia* and other *Vitis* genotypes used as rootstocks (Bodor *et al.*, 2010; Zoghlami *et al.*, 2013). The seedlings of these crosses have evolutionary benefit, as they can inherit phylloxera resistance from the non-*sylvestris* parent. Optimal collection sites are far away from commercial vineyards and have never been used for grape production.

The challenge in accession identification is to determine the subspecies to which the accession belongs. The most important morphological difference between the woodland grape and the European grapevine is that the former is dioecious and the latter is monoecious (Ocete *et al.*, 2015).

The molecular analysis of the different populations of woodland grapes began about a decade ago (Arroyo-García *et al.*, 2006; This *et al.*, 2006). In most of the cases, simple sequence repeats (SSR) markers were used for characterisation (Biagini *et al.*, 2012; Biagini *et al.*, 2014; Bitz *et al.*, 2015). Some characteristic "*sylvestris*" alleles were identified, but they were only characteristic in a specific population (Doulati Baneh *et al.*, 2015).

In a recent study, we compared the SSR profiles of 32 woodland grapes to those of 16 European grapevine varieties and 20 rootstocks. Morphology and SSR analyses suggested that the analysed *Vitis vinifera* ssp. *sylvestris* Gmelin accessions were true-to-type (Jahnke *et al.*, 2016). In this report, the results of the acid phosphatase isoenzyme analyses of the same woodland grape accessions are presented and compared to our previous results.

Material and methods

1. *Vitis* accessions

68 *Vitis* accessions, 16 *Vitis vinifera* ssp. *sativa* cultivars, 32 *Vitis vinifera* ssp. *sylvestris* genotypes and 20 others (mainly used as rootstocks) were analysed (Table 1).

2. SSR analysis

Dormant canes were collected in January 2016 and subsequently stored in plastic bags at 4 ˚C until processing, within 2 days. Active enzymes were extracted from the dormant canes of the 68 accessions as described by Arulsekar and Parfitt (1986). Vertical polyacrylamide gel electrophoreses were carried out and gels were stained for acid phosphatase as described by Royo *et al.* (1997). Results were evaluated visually. Isozyme bands were digitally scored (1-present, 0-absent). Chi-square test of independence and contingency coefficient calculation were carried out using Microsoft Excel.

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Table 2. The banding patterns of acid phosphatase (1-present ; 0-absent)

No.	Accession ID*	ACP1	ACP2	ACP3	ACP4	ACP5	ACP6	ACP7	ACP8
1	Sziren	1	1	1	1	1	1	1	0
2	Trilla	1	1	1	1	1	1	1	1
3	Gesztus	1	1	1	1	1	1	1	1
4	Heureka	1	1	1	1	1	1	1	0
5	Generosa	1	1	1	1	1	1	1	0
6	Kecskemet_7	1	1	1	1	1	1	1	0
7	Cserszegi_fuszeres	1	1	1	0	1	1	1	1
8	Irsai_Oliver	1	1	1	1	1	1	1	0
9	Kovidinka	1	1	1	0	1	1	1	1
10	Pinot_gris	1	1	1	0	1	1	1	0
11	Ezerjo	1	1	0	1	1	1	1	0
12	Pozsonyi_feher	1	1	1	1	1	1	1	1
13	Kadarka	1	1	1	1	1	1	1	1
14	Muscat_Lunel	1	1	1	1	1	1	1	0
15	Muscat_ottonel	1	1	1	0	1	1	1	0
16	Piros_tramini	1	1	1	0	1	1	1	0
17	S1	1	1	1	0	1	1	1	0
18	S4_1	1	1	1	0	1	1	1	0
19	S4_2	1	1	1	0	1	1	1	0
20	S4_3	1	1	1	1	1	1	1	0
21	S6_1	1	1	1	1	1	1	1	0
22	S6_2	1	1	1	1	1	1	1	0
23	S6_4	1	1	0	1	1	1	1	0
24	S7	1	1	1	1	1	1	1	0
25	B1	1	1	1	1	1	1	1	0
26	B2	1	1	1	1	1	1	1	0
27	B5	1	1	1	1	1	1	1	0
28	B10	1	1	1	0	0	0	0	0
29	B12	1	1	1	1	1	1	1	0
30	B13	1	1	1	1	1	1	1	0
31	B16	1	1	1	0	0	0	0	0
32	B19	1	1	1	0	0	0	0	0
33	B21	1	1	1	1	1	1	1	0
34	B24	1	1	0	1	1	1	1	0
35	B26	1	1	1	0	0	0	0	0
36	B27	1	1	1	0	0	0	0	0
37	B30	1	1	1	0	0	0	0	0
38	B31	1	1	1	0	0	0	0	0
39	B33	1	1	1	0	1	1	1	0
40	B34	1	1	1	0	0	0	0	0
41	B36	1	1	1	0	0	0	0	0
42	B37	1	1	1	0	1	1	1	0
43	B41	1	1	1	0	1	1	1	0
44	B47	1	1	1	1	1	1	1	0
45	B48	1	1	1	1	1	1	1	0
46	B49	1	1	1	0	1	1	1	0
47	B50	1	1	0	1	1	1	1	0
48	B51	1	1	1	0	1	1	1	0
49	V._berl._R1	1	1	1	1	1	1	1	0
50	V._rup._FW3	1	1	1	1	1	1	1	0
51	V._rup._T	1	1	1	1	1	1	1	0
52	V._cord.	1	1	1	1	1	1	1	0
53	V._rip._GdM	1	1	1	1	1	1	1	0
54	Aramon_rup_G1	1	1	1	1	1	1	1	0
55	V._vip._Ggb	1	1	1	1	1	1	1	0
56	V._rup._FW1	1	1	1	1	1	1	1	0
57	Jacquez	1	0	1	0	1	1	1	0
58	Vialla	0	1	1	0	1	1	1	0
59	V._cin._Arnold	0	1	1	0	1	1	1	0
60	V._aest._S.	0	1	0	1	1	1	1	0
61	V._sol.	1	1	1	1	1	1	1	0
62	V._rup._FW2	1	1	1	1	1	1	1	0
63	V._berl._R107	1	1	1	1	1	1	1	0
64	Aramon_rup_G2	1	1	1	1	1	1	1	0
65	N._Mex.	1	1	1	1	1	1	1	0
66	T5C	1	1	1	1	1	1	1	0
67	SO4	1	1	1	1	1	1	1	0
68	5BB	1	1	1	1	1	1	1	0

