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Evaluation of Lime-induced Chlorosis Tolerance in New Rootstock Hybrids of Grapevine

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Summary

The adaptation to site pedological and geological conditions belongs to the most important requirements that concern grapevine rootstocks. The tolerance to the lime-induced chlorosis represents a very important property of rootstock plants.

This paper presents the evaluation of tolerance of 20 new rootstock hybrids and 4 rootstock varieties to this type of chlorosis. The evaluation was performed within the framework of a pot experiment in 2007. In hybrids and rootstock varieties the following parameters were studied: length of annual shoots, chlorophyll content index (CCI) and leaf chlorosis score. The evaluated rootstock hybrids were combinations of North American *Vitis* spp. (*V. berlandieri, V. riparia, V. rupestris,* and *V. cinerea*) and the East Asian species *Vitis amurensis*.

A very high and/or high tolerance to lime-induced chlorosis was demonstrated in hybrids 16-1-6; 17-1-6; 17-1-9; 17-2-10; 17-3-6; 17-6-2; 17-8-2; 9-20-1 and 17-12-1. Groups D and F, the most tolerant hybrids originated from combinations with *V. berlandieri, V. riparia* and *V. cinerea*. The evaluation demonstrated that, as compared with varieties originating from the crossing *V. berlandieri* x *V. riparia*, the rootstocks originating from crossings with *V. cinerea* were less tolerant. Hybrids originating from *V. rupestris* showed also only a medium tolerance to lime.

From the methodological viewpoint of the evaluation of chlorosis tolerance the correlation existing between the chlorophyll content in leaves and the chlorosis evaluation performed on the base of „leaf chlorosis score" is very important (r = −0.84). This means that chlorophyll content index (CCI) and leaf chlorosis score can be used when evaluating the tolerance of rootstocks to chlorosis.

Key words. chlorosis tolerance – grapevine – hybrid – lime – rootstocks – variety – *Vitis* spp.

Introduction

A successful growing of grapevine is very dependent on a proper selection of rootstock varieties. Grapevine is commonly grown under very different pedological and climatic conditions. The adaptation of rootstocks to calcareous soils represents an important property of grapevine plants and for that reason the resistance to lime-induced chlorosis plays an important role.

Chlorosis is a widespread physiological deficiency affecting plants cultivated in soils with a high content of calcium carbonate and/or lime. This deficiency causes either yellowing of intervein areas or a loss of chlorophyll from the leaf blade (Ostrovskaya et al. 1990).

Lime-induced chlorosis affects also yields and quality of grapevine plants growing in many calcareous areas world-wide (Bavaresco et al. 1994).

Lime-induced iron chlorosis, i. e. the condition of a reduced availability of soluble iron for the vine due high concentrations of bicarbonate ions in calcareous soils, can seriously impair the health condition of grapevine plants.

Lime-tolerant grapevine rootstocks show some specific physiological mechanism enabling them to overcome chlorosis when grown on calcareous soils, including an improvement of root Fe uptake (Varanini and Magioni 1982; Bavaresco et al. 1991).

Breeding efforts to get proper genotypes included also successful crossings of wild grapevine species and for that reason some chlorosis-tolerant rootstocks are now available for the grapevine growers in many calcareous areas world-wide (Fregoni 1980; Pouget 1980; Bavareso et al. 1993).

When breeding and selecting rootstock varieties above, all North American *Vitis* spp., *V. riparia, V. rupestris* and *V. berlandieri* are used. *V. riparia* and *V. rupestris* are not very tolerant to calcareous soils. *V. berlandieri* is widely recognized for its good adaptation to calcareous soils. *V. vinifera* is a species, which is tolerant to calcareous soils (Cousins 2005).

In spite of this fact, however, the testing and selection of new rootstock varieties with an increased tolerance to this lime-induced chlorosis is very important because there are many wine growing regions with calcareous sites where the symptoms of chlorosis occur very often. A combination of phylloxera resistance with a good adaptation to local site conditions is also very important.

The evaluation of plants in pot experiments is a frequently used method. The tolerance of vines to chlorosis was evaluated in pot experiment also in the past (see e.g.

reports published by Bavaresco et al. 1993, 1994; Ksouri et al. 2005).

