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SUITABILITY OF THE DATE OF GRAPEVINE TISSUE SAMPLING FOR TESTS OF GFLV AND ARMV PRESENCE USING ELISA METHOD

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Abstract

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Grapevine fanleaf virus (GFLV) and *Arabis mosaic virus* (ArMV) are two important agents from the Nepoviruses genus. At present, serodiagnostic method is one of routine methods of virus pathogens testing also in grapevines. The aim of this study was to determine a suitable date of grapevine tissue sampling using the ELISA method and to follow visible symptoms of GFVL and ArMV infestation of vines. Positive results of tests for the occurrence of GFLV were obtained in the following growth stages: B (intensive growth – growth apex), C (beginning of flowering – young leaves) and D (beginning of flowering – inflorescence). Samples evaluated by ELISA method as positive for ArMV were found out in growth stages B (intensive growth – growth apex) and C (beginning of flowering – inflorescence). In all other stages the results of performed tests were negative. It is concluded that the stage B (intensive growth – growth apex) seems to be the most suitable for sampling of grapevine tissue.

grapevine, grapevine virus disease, GFLV, ArMV, ELISA method, visual symptoms, term of sampling

In the territory of former Czechoslovakia, virus pathogens of grapevine were for the first time described by Straňák in 1912. He defined is as a grapevine mosaic and emphasised that this disease was not a physiological disorder (Bojňanský, 1963).

At present, 55 viruses are known, which can cause infection of grapevine plants (Martelli, 2003).

Viruses are submicroscopic particles of different sizes and shapes, which cause physiological changes in plant tissues. These changes are thereafter manifested as visible symptoms, e.g. changes in colour and shape of leaves and/or even the dying-off of plants. Within the plant organism they induce histological, cytological and also metabolic changes (Ipach, 2004).

A practical evaluation of malignity of virus diseases is performed on the base of an evaluation of percentages of individual viruses occurring in the vineyard. The infected material is then evaluated with regard to harmful effects of virus diseases on the photosynthesis, formation of active root zone, formation of assimilation apparatus and also to some secondary effects, e.g. yield depression, reduction of grape quality and deterioration of quality of wine as the final product (Martelli, 1978).

Grapevine fanleaf virus (CFLV) and *Arabis mosaic virus* (ArMV) are two important agents from the Nepoviruses genus and the serodiagnostics is one of routine methods used for testing of virus pathogens also in grapevines.

After its introduction into the virological practice, ELISA became the main diagnostic method which is currently used in practice for the detection of the economically most important viral diseases – Grapevine fanleaf virus (GFLV), Grapevine leafroll viruses (GLRV) and Arabis mosaic virus (ArMV) (Vanek, 1992).

GFLV can be detected routinely by ELISA of various grapevines extracts (leaves collected during spring, rootlets, cortical scrapings from mature canes, petioles) (Andret-Link, et al., 2004).

Poljuha et al. (2004) was interested for observation of 6 viral pathogens, inclusive GFLV and ArMV, with ELISA method.

Vanek (1992) published data about the date of plant tissue sampling, suitability of individual parts of the plant and about the distribution of viruses inside the plants of grapevine.

The aim of this study was to determine a suitable date of grapevine tissue sampling using the ELISA method and to follow visible symptoms of GFVL and ArMV infection of vines.

MATERIAL AND METHODS

The evaluation of the suitability of the date of sampling for tests of GFLV and ArMV by means of ELISA method was carried out in years 2001–2004.

Samples of grapevine varieties and clones were obtained in vineyards of the Grapevine Breeding Station in Polešovice, Czech Republic. Within the whole study period the same varieties and clones were followed and their survey is presented in Tab. III.

Viruses were detected using ELISA method. Commercial antisera (Bioreba, Switzerland) against GFLV and ArMV were used.

The ELISA tests were performed according to the methodology described by Komínek (2001).

Plant parts and dates used for the evaluation of GFLV and ArMV occurrence are presented in Tab. I.

A visual evaluation of symptoms of GFLV and ArMV infection was performed on the base of known symptoms of these two viruses. Symptoms for visual evaluation are in Tab. II.

I: *Term of sampling in individual phenological stages, sampled plant parts and tests performed in individual years*

Name	Phenological stage	Plant part	2001	2002	2003	2004
A	Dormancy	Germinating winter buds	X	X	X	X
B	Intensive growth	Growth apex	-	-	X	X
C	Beginning of flowering	Young leaves	X	X	X	X
D	Beginning of flowering	Inflorescence	X	-	X	X
E	Pea size of berries	Young leaves	X	-	X	-
F	Maturity	Growth apex	-	X	-	-
G	Maturity	Mature leaves	-	X	-	-

Date of evaluation of visual symptoms was July – September in every research year.

