Orchid Viruses of Natural Ukrainian Flora

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Abstract

Virus infection greatly affects to the normal growth and reproductive intensity of orchid species in greenhouses. Peculiarities of ornamental orchid viruses has become known in different countries of the world while there is nothing known about orchid virus infection in natural flora of Europe, for example in Ukraine. After extensive virus detection of natural orchids of Ukraine (Carpathians, Crimea), some viruses infecting these plants in their natural inhabitance were determined.

Keywords: orchid virus; terrestrial orchids; indirect ELISA; DAS-ELISA

INTRODUCTION

Most of the European endemic orchids belong to rare and disappearing species and their affection by viruses is the consisting part of the problems of plant biodiversity studying and preservation.

Infection of *Orchis* spp. by *Tobacco rattle virus* (USA) and *Turnip mosaic virus* (Germany) has been detected in terrestrial zone, and new member of *Potyvirus* genera has been determined as pathogen of *Cypripedium calceolus* in Germany in 1986 (LESEMANN & VETTEN 1985.). Besides, it was registered that some representatives of *Potyvirus* genera (*Clover yellow vein virus* – CYVV and *Bean yellow mosaic virus* – BYMV) affected orchids in natural European ecosystems (ZETTLER *et al*. 1990). Also, investigation of orchid samples for presence of other viruses, such as *Arabis mosaic virus* (ArMV) and *Tomato aspermii virus* (TAV), was conducted because these viruses were detected in agrocenoses surrounding the place of sampling. *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV) are known to be the most prevalent viruses infecting cultivated tropical orchids (PEARSON & COLE 1986), while there are no confirmed reports of natural infection by either CymMV or ORSV of wild tropical orchids (ZETTLER *et al*. 1978; KOBAYASHI & KAMEMOTO 1989). Concerning orchids from natural European flora, there is not data on their affection by CymMV and ORSV, too.

Single accidents of virus infection in orchids of temperate climate proves the necessity of wider and purposeful testing of orchids for virus presence in this part of Europe.

MATERIALS AND METHODS

Plants of *Comperia, Dactylorhisa, Epipactis, Gymnadeae, Himantoglossum, Limodorum, Listera, Neotia, Orchis, Ophris* and *Orchis* species from Ukrainian natural ecosystems (Carpathians, Crimea) were the objects of this research (Figure 1 and Table 1). Moreover, plants of *Plantathera* and *Cypripedium* collected from Kanev National Reserve and collection of Grishko' National Botanic Garden were analyzed, too (Figure 1 and Table 1). Plant leaves were homogenized in 0.1M PBS, pH 7.4. Homogenate was then squashed through the caprone filter and centrifuged at low speed (4000 rpm) for 20 min. Virus identification was carried out using standard indirect ELISA and DAS-ELISA with polyclonal antiserums to TAV, ArMV, TRV, CYVV, BYMV, TuMV, TMV isolates and to mixture of CymMV and ORSV on Labsystem polystirol plates. Absorbance at 405 nm was measured using MR 700 "Dynatec" reader in 60 min after the addition of substrate. Samples were counted as posi-

Table 1. Results of orchid testing from Ukrainian natural ora

Plant sample	Year	Sampling points	Reaction with antiserum to							
			TRV	BYMV	CYMV	TAV	ArMV	TuMV	ORSV + CymMV	TMV
1. *Dactylorhisa sambucina*	2002	Khusta National Reserve	–	–	–	+	–	–	–	–
2. *Dactylorhisa incarnata*	2002	Khusta National Reserve	–	–	–	+	–	–	–	–
3. *Dactylorhisa majalis*	2002	village Kniaginia	+	–	–	+	+	–	+	–
4. *Dactylorhisa sambucina*	2002	village Kniaginia	–	–	–	+	+	–	–	–
5. *Dactylorhisa majalis*	2002	village Kolochava	+	–	–	+	+	–	+	–
6. *Listera ovata*	2002	village Kniaginia	+	–	–	+	+	–	+	+
7. *Cymnoadea conopsea*	2002	village Kniaginia	+	–	–	+	+	–	–	–
8. *Orchis mascula*	2002	village Krepkoe	–	–	–	–	–	–	–	–
9. *Orchis punctulata*	2002	village Krepkoe	+	–	–	–	–	–	+	–
10. *Orchis morio*	2002	village Krepkoe	+	+	–	+	–	–	–	–
11. *Orchis purpurea*	2002	village Krepkoe	–	–	–	+	–	–	–	–
12. *Ophrys taurica*	2002	village Krepkoe	–	–	–	–	–	–	+	–
13. *Dactylorhisa majalis*	2002	Shaudan	–	–	–	–	–	–	+	–
14. *Dactylorhisa maculata*	2002	Shaudan	+	–	–	–	–	–	+	–
15. *Himantoglossum caprinum*	2000	Laspi	+	–	–	–	–	–	+	–
16. *Comperia comperana*	2000	Cape Sarych	–	–	–	+	+	–	–	–
17. *Neottia nidus-avis*	2000	village Kuybyshevo	+	–	–	+	+	–	+	+
18. *Limodorum abortirum*	2000	Cape Sarych	–	–	–	+	–	–	+	+
19. *Epipactis helleborine*	2000	Cape Sarych	–	–	–	–	–	–	+	+
20. *Epipactis helleborine*	2000	Sevastopol	–	–	–	+	–	–	+	+
21. *Orchis pupurea*	2000	Sevastopol	+	–	–	+	+	+	+	–
22. *Plantathera bifolia*	2001	Kanev National Reserve	–	–	–	+	–	–	–	–
23. *Cypripedium* sp.	2001	Grishko' National Botanical Garden, Kiev	+	+	–	+	–	–	+	+

