ÞEMËS ÛKIO MOKSLAI. 2004. Nr. 1. P. 19–23

Viral diseases of *Poaceae* family plants

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Plants of common cocksfoot (*Dactylis glomerata* L.), tufted hairgrass (*Deschampsia cespitosa* (L.) P. Beauv.), meadow fescue (*Festuca pratensis* Huds.), perennial ryegrass (*Lolium perenne* L.), *Festulolium loliaceum* (Huds.) P. Fourn. (*Festuca pratensis* L. × *Lolium perenne* L.) showing symptoms of mosaic spotting, chlorotic and necrotic streaks on leaves and stems were detected at the Plant Breeding Centre of Lithuanian Institute of Agriculture and also at the Vilnius station for investigation of plant cultivars. Virus isolates were investigated by the methods of test-plants, electron microscopy and DAS-ELISA.

According to the results of symptomatology on host- and test-plants, virion morphology, transmission of viral infection by mechanical inoculation and aphides, and DAS-ELISA, for the first time in Lithuania the following viruses were identified: *Cocksfoot streak potyvirus*, *Festuca necrosis closterovirus* and *Ryegrass mosaic rymovirus.*

Key words: *Poaceae* family, virus identification, virus diseases, *Closterovirus*, *Potyvirus*

INTRODUCTION

Forage feed plants belong to a group of plants highly important for the nutrition of domestic and wild animals. Many of these plants are grown in plowed fields or represent cultivated and spontaneous inhabitans of meadow, pasture and roadside plant populations. During the vegetation period the yield of perennial forage grass is influenced not only by the ecological factors, but also by diseases caused by pathogens.

Investigating virus diseases of graminaceous plants, isolated and identified were three viruses from cocksfoot, fescue and ryegrass: *Cocksfoot streak potyvirus* (CSV), *Festuca necrosis closterovirus* (FNV) and *Ryegrass mosaic rymovirus* (RGMV).

The CSV disease was first described and the agent of disease identified by Smith and Storey in 1952 [4]. The agent of the disease (CSV) belongs to the genus potyvirus, has filamentous particles with a clear modal length of 750 nm and 13 nm wide. CSV is transmitted by mechanical inoculation with sap, in non-persistent way by aphids and is not transmitted by seed [4]. A characteristic symptom of disease caused by CSV on cocksfoot and other species of the genus is pronounced streaked stripes, after which the disease was named. The virus spreads in England, Holland, France, Germany, Sweden, USA [14] and Russia [17]. CSV is an economically important plant pathogen. It infects a number of grass species and, depending on the host, can cause forage yield losses of 10 to 60% [14].

FNV was first observed in Central Europe. The virus was investigated and characterized by H. E. Schmidt et al. in South Germany in 1963 [8]. The agent of the disease belongs to the genus *Closterovirus* and has filamentous flexuous particles with a clear modal length of 1725 nm and 18 nm wide [9]. Natural virus hosts include *Festuca ptatensis, F. arundinaceae* and *Lolium perenne.* Necrosis, reddening and plant death from the roots upward characterize the symptoms on infected plants. Virus is not seed-borne, does not spread by contact, and is not transmitted by mechanical inoculation, but is transmitted by *Rhopalosiphum padi* L. aphides [14].

RGMV is presently classified as the type species of the mite-transmitted genus *Rymovirus* of the family *Potyviridae.* On the basis of its transmission, it is grouped together with *Wheat streak mosaic* (WSMV), *Agropyron mosaic virus* (AGMV), *Hordeum mosaic virus* (HMV), and *Oat necrotic mottle virus* (ONMV) [12]. RGMV is widely distributed where ryegrass is grown – in the USA, Canada, several European countries, Australia, New Zeland and South Africa [11], Czech Republic, Slovakia [15]. Virus can decrease the yield of pastures of Italian ryegrass (*Lolium multiflorum* Lam.) and perennial ryegrass (*L. perenne* L.) by 30–50% [11]. The symptoms are caused by different (from faint to severe) mosaic with chlorotic and necrotic streaking, especially in perenial ryegrass [4]. The host range of RGMV is restricted to festucoid species within the

family *Poaceae*. Disease diagnosis can be performed by mechanical inoculation of *L. perenne, L. multiflorum, D. glomerata* and *Avena sativa* plants [14]. RGMV is thought to be transmitted by the eriophyid mite *Abacarus hystrix* (Nalepa) [7]. RGMV particles are flexuous rods about 705 nm long and 15–18 nm in diameter [11].