II: *Visual symptoms used for the evaluation of grapevines under field conditions*

Virus disease	Symptoms
Grapevine fanleaf virus (GFLV)	Sharp and markedly cogged leaf edges, opened petiole sinus, fanwise arranged venation, sinuous leaf edges, short internodes, fasciation
Arabis mosaic virus on grapevine (ArMV)	Leaf deformations similar to those of GFLV. Fanwise arranged venation and marked indentation. Atypical shape of leaves. Thin annual shoots and short internodes.

RESULTS AND DISCUSSION

GFLV is a widespread nepovirus, which causes degeneration of grapevines. It reduces yields by as much as 80% and influences also the quality of berries (Martelli and Savino, 1990).

Results of evaluation performed in different growth stages on the base of ELISA test and occurrence of visual symptoms for GFLV are presented in Tab. III.

III: *Effect of date of sampling and plant part on the presence of GFLV (ELISA testing – + positive, – negative, visual symptoms – Y– yes, N – no)*

Sample No.	Variety/Clone	Phenological stage and sampled plant part							Visual symptoms
		A	B	C	D	E	F	G	
66	Pola	–	–	–	–	–	–	–	N
65	Pola	–	+	+	–	–	–	–	N
5	Pola	–	–	–	–	–	–	–	N
6	Pola	–	–	–	–	–	–	–	N
101	Pola	–	+	+	+	–	–	–	Y
70	Olsava	–	+	+	–	–	–	–	Y
71	Olsava	–	+	+	+	–	–	–	Y
72	Olsava	–	+	+	–	–	–	–	Y
73	Olsava	–	+	+	–	–	–	–	Y
90	Olsava	–	+	+	+	–	–	–	Y
91	Olsava	–	+	+	+	–	–	–	Y
92	Olsava	–	+	+	+	–	–	–	Y
13	Muscat moravsky	–	–	–	–	–	–	–	N
75	Muscat moravsky	–	–	–	–	–	–	–	N
76	Muscat moravsky	–	–	–	–	–	–	–	N
19	Chardonnay/ 158/7	–	+	+	–	–	–	–	Y
22	Chardonnay/ 158/7	–	+	+	+	–	–	–	Y
23	Chardonnay/ 155/6	–	+	+	–	–	–	–	Y
25	Chardonnay/ 155/6	–	+	+	–	–	–	–	Y
26	Chardonnay/ 155/6	–	+	+	–	–	–	–	Y
27	Chardonnay/ 156/4	–	+	+	–	–	–	–	Y
28	Chardonnay/ 156/4	–	+	+	+	–	–	–	Y
31	Chardonnay/ 160/1	–	+	+	–	–	–	–	Y
32	Chardonnay/ 160/1	–	+	+	–	–	–	–	Y
33	Chardonnay/ 160/1	–	+	+	+	–	–	–	Y
35	Chardonnay/ 161/6	–	+	+	–	–	–	–	Y
36	Chardonnay/ 161/6	–	+	+	–	–	–	–	Y
37	Chardonnay/ 161/6	–	+	+	–	–	–	–	Y
39	Zweigeltrebe	–	+	+	–	–	–	–	Y
40	Zweigeltrebe	–	+	+	–	–	–	–	Y
42	Zweigeltrebe	–	+	+	–	–	–	–	Y
43	Zweigeltrebe	–	+	+	–	–	–	–	Y
Positive in %		0%	81.25%	81.25%	25.00%	0%	0%	0%	

Tab. III. indicates that positive results for GFLV were obtained in the following growth stages: B (intensive growth – growth apex), C (beginning of flowering – young leaves) and D (beginning of flowering – inflorescence). On other dates of sampling only negative results were recorded in all samples under study.

ArMV damages not only in grapevine but also other cultural plants. This virus is widely distributed in vineyards throughout Europe.

Results of evaluation performed in different growth stages on the base of ELISA test and occurrence of visual symptoms for ArMV are presented in Tab. IV.

IV: *Effect of date of sampling and plant part on the presence of ArMV (ELISA testing – + positive, – negative, visual symptoms – Y– yes, N – no)*