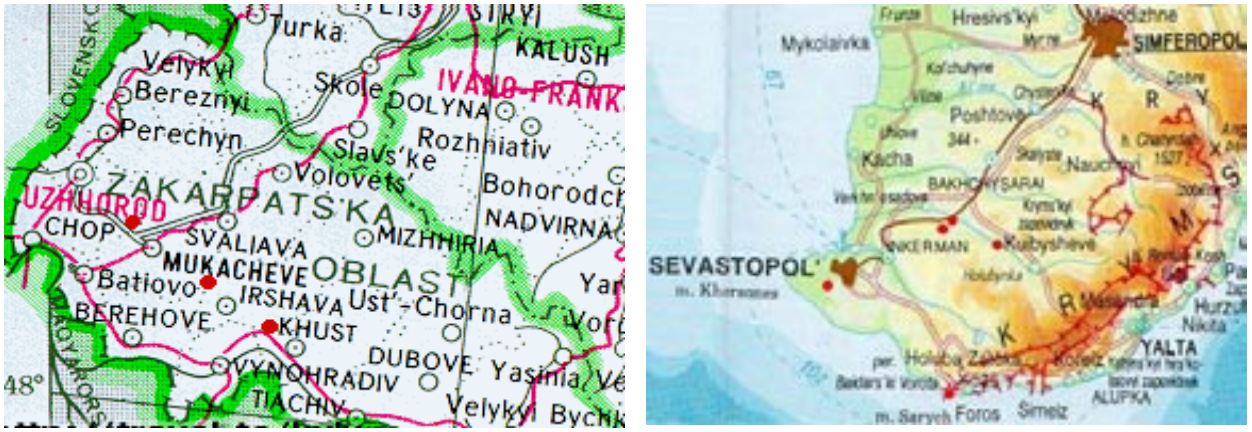

Figure 1. Orchid sampling points for Ukrainian national ecosystems

tive in case of their twofold density comparing to that of virus-free negative controls.

RESULTS

Results of DAS-ELISA show that TAV and ArMV are the most widespread orchid viruses from Carpathien's natural flora (Table 1). Besides, it was demonstrated that natural Crimean orchids were infected by viruses related to TRV and TAV (Table 1). Taking into accounting the fact of frequent positive reactions of sample with antiserum to the mixer of CymMV and ORSV, and comparetively rare positive reactions with antiserum to TMV (related to ORSV) (Table 1), we can make an assumption that most of natural Crimean orchids are infected by CymMV.

As a result of conducted testing, it was revealed that plants belonging to *Listera ovata* (village Kniaginia), *Neottia nidus – avis* (village Kuybyshevo), *Orchis purpurea* (Sevastopol) and *Cypripedium* spp. (from collection of Grishko' National Botanical Garden, Kiev) are the most affected orchid species.

DISCUSSION

Examination of Ukrainian natural orchids proved that viruses related to TAV, CymMV, ArMV and TRV are the most widespread viruses. High degree of TAV and ArMV infection of Carpathian orchids can be explained by their wide spreading in neighbour agrocenosis.

Therefore, it was determined that most orchids from Ukrainian natural ecosystem were infected with viruses, related to *Tobravirus, Cucumovirus* and *Potexvirus* genera. This results differ from data presented by Lesemann and Vetten for orchids in Germany, where affection of orchids by viruses from *Potyvirus* genera was registered (LESEMANN & VETTEN 1985). It should be noted that orchids which are under threat of extinction are mostly contaminated by viruses. Besides, a tendency was shown towards the for increasing of virus concentration in samples, collected near cities.

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