The aim of this paper is to provide results of our tests and evaluation of tolerance of new, phylloxera-resistant hybrids to this physiological deficiency. Based on the results obtained it is then possible to recommend a suitable method of evaluation. Experimental results can be used when selecting parent varieties and species for breeding of new rootstock cultivars.

Materials and Methods

Testing and evaluation of rootstock plants were performed in the container room of the Faculty of Horticulture, Mendel University of the Agriculture and Forestry Brno, in Lednice na Morave. The experiment was established in 2006 and the first evaluation was performed in 2007.

In this experiment, rootstock varieties registered in the Czech Republic (Kober 5 BB, Craciunel 2, Teleki 5C, SO4) were evaluated together with 20 new rootstock hybrids showing a high degree of resistance to phylloxera (Pavloušek and Michlovský 2007). These hybrids are presented in Table 1.

One-year-old wood was cut and rooted in a glasshouse in January 2006. The experiment with $CaCO_3$ application and evaluation of tolerance to chlorosis was performed in 2007. The experiment was established in containers with a volume of 4 litres; each container was under drip irrigation and the soil humidity was maintained on percentages approaching to values of field capacity (Bavaresco et al. 1994).

Pots contained the substrate Klassman with the following parameters: pH = 5.2; N = 210 mg l^{-1}; P_2O_5 = 240 mg l^{-1}; K_2O = 270 mg l^{-1} and Mg = 120 mg l^{-1}. The experimental substrate was enriched with 10 % $CaCO_3$ (variant V1) and 20 % $CaCO_3$ (variant V2) (see Kocsis et al. 1998). Control plants (C) were grown in a pure Klassman substrate (i.e. without $CaCO_3$).

All evaluations were performed 120 days after the onset of bud burst of experimental plants. The length (in cm) of the main annual shoot was measured in all plants. The content of chlorophyll in leaves was evaluated by means of chlorophyll content index next only CCI. For this evaluation the apparatus CCM-200 (Chlorophyll Content Meter) was used.

The CCM 200 is an absorbance meter used for a non-destructive estimation of the chlorophyll content in the leaf tissue. The CCM-200 weights 180 g, has a 0.71 cm^2 measurement area, and calculates a chlorophyll content index (CCI) on the base of absorbance measurements at 660 and 940 nm.

The visual evaluation of leaf chlorosis score was performed using the IPGRI scale (1997) where 1 = very high tolerance to chlorosis and 9 = very little tolerance to chlorosis.

In each experimental variant there were always 15 plants (either of hybrids or of cultural varieties).

The statistical analysis involved estimation of averages and standard deviations. The obtained results were evaluated also by means of variance analysis (ANOVA) and the Tukey test at the significance level of $P<0.01^{**}$. All correlations were calculated using the Pearson's method.

Results and Discussion

The main function attributed to grapevine rootstocks has so far been that of ensuring adequate resistance against phylloxera and tolerance of other limiting soil and climate factors such as active limestone, aridity, soil compaction and salinity (Scalabrelli et al. 2003).

To evaluate the chlorosis tolerance the following parameters were used: length of annual shoots, CCI and leaf chlorosis score. The use of these parameters corresponded with the observations described below

An increased content of lime in soil can show a negative effect on the production of fresh matter and chlorophyll and may also induce chlorosis of leaves. These facts were corroborated also by Bavaresco et al. (2005) who mentioned that calcareous soils showed a marked effect of the occurrence of chlorosis in young leaves. These symptoms could be observed above all in the middle of the growing season. The older leaves showed less pronounced symptoms of chlorosis.

Marschner (1995) also mentioned that the growth rate of sink tissues and such organs as the roots, shoot apex, fruits and storage organs can be limited by supply of photosynthates from the source leaves or by a limited capacity of the sink to utilize the photosynthates.

Table 1. Varieties, hybrids and their parents.