1For more information about accessions see Table 1

Results

The isozyme banding patterns of acid phosphatase are presented in Table 2.

Gel photos of *Vitis vinifera* ssp. *sylvestris* and *Vitis vinifera* ssp. *sativa* accessions are presented in Figures 1 and 2, respectively.

The acid phosphatase isoenzyme patterns consist of 2 zones. The presence of a maximum of 4 bands in the faster migrating region represents a distinct locus. This region consists of 3 or 4 bands in the case of *Vitis vinifera* ssp. *sativa* cultivars and 3 bands for all analysed rootstocks and the majority of woodland grapes, but is absent in some *Vitis vinifera* ssp. *sylvestris* accessions.

Discussion

Acid phosphatases, which are involved in phosphorus metabolism in plants (Tadano and Sakai, 1991), usually have a high degree of polymorphism. These enzymes are usually glycoproteins, needing other enzymes to add the glycoprotein part. Empirical data suggests that in most cases changes in electrophoretic mobility are caused by changes in the DNA sequence of the structural genes, although it cannot be excluded that some of the polymorphism can be traced back to the polymorphism of the processing enzymes (Weeden and Wendel, 1989).

Acid phosphatases in plants are usually monomeric or dimeric with 2-4 isozymes and different subcellular localisation (de Cherisey *et al.*, 1985 in Weeden and Wendel, 1989). Based on the present results and our previous study (Jahnke et al., 2009), acid phosphatase in grape (*Vitis vinifera* L.) has 2 zones of activity. The slower migrating zone has a maximum of 4 bands (1-4 in Table 2). The patterns of this zone can be interpreted as two (duplicated) loci with 4 alleles and monomeric enzyme. The faster migrating zone can be interpreted as a single locus coding 2 subunits (dimeric enzyme). In most of the Pontican European grapevine cultivars (*Vitis vinifera* ssp. *sativa* proles *pontica*), this locus is duplicated, which gives a special 4-band pattern in this zone. In about 50 percent of the woodland grape (*Vitis vinifera* ssp. *sylvestris*) genotypes, this locus is absent (null allele). This means that this type of acid phosphatase is not essential for the plant.

"The changes in the number and expression of loci in the course of phylogenesis suggest what evolutionary processes may have taken place" (Basaglia, 1989). Taking into account that acid phosphatases are involved in phosphorus metabolism and energy transfer, this extra locus can be advantageous and can play a remarkable role in the domestication of the grape. This phenomenon can be used as a marker in future studies.

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