The aim of this investigation was to evaluate virus infection in gramineous plants, to identify agents, to define experimental host range, symptoms, morphology of virions, to preparate antigens.

MATERIALS AND METHODS

Material for investigation was collected at the Vilnius Station for investigation of plant cultivars and at the Plant Breeding Centre of Lithuanian Institute of Agriculture, Dotnuva, Akademija in 1998–2002. The samples were collected from gramineous plants showing viral symptoms on leaves and stem. The experimental work was carried out in the greenhouse and at the Laboratory of Plant Viruses of the Institute of Botany. Viruses were identified by test-plant reaction and virus particle morphology [4, 14] and DAS-ELISA [5].

Test-plants were grown and inoculated in greenhouse conditions. Fifteen plants species of the families *Amaranthaceae, Chenopodiaceae, Poaceae* and *Solanaceae* were inoculated with the study viruses. The following test-plants were inoculated: *Agrostis stolonifera* L., *Avena sativa* L., *Chenopodium amaranticolor* Coste et Reyn., *C quinoa* Willd., *Dactylis glomerata* L., *Festuca pratensis* Huds., *Gomphrena globosa* L., *Hordeum distichon* L., *Lolium perenne* L., *Nicotiana debneyi* Domin., *Phleum pratense* L., *Poa palustris* L., *P. pratensis* L., *Secale cereale* L., *Zea mays* L. The inoculum for mechanical sap inoculation was prepared by grinding infected leaves in 0.1 M phosphate bufer pH 7.1. Monocotyledonous test-plants were inoculated at the stage of three leaves and dicotyledonous test-plants at the four-leave stage. Presence of virus particles was observed in the preparations, using a JEM-100S electron microscope after negative staining with 2% uranyl acetate solution [6].

The viruses isolates were purified from infected leaves according to the modified methods based on coksfoot [10] and fescue [1].

RESULTS AND DISCUSSION

Cocksfoot streak virus. CSV has been isolated and identified from naturaly infected *Dactylis glomerata* plants showing light-green or yellow streaks on leaves and stems. Possibility of the disease agent transmission by mechanical sap inoculation was ivestigated inoculating 15 test-plants belonging to 4 families. The infection was succesfully transmited to test-plants of five species: *A. sativa* '*Jaugila*', '*Edit*', '*Jak*', '*Jovar*', *H. distichon* '*Anni*', '*Ula*', *D. glomerata* '*Asta*', *L. perenne* '*Sodrë*', '*Þvilgë*', *P. pratense* '*Vëlenis*'. Test-plants from another 10 species (*A. stolonifera, C. amaranticolor, C. quinoa, F. pratensis, G. globosa, N. debneyi, P. palustris, P. pratensis, S. cereale, Z. mays*) did not react to inoculation. From the monocotyledonous plants tested, *A. sativa* and *D. glomerata* were most susceptible to virus infection. First symptoms of chlorotic streaks on oat leaves appeared within 18 days after inoculation. Later necrotic and chlorotic streaks appeared on leaves and stems. Sometimes necrosis started from the tip and extended to the basal part of leaves, leading to their premature death. Virus infection was successfully transmitted to *D. glomerata* '*Asta*' plants, which showed yellowish-greenish streaking and chlorosis on leaves.

Electron microscopy of negatively stained leaf dip preparations made from naturally infected cocksfoot plants and infected test-plants revealed the presence of flexuous filamentous particles measuring 750 nm in length. Such particle morphology is characteristic of C*ocksfoot streak potyvirus.*

Purified virus preparations had A_{max} at 260 nm and A_{min} at 240 nm. The ratio of absorbance at 260/280 was 1.02. The yield of the purified virus, taking into account potyvirus specific absorbance $A_{260}^{0.1\%}$ = 2.04, was calculated to be 23.3 mg/100 g of infected plant tissue. Electron microscopy of the purified virus revealed flexuous filamentous particles about 750 nm in length (Fig. 1).