Symbol	Parentage	Hybrids and varieties
A	(Teleki 5 C x Börner) x Bruci	16-1-6, 16-1-7, 16-2-5
B	BV-9-20-4 x BV-8-20-6 (Teleki 5 C x Börner) x (Peking 1 x Bruci)	16-10-1, 16-10-3
C	Binova x Börner	17-1-6, 17-1-9, 17-2-3, 17-2-7, 17-2-10, 17-3-1, 17-3-6, 17-6-2, 17-6-7, 17-6-9
D	Binova x [(Binova x Teleki 5C) x Börner]	17-18-2
E	(Binova x Aurelius) x (Peking 1 x Bruci)	16-12-6
F	Teleki 5 C x Börner	9-20-1
G	BV-9-21-6 x BV-8-20-6 (Teleki 5 C x Börner) x (Peking 1 x Bruci)	17-12-1, 17-13-10
H	*V. berlandieri* x *V. riparia*	Kober 5 BB, Craciunel 2, Teleki 5C, SO4

V. = *Vitis*, Bruci = [(*V. berlandieri* x *V. rupestris*) x *V. cinerea*]

Table 2. Results of two-way ANOVA of plant characteristics by treatment (T) and variety (V) and statistical significance**P<0.01.

Dependent variables	Treatment (T)	Variety (V)	T x V
Shoot length	507.49**	171.93**	7.10**
CCI	1788.41**	199.20**	37.64**
Leaf chlorosis score	403.07**	24.57**	8.10**

In our experiment a statistically highly significant effect of the experimental treatments – variants and the varieties on all parameters under study was demonstrated. Results of variance analysis (ANOVA) are presented. (Table 2)

Shoot length

When evaluating plants in individual experimental variants it was observed that an increased content of $CaCO_3$ in soil reduced the length of shoots. Results of evaluation of individual hybrids and registered varieties are presented. (Table 3) The lowest growth increments were recorded in hybrids 17-2-3, 17-3-1 and 17-6-9 for average value of the three variants.

Results of evaluation of rootstock hybrids according to their pedigree are presented in Table 4. Statistically highly significant differences were demonstrated in all experimental variants (P<0.01). As far as the objective evaluation of growth parameters is concerned it can be said, that the presentation of concrete percentual differences between controls (C) and variants V1 and V2 is also significant.

The most marked growth inhibition due to the application of $CaCO_3$ was recorded in hybrids in group A, which differed from all other groups; this difference was statistically significant. On the other hand the less marked growth inhibition was observed in group A.

Alcantara et al. (2003) also used growth parameters when evaluating the occurrence of chlorosis in olive trees; these authors demonstrated varietal differences in tolerance of plants to Fe deficiency. Cinelli et al. (2003) performed a similar evaluation of chlorosis in peach and pear varieties.

Our experimental results correspond with data published by Bavaresco et al. (1993) who mentioned that the growth of shoots was influenced by soil conditions and that it was inhibited in plants growing on calcareous soils.

Chlorophyll content index (CCI)

Measurements of chlorophyll content are very important above all in those plants, which are in stress situations.

Penuelas and Filella (1998) mention that pigmentation can be directly related to stress physiology, as concentrations of carotenoids increase and chlorophyll generally decrease under stress conditions and during senescence.

Table 3. Evaluation of shoot length in rootstock hybrids and registered varieties (in cm) and their standard deviations.

Hybrid	Pedigree	Control (C)	Variant V1	Variant V2
16-1-6	A	121.5 ± 2.9	119.8 ± 4.1	97.5 ± 6.5
16-1-7	A	132.8 ± 2.9	97.0 ± 6.8	77.5 ± 6.5
16-2-5	A	116.5 ± 2.4	94.0 ± 3.4	66.5 ± 6.6
16-10-1	B	147.8 ± 3.9	118.0 ± 5.0	109.0 ± 8.4
16-10-3	B	144.3 ± 3.8	111.5 ± 3.1	99.8 ± 3.7
17-1-6	C	100.0 ± 3.6	87.0 ± 5.7	79.8 ± 6.9
17-1-9	C	95.3 ± 5.3	77.5 ± 6.5	77.5 ± 6.5
17-2-3	C	78.5 ± 1.9	70.0 ± 7.1	47.5 ± 6.5
17-2-7	C	100.0 ± 2.8	80.0 ± 7.1	59.8 ± 5.6
17-2-10	C	74.3 ± 2.5	68.3 ± 4.7	72.5 ± 2.9
17-3-1	C	69.5 ± 1.9	59.0 ± 6.2	48.8 ± 6.3
17-3-6	C	89.8 ± 3.1	58.5 ± 3.7	58.8 ± 6.0
17-6-2	C	109.5 ± 1.9	98.8 ± 6.3	88.0 ± 6.3
17-6-7	C	79.8 ± 1.3	57.3 ± 6.1	50.0 ± 10.8
17-6-9	C	58.8 ± 3.5	39.5 ± 3.3	48.8 ± 8.5
17-8-2	D	119.5 ± 2.6	97.0 ± 5.7	98.8 ± 6.3
16-12-6	E	134.3 ± 4.4	118.3 ± 7.7	98.8 ± 8.5
9-20-1	F	99.0 ± 6.8	77.3 ± 6.6	77.5 ± 6.5
17-12-1	G	134.5 ± 4.2	123.3 ± 5.6	117.5 ± 6.5
17-13-10	G	119.0 ± 5.8	110.0 ± 8.2	87.5 ± 5.6
Kober 5 BB	H	128.3 ± 8.9	118.8 ± 8.5	99.3 ± 6.5
SO 4	H	90.3 ± 9.0	86.3 ± 4.8	87.5 ± 6.5
Teleki 5 C	H	103.8 ± 4.8	88.3 ± 7.7	81.3 ± 8.5
Craciunel 2	H	123.8 ± 8.5	114.8 ± 4.1	103.8 ± 4.4