Sample No.	Variety/Clone	Phenological stage and sampled plant parts							Visual symptoms
		A	B	C	D	E	F	G	
66	Pola	–	+	+	–	–	–	–	Y
65	Pola	–	–	–	–	–	–	–	N
5	Pola	–	–	–	–	–	–	–	N
6	Pola	–	+	–	–	–	–	–	Y
101	Pola	–	–	–	–	–	–	–	N
70	Olsava	–	+	–	–	–	–	–	Y
71	Olsava	–	+	+	–	–	–	–	Y
72	Olsava	–	+	+	–	–	–	–	Y
73	Olsava	–	+	+	–	–	–	–	Y
90	Olsava	–	+	+	–	–	–	–	Y
91	Olsava	–	+	–	–	–	–	–	Y
92	Olsava	–	+	–	–	–	–	–	Y
13	Muscat moravsky	–	–	–	–	–	–	–	N
75	Muscat moravsky	–	–	–	–	–	–	–	N
76	Muscat moravsky	–	–	–	–	–	–	–	N
19	Chardonnay/ 158/7	–	–	–	–	–	–	–	Y
22	Chardonnay/ 158/7	–	+	+	–	–	–	–	Y
23	Chardonnay/ 155/6	–	+	–	–	–	–	–	Y
25	Chardonnay/ 155/6	–	+	–	–	–	–	–	Y
26	Chardonnay/ 155/6	–	–	–	–	–	–	–	Y
27	Chardonnay/ 156/4	–	+	+	–	–	–	–	Y
28	Chardonnay/ 156/4	–	+	+	–	–	–	–	Y
31	Chardonnay/ 160/1	–	+	+	–	–	–	–	Y
32	Chardonnay/ 160/1	–	+	+	–	–	–	–	Y
33	Chardonnay/ 160/1	–	+	+	–	–	–	–	Y
35	Chardonnay/ 161/6	–	+	+	–	–	–	–	Y
36	Chardonnay/ 161/6	–	+	–	–	–	–	–	Y
37	Chardonnay/ 161/6	–	+	+	–	–	–	–	Y
39	Zweigeltrebe	–	+	–	–	–	–	–	Y
40	Zweigeltrebe	–	+	–	–	–	–	–	Y
42	Zweigeltrebe	–	+	–	–	–	–	–	Y
43	Zweigeltrebe	–	+	–	–	–	–	–	Y
Positive in %		0%	75.00%	40.62%	0%	0%	0%	0%	

Tab. IV. indicates that positive results for ArMV were obtained in the following growth stages: B (intensive growth – growth apex) and C (onset of flowering –young leaves). On other dates of sampling only negative results were recorded in all samples under study.

Results analysis of variance (ANOVA) demonstrated statistically highly significant effect of the phenological stage on results of ELISA tests (P = 0.05 and 0.01). Tabs V. and VI. present results of Tukey test on significance levels of 95% and 99%. These results corroborate a highly significant effect of phenophases B, C and D for GFLV and of B and C in case of ArMV infections.

V.: *Effect of the phenological stage on results of ELISA test in plants infected by GFLV (*95%, **99%)*

	A	B	C	D	E	F	G
A		**	**	**			
B	**			**	**	**	**
C	**			**	**	**	**
D	**	**	**		**	**	**
E		**	**	**			
F		**	**	**			
G		**	**	**			

VI.: *Effect of the phenological stage on results of ELISA test in plants infected by ArMV (*95%, **99%)*

	A	B	C	D	E	F	G
A		**	**				
B	**		**	**	**	**	**
C	**	**		**	**	**	**
D		**	**				
E		**	**				
F		**	**				
G		**	**				

Bovey et al. (1980), Walter et al. (1993), Dovas et al. (2003) and Komínek et al. (2003) carried out serological testing of GFLV by means of ELISA test.

For testing and identification of virus diseases different methods were used. Some of them are more reliable than others. A visual evaluation can be used when deciding if the plant is healthy or not. However, this is the least reliable method how to decide if the grapevine plants are infected or not.

Results of visual evaluation of GFLV and ArMV symptoms recorded in vineyards of the Grapevine Breeding Station in Polešovice are presented also in Tabs III. and IV.. Visual symptoms were followed during the whole growing season.

Bouyahia et al. (2003) studied the effects of sampled plant parts on results of testing of the occurrence of viroses by means of ELISA method and recommended to use wood and leaves, particularly those with symptoms of viral infestation.

In our study results obtained within the period of several years corroborated data published by Bouyahia et al. (2003), Weber et al. (2002). The most suitable stage of sampling and part of plant seem to be the stage of intensive growth (B) and the growth apex, respectively.

Weber et al. (2002) mentioned that diagnostic symptoms of viral diseases could occur only in some growth stages. He also recommended testing plants for nepoviruses (GFLV) in the spring season because it is possible to use young growth apices. As the virus is sensitive to heat, its concentration in plants decreases and in summer is too low and undetectable.

The suggested method of sampling can be therefore used for routine tests of the occurrence of nepoviruses by means of the ELISA test.

SUMMARY

The term of grape tissues sampling is very important for testing of nepoviruses with ELISA method. The aim of this research work was evaluated the testing of GFLV and ArMV in different plant parts and different phenological stages.

It is concluded that for both virus disease is the stage B (intensive growth – growth apex) seems to be the most suitable for sampling of grapevine tissue.

SOUHRN

Vhodnost termínu odběru vzorku révy vine pro testování GFLV a ArMV využitím ELISA metody

Při hodnocení výskytu virových chorob metodou ELISA je u nepovirů velmi významný termín odběrů vzorků. Cíle této práce bylo vyhodnotit výskyt GFLV a ArMV v různých fenofázích a různých částech rostliny.

Z pohledu obou virových chorob se zdá jako nejvhodnější termín odběru stadium B (intenzivní růst – růstový vrchol).

réva vinná, virové choroby révy vine, GFLV, ArMV, metoda ELISA, vizuální příznaky, termín odběru

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