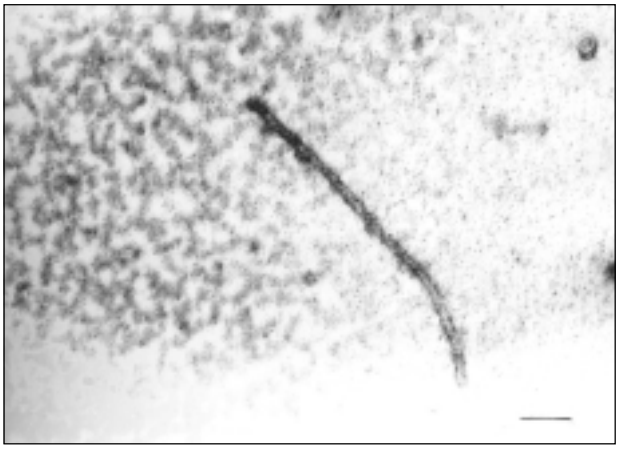

Fig. 1. *Cocksfoot streak potyvirus* particles in purified EM preparation. Bar represents 100 nm

Festuca necrosis virus. FNV isolates (Is.) used in this study were collected from naturally infected plants of *Festuca pratensis* Huds. (Is. F0102) and

Festulolium loliaceum (Huds.) P. Fourn. (*Festuca pratensis* L. × *Lolium perenne* L.) (Is. F/L0203) showing chlorotic and necrotic symptoms. Electron microscopy of negatively stained leaf dip preparations made from naturally infected *Festuca* and *Festulolium* plants revealed the presence of filamentous flexuous particles measuring 1700 nm in length (Fig. 2). Such particle morphology is characteristic of *Festuca necrosis closterovirus*. Transmission possibility of the disease agent by mechanical sap inoculation was investigated inoculating the following test-plants: *A. stolonifera* '*Guoda*', *A. sativa* '*Edit*', '*Jak*', '*Jaugila*', '*Jovar*', *D. glomerata* '*Asta*', *F. pratensis* '*Dotnuva*', *H. distichon* '*Anni*', '*Rataj*', *L. perenne* '*Sodrë*', *P. pratense* '*Velenis*', *T. aestivum*, *Z. mays* '*Pionier*', *C. amaraticolor*, *C. quinoa*, *G. globosa*. The test-plants did not react to inoculation. The virus was not transmissible by mechanical inoculation to test-plants.

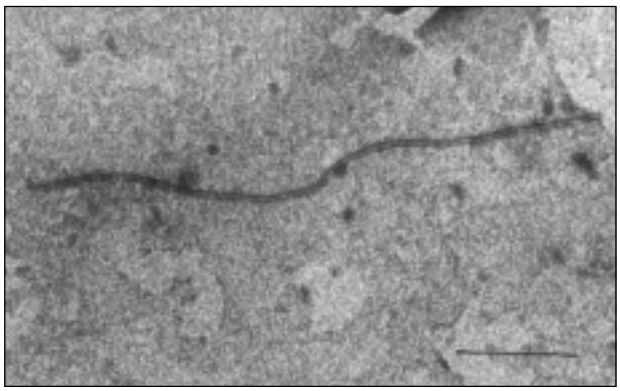

Fig. 2. *Festuca necrosis closterovirus* particles in *Festuca* L. plants. Bar represents 500 nm

The agent of the disease was transmitted by *Rhopalosiphum padi* L. aphides to *Avena sativa* plants. Transmission was carried out by Dr Frank Rabenstein (Federal Centre for Breeding Research on Cultivated Plants, Institute for Resistance Research and Pathogen Diagnostics, Aschersleben, Germany).

Purified virus preparations had A_{max} at 260 nm and A_{min} at 240 nm. The ratio of absorbance at 260/280 was 1.2. The yield of the purified virus, taking into account closterovirus specific absorbance $A^{0.1\%}_{260}$ = = 2.27 (Bar-Joseph, Smookler, 1976), was calculated to be 22 mg/1 kg of infected plant tissue.

Ryegrass mosaic virus. The RGMV was isolated from naturally infected *Lolium perenne*, *Festuca pratensis*, *Festulolium* '*Punia*' cultural plants and *Deschampsia cespitosa*, showing symptoms of chlorotic flecking, mosaic and necrotic streaks.

Thirteen test-plant species of the families *Poaceae* and *Chenopodiaceae* were experimentally inoculated with RGMV. Fifteen days after inoculation, some plants of *Poaceae* schowed disease symptoms on leaves. The test-plants most susceptible for virus infection were *A. sativa, L. perenne, F. pratensis, D. glomerata. A. sativa* '*Jaugila*' developed systemic mosaic, necrotic streaking in inoculated leaves. Virus infection was transmitted to *D. glomerata* '*Asta*', *F. pratensis* '*Dotnuva*' and *L. perenne* '*Sodrë*', '*Þvilgë*', which developed systemic mosaic, chlorotic flecking and streaking on leaves. *A. stolonifera, P. palustris* and *P. pratensis* were less sensible. The test plants *C. amaranticolor, C. quinoa, H. distichon, S. cereale, T. aestivum, Z. mays* reacted to none of the virus isolates.