Table 4. Evaluation of shoot length (in cm) in individual groups of rootstock hybrids as dependent on their pedigree, differences between controls on the one hand and individual variants on the other and a statistictic comparison of these differences **P<0.01.

Pedigree	Control (C)		Variant V1		Variant V2	
	(cm)	(%)	(cm)	(%)	(cm)	(%)
A	123.6	100	103.6	83.8 ab	80.5	65.1 a
B	146.0	100	114.8	78.6 a	104.4	71.5 b
C	85.6	100	69.6	81.1 ab	63.1	73.6 b
D	119.5	100	97.0	81.2 ab	98.8	82.6 c
E	134.3	100	118.3	88.1 b	98.8	73.6 b
F	99.0	100	77.3	78.0 a	77.5	78.3 c
G	126.8	100	116.6	92.0 c	102.5	80.9 c
H	111.5	100	102.0	91.5 c	92.9	83.4 c

Table 5. Evaluation of CCI values in rootstock hybrids and registered rootstock varieties, their standard deviations and statistically significant intervarietal differences within individual pedigree groups at the significance level of **P<0.01.

Hybrid	Pedigree	Control (C)	Variant V1	Variant V2
16-1-6	A	20.3 ± 0.6 c	18.3 ± 0.3 c	17.3 ± 0.4 c
16-1-7	A	18.3 ± 0.3 b	15.1 ± 0.4 b	11.0 ± 0.4 b
16-2-5	A	15.3 ± 0.5 a	11.1 ± 0.5 a	9.8 ± 0.4 a
16-10-1	B	22.1 ± 0.8 a	15.3 ± 0.4 b	12.1 ± 0.5 b
16-10-3	B	16.3 ± 0.4 a	10.5 ± 0.5 a	8.7 ± 0.7 a
17-1-6	C	20.3 ± 0.6 ab	18.6 ± 0.4 d	18.6 ± 0.2 e
17-1-9	C	19.7 ± 0.5 a	18.8 ± 0.5 d	16.7 ± 0.5 d
17-2-3	C	22.1 ± 0.6 cd	16.4 ± 0.6 bc	10.1 ± 0.6 b
17-2-7	C	23.2 ± 0.5 de	13.3 ± 0.5a	10.0 ± 0.4 b
17-2-10	C	24.3 ± 0.6 e	23.3 ± 0.6 g	21.1 ± 0.4 f
17-3-1	C	22.2 ± 0.6 cd	15.2 ± 0.5 b	9.7 ± 0.6 ab
17-3-6	C	24.0 ± 0.8 e	22.0 ± 0.4 f	20.1 ± 0.6 f
17-6-2	C	21.1 ± 0.5 bc	20.8 ± 0.6 e	18.8 ± 0.3 e
17-6-7	C	20.2 ± 0.4 b	13.1 ± 0.4 a	8.8 ± 0.3 a
17-6-9	C	21.3 ± 0.5 abc	16.7 ± 0.7 c	12.2 ± 0.5 c
17-8-2	D	20.5 ± 0.7	20.2 ± 0.3	18.7 ± 0.5
16-12-6	E	23.0 ± 0.6	15.4 ± 0.5	9.4 ± 0.4
9-20-1	F	23.3 ± 0.5	21.5 ± 0.9	22.0 ± 0.4
17-12-1	G	21.1 ± 0.5 a	18.6 ± 0.4 a	17.8 ± 0.4 a
17-13-10	G	22.3 ± 0.4 b	15.7 ± 0.5 b	9.6 ± 0.5 b
Kober 5 BB	H	22.3 ± 0.5 b	21.3 ± 0.3 c	17.8 ± 0.4 b
SO 4	H	20.1 ± 0.5 a	18.8 ± 0.5 b	18.3 ± 0.4 b
Teleki 5 C	H	19.6 ± 0.5 a	15.5 ± 0.6 c	10.0 ± 0.4 a
Craciunel 2	H	21.2 ± 0.5 b	20.3 ± 0.5 a	20.1 ± 0.5 c