Electron microscopy analysis revealed the presence of viral particles in tested specimen grid preparations prepared from naturally infected fescues and festulolium plant samples or from inoculated monocotyledonous plants. Virus particles are flexuous filaments about 700 nm., specific for potyviruses (Fig. 3). RGMV infection in host-plants and in inoculated test-plants expressing systemic mosaic was comfirmed by the DAS-ELISA test.

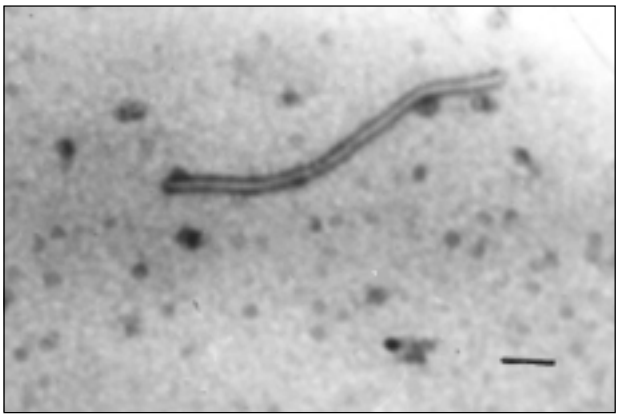

Fig. 3. *Ryegrass mosaic rymovirus* particles in *Festulolium* cv. '*Punia*' plants. Bar represents 100 nm

In this work, we describe three viruses identified from gramineous forage plants. The virus disease called "cocksfoot streak" was first described in Lithuania in 2000 [16]. The agent of this disease, CSV, transmissible by aphids, has a narrow host range in the *Poaceae*. Host range studies and symptoms caused by the virus isolated from cocksfoot in *A. sativa, D, glomerata, H. distichon* and L. *perenne* are in accordance with the descriptions on CSV [4, 14]. Electron microscopy of the purified virus revealed flexuous filaments about 750 nm in length, as had been reported by other investigators [4, 14, 17].

The disease caused by FNV is characterized by symptoms of chlorosis and necrosis on infected *F. pratensis* and *Festuca* × *Lolium* hybrids plants. FNV was found in *Festuca* × *Lolium* hybrids expressing these symptoms in mixed infection with RGMV.

However, in Germany in *Festuca* × *Lolium* hybrids with such symptoms RGMV has often been isolated, but closterovirus-like particles have never been observed (F. Rabenstein, F. Matzck, unpublished). On the basis of host-range symptoms, particle size and morphology, and virus transmission by aphides the virus is considered to be identical with the *Festuca necrosis virus* identified and described in Germany [4].

RGMV causing mosaic disease on ryegrass was found in Lithuania in 2002. Symptoms of the disease caused by RGMV resemble those caused by other viruses affecting gramineous plants – light green to yellow mosaic, chlorotic and necrotic streaks. European RGMV isolates differ in virulence, whereas all isolates from the USA and Canada constitute a group of mild strains [13]. The host range and symptoms of our virus isolates was similar to the one described for RGMV [15]. Observation of virus particles in crude preparations on infected host plants revealed the presence of filamentous particles about 670–700 nm long. The modal length of RGMV investigated in Czechoslovakia was estimated to be 665–700 nm [15], in Germany 650–750 nm [3], in Canada 705 nm [13]. In the description of the virus, the modal length of 700 nm is indicated [4].