Results of CCI evaluation in individual hybrids are presented (Table 5). Values of CCI in individual pedigree groups are presented in Fig. 1.

Very interesting results were obtained when evaluating individual pedigree groups. In both experimental variants (V1 and V2), hybrids in the group B were significantly differed from other groups, except A(V1) and E (V2). This group showed a very low value of CCI and this may indicate a high sensitivity of these hybrids to stress and/or chlorosis. On the other hand, hybrids in group F were also significantly different from all other groups: in both variants, however, they showed very high values of CCI so that these plants may be considered as tolerant to stress situations.

When comparing hybrids occurring within individual pedigree groups, significant differences were found out above all in groups B and C (Table 5).

Leaf chlorosis score

Pouget and Ottenwaelter (1978) evaluated as the first authors visual symptoms of leaf chlorosis. The same

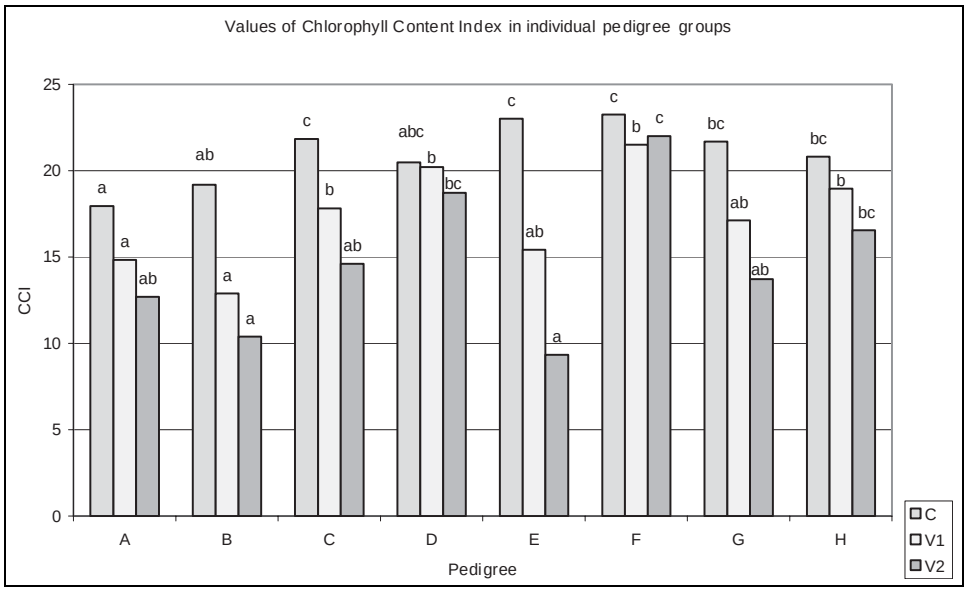


Fig. 1. Values of CCI in rootstock hybrids and registered rootstock varieties and a statistical analysis of differences at the significance level of P<0.01.

method of visual evaluation was thereafter used also by BAVARESCO et al. (1993, 1994); KSOURI et al. (2005, 2007).

Values of chlorosis leaf score in individual hybrids under study are presented in Table 6.

In the variant V1 (with the $CaCO_3$ content of 10 %) a very high tolerance was observed in hybrids 16-1-6, 17-1-9, 17-2-10, 17-3-6, 17-6-2, 17-8-2 and 9-20-1, and in the registered rootstock varieties SO4 and Craciunel 2. These hy-

Table 6. Evaluation of chlorosis symptoms in rootstock hybrids and registered rootstock varieties, their standard their standard deviations and statistically significant intervarietal differences within individual pedigree groups at the significance level of (^{abc}) as well as among varieties in general (abcdef) at the significance level of **P<0.01.

Hybrid	Pedigree	Control (C)	Variant V1	Variant V2
16-1-6	A	1.0	^{a} 1.5 a	^{a} 3.5 abcde
16-1-7	A	1.0	^{b} 3.5 abcd	^{ab} 5.0 cdef
16-2-5	A	1.0	^{b}4.5 bcd	^{b} 6.5 f
16-10-1	B	1.0	^{a} 2.5 abc	^{a} 5.5 def
16-10-3	B	1.0	^{b} 5.0 cd	^{a} 6.5 f
17-1-6	C	1.0	^{bc} 3.5 abcd	^{a} 3.5 abcde
17-1-9	C	1.0	^{a} 1.0 a	^{a} 3.5 abcde
17-2-3	C	1.0	^{bc} 3.5 abcd	^{b} 6.5 f
17-2-7	C	1.0	^{c} 5.5 d	^{b} 6.5 f
17-2-10	C	1.0	^{ab} 1.5 a	^{a} 3.5 abcde
17-3-1	C	1.0	^{c} 5.5 d	^{b} 6.0 ef
17-3-6	C	1.0	^{ab} 1.5 a	^{a} 3.0 abcd
17-6-2	C	1.0	^{a} 1.0 a	^{a} 2.5 abc
17-6-7	C	1.0	^{c} 5.5 d	^{b} 6.0 ef
17-6-9	C	1.0	^{bc} 3.5 abcd	^{a} 4.5 bcdef
17-8-2	D	1.0	2.0 ab	2.0 ab
16-12-6	E	1.0	5.5 d	5.5 def
9-20-1	F	1.0	1.5 a	1.0 a
17-12-1	G	1.0	^{a} 3.5 abcd	^{a} 2.5 abc
17-13-10	G	1.0	^{b} 6.0 d	^{b} 5.5 def
Kober 5 BB	H	1.0	^{ab} 2.0 ab	^{a} 2.5 abc
SO 4	H	1.0	^{a} 1.0 a	^{a} 1.5 a
Teleki 5 C	H	1.0	^{b} 3.5 abcd	^{b} 5.5 def
Craciunel 2	H	1.0	^{a} 1.0 a	^{a} 1.5 a

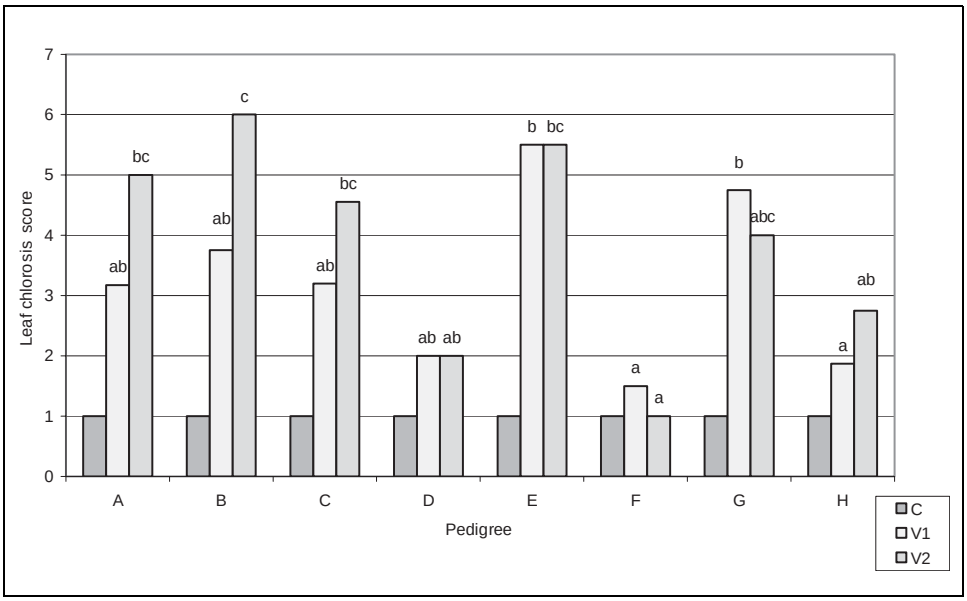


Fig. 2. Evaluation of leaf chlorosis score in individual pedigree groups of rootstock hybrids and a statistical analysis of differences at the significance level of P<0.01.