CONCLUSIONS

1. The virus isolated from the cocksfoot plants showing light-green or yellow streaks on leaves and stems, according to data on virion morphology, transmissibility by mechanical inoculation, reaction on test-plants was identified as *Cocksfoot streak potyvirus.*

2. According to symptomatology on host-plants (chlorotic and necrotic streaks), virion morphology, transmission of virus infection by aphides (*Rhopolosiphum padi* L.) and not non-transmission by mechanical inoculation, the virus isolated from *Festuca pratensis* Huds., *Festulolium loliaceum* (Huds.) P. Fourn., was identified as *Festuca necrosis closterovirus.*

3. According to the morphology of virus particles (~700 nm), host-range, symptoms on test-plants (systemic mosaic, chlorotic and necrotic flecking and striping) and DAS-ELISA data, the virus isolated from *Lolium perenne* L., *Festuca pratensis* Huds., *Festulolium loliaceum* (Huds.) P. Fourn. and *Deschampsia cespitosa* (L.). P. Beauv. with leaf mosaic, chlorotic and necrotic streak symptoms is identical to *Ryegrass mosaic rymovirus* from the genus *Rymovirus.*

ACKNOWLEDGEMENTS

We thank Habil. Dr. A. Svirskis (Plant Breeding Centre of Lithuanian Institute of Agriculture, Dotnuva, Akademija) and agronomist V. Stanionytë (Station for investigation of plant cultivars, Vilnius) for providing various cereal cultivar seed plants.

Received
14 May 2003

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POACEAE ÐEIMOS VARPINIØ AUGALØ VIRUSINËS LIGOS

S a n t r a u k a

Nuo 1998 m. Botanikos institute, Fitovirusø laboratorijoje, pradëtos tirti varpiniø augalø virusinës ligos. Vilniaus augalø veisliø tyrimo stoties bei Lietuvos þemdirbystës instituto Selekcijos centro ávairiø veisliø varpiniø þoliø bandyminiuose laukeliuose, taip pat atskirø pasëliø laukuose bei pakelëse aptikti tikrojo eraièino (*Festuca pratensis* Huds.), daugiametës svidrës (*Lolium perenne* L.), paprastosios eraièinsvidrës (*Festulolium loliaceum* (Huds.) P. Fourn. (*Festuca pratensis* L. × *Lolium perenne* L.), paprastosios ðunaþolës (*Dactylis glomerata* L.), kupstinës ðluotsmilgës (*Deschampsia cespitosa* (L.) P. Beauv) augalai su stebimais virusinës ligos simptomais – mozaikiniu dëmëtumu, chlorotiniais ir nekrotiniais dryþeliais lapuose ir stiebuose. Iðskirti virusø izoliatai, kurie buvo tiriami augalø-indikatoriø, elektroninës mikroskopijos, DAS-ELISA metodais. Buvo tiriami infekcijos uþkrëtimo bûdai (mechanine inokuliacija, perneðimas amarais). Atliktø tyrimø duomenimis, Lietuvoje pirmà kartà buvo identifikuoti trys varpinius augalus paþeidþiantys virusai: ðunaþolës dryþligës (*Cocksfoot streak potyvirus*), eraièino nekrozës (*Festuca necrosis closterovirus*) ir svidrës mozaikos (*Ryegrass mosaic rymovirus*).

Raktaþodþiai: *Poaceae* ðeima, identifikacija, virusinës ligos, *Closterovirus Potyvirus*

Лайма Урбанавичене

ВИРУСНЫЕ БОЛЕЗНИ ЗЛАКОВЫХ РАСТЕНИЙ, ПРИНАДЛЕЖАЩИХ СЕМЕЙСТВУ *POACEAE*

Р е з ю м е

В 1998 г. в Лаборатории фитовирусов Института ботаники начаты исследования вирусных болезней злаковых растеий. Обследованы растения на опытных полях Вильнюсской сортоиспытательной станции, на селекционном центре растений Литовского института земледелия, а также на отдельных посадках и обочинах. Обнаружены растения овсяницы луговой (*Festuca pratensis* Huds.), райграса пастбищного (*Lolium perenne* L.), ежи сборной (*Dactylis glomerata* L.) и луговика дернистого (*Deschampsia cespitosa* (L.) P. Beauv.) с симптомами вирусной инфекции: мозаичная пятнистость, хлоротические и некротические штрихи на листьях и стеблях. Выделены вирусные изоляты, которые идентифицированы методами растений-индикаторов, электронной микроскопии и DAS-ELISA. Исследовались способы переноса инфекции механической инокуляцией и тлями.

В результате наших исследований в Литве впервые на злаковых травах идентифицированы три вируса: полосатости ежи (*Cocksfoot streak potyvirus*), некроза овсяницы (*Festuca necrosis closterovirus*) и мозаики райграса (*R yegrass mosaic rymovirus*).

Ключевые слова: семейство *Poaceae*, идентификация, вирусные болезни, *Closterovirus*, *Potyvirus*