brids and varieties were statistically significantly different from other evaluated hybrids and varieties. On the other hand, a low tolerance of chlorosis was demonstrated in the following hybrids: 16-2-5, 16-10-3, 17-2-3, and 17-2-7.

As far as the growing of grapevine in localities with calcareous soils is concerned, results of the evaluation in variant V2 are rather important. The highest (and statistically significant) difference was found in the hybrid 9-20-1, which originated from the crossing Teleki 5C x Börner. Its value of tolerance was comparable with those of registered rootstock varieties SO4 and Craciunel 2. In this context SCHMIDT et al. (2008) mentioned the suitability of *V. berlandieri* as a very good partner for breeding and selection of rootstocks tolerant to lime. *V. berlandieri* is also present in the pedigree of hybrid 9-20-1 and this may be the cause of its high tolerance to chlorosis. In many other hybrids, however, only medium and/or low degree of tolerance was found (Table 6).

When comparing hybrids existing within individual pedigree groups, significant differences were observed above all within the group C (Table 6).

Results of the evaluation of chlorosis tolerance in individual pedigree groups are presented in Fig. 2. The highest tolerance to chlorosis (and thus also to the content of lime in soil) was demonstrated in hybrids from groups F and D; these values were comparable with those obtained in the group of hybrids originating from the crossing *V. berlandieri* x *V. riparia* (H).

From the viewpoint of the chlorosis tolerance evaluation the observed correlation between leaf chlorosis score and value of CCI is very important. Value of Pearson correlation was r = −0.84.

KSOURI et al. (2005), as well, demonstrated a marked negative correlation existing between the content of chlorophyll in leaves and leaf chlorosis score (r = −0.87).

In another study (KSOURI et al. 2007), the existence of a relationship between symptoms of leaf chlorosis and the decreased content of chlorophyll was also proved.

As far as the existence of correlations between other parameters under study was concerned, the obtained results were not significant. Pearson correlations between the shoot length on the one hand and leaf chlorosis and CCI were r = −0.37 and r = 0.28, respectively.

The evaluation of chlorosis tolerance revealed statistically significant differences between hybrids originating from the crossing *V. berlandieri* x *V. riparia* (H) on the one hand and all other pedigree groups except D on the other (Fig. 2).

The species *V. berlandieri*, which was described by MAY (1994) as resistant to chlorosis, was present in many combinations under study. On the other hand, however, the species *V. cinerea* was present in all hybrid combinations, either as the rootstock Börner or as the combination called Bruci [(*V. berlandieri* x *V. rupestris)* x *V. cinerea*]. SCHMIDT et al. (2005) did not recommend the rootstock Börner for soils with higher pH values and a high content of active lime in soil.

Hybrids with a very high tolerance to chlorosis originated from parent combinations D [Binova x (Binova x Teleki 5C) x Börner] and F [Teleki 5 C x Börner]. These hybrids originated from combinations of *V. berlandieri, V. riparia* and *V. cinerea*. Hybrid combinations with *V. rupestris* and *V. amurensis* showed mostly only a medium tolerance to chlorosis. A simple hybrid combination Binova x Börner showed also a medium tolerance to chlorosis. These findings corroborate results published by COUSINS (2005) who also mentioned a lower tolerance of *V. rupestris* to chlorosis.

Conclusions

Our evaluation demonstrated that, as compared with rootstocks originating from the crossing *V. berlandieri* x *V. riparia*, the chlorosis tolerance of rootstocks with either *V. cinerea* or *V. rupestris* in their pedigrees was reduced.

The obtained results indicate that values of CCI and the leaf chlorosis score can be used when evaluating the tolerance of rootstocks to lime-induced chlorosis.

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