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ORAPID COMMUNICATION

Turnip mosaic virus in rhubarb grown on farms in Poland

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Abstract

The aim of this study was the detection and molecular characterization of newly identified turnip mosaic virus (TuMV) isolates infecting rhubarb in Poland. The presence of the virus in rhubarb was confirmed by ELISA and RT-PCR techniques. The specificity of the obtained products was verified by Sanger sequencing. Two sequences (1,077 nt) of the TuMV coat protein gene and the 3'-terminal non-coding region were uploaded to the Genbank database (access no. MG882689 and MG882690). The phylogenetic analysis was performed based on the coat protein gene sequences of two new Polish isolates from rhubarb and 43 other TuMV sequences retrieved from the Genbank. The isolates studied were grouped with an isolate from rhubarb (AB701709) found in the UK (98% nucleotide identity). It is the first phylogenetic analysis of TuMV isolates infecting rhubarb in Poland.

Keywords: phylogenesis, *Rheum rhabarbarum*, RT-PCR, turnip mosaic virus (TuMV)

Introduction

Rhubarb (*Rheum rhabarbarum* L.) is an edible herbaceous perennial belonging to the knotweed family (Polygonaceae). Rhubarb is native to Asia, including Siberia. Originally it was cultivated only as an ornamental plant, but later, it became common for its medicinal properties. It was brought to Europe in the 16th century, and its cultivation started 200 years later. The first European rhubarb plantations were established in Italy. It arrived in Poland in the eighties of the 19th century. In recent years, rhubarb has become increasingly popular in Poland, but its cultivation is concentrated mainly on small and medium (up to 5 ha) organic farms. Rhubarb is a vegetatively propagated perennial, and viruses can affect it at any stage of growth, causing yield losses due to abnormal plant growth, loss of vigor, or leaf discoloration (Walkey and Cooper 1972; Thomas 2011). To date, the following have been described on rhubarb: turnip mosaic virus (TuMV), arabis mosaic virus (ArMV), cherry leaf roll virus (CLRV), cucumber mosaic virus (CMV), strawberry latent ringspot virus (SLRV) (Stace-Smith and Jacoli 1967; Tomlinson and Walkey 1967; Robertson and Lanson 2005; Komorowska *et al.* 2018). Turnip mosaic virus, belonging to the genus *Potyvirus*, infects many plant species, mainly in the family *Brassicaceae*. It is probably the most widespread and significant virus infecting cultivated and ornamental plants in this family, occurring worldwide, including temperate and tropical regions of Africa, Asia, Europe, Oceania, and North and South America (Ohshima *et al.* 2002). TuMV ranked second after cucumber mosaic virus as the most important virus infecting field-grown vegetables in a survey of viral diseases in 28 countries and regions (Tomlinson 1987). TuMV is transmitted with propagating material and in a non-persistent manner by more than 50 species of aphids. One of them is *Myzus persicae*, the peach-potato aphid, the most important vector of the virus (Hamlyn 1953). The virus is not seed-born. The reservoir of TuMV may be wild plants of the genus *Brassica*. The virus particles are filamentous,

700–750 nm long and 12 nm in diameter. The viral genome is a single strand of RNA with positive polarity [ss(+)RNA] and a size of approximately 10 kb. The genome contains a single reading frame, and translation results in a single polyprotein undergoing proteolysis, from which 10 proteins are formed (Riechmann *et al.* 1992). Phylogeographical analysis of the entire genus *Potyvirus* (Gibbs and Oshima 2010) showed that the genus, like TuMV, originated in western Eurasia and/or North Africa and probably evolved from a virus of monocotyledonous plants. In the first year of infection, small rings 2-10 mm in size, surrounded by a reddish border, appear on the leaves of infected rhubarb plants. As the disease progresses, the spots cluster into bigger, irregular structures, leading to reddening of the leaf blade (Fig. 1). Infected plants are smaller than healthy plants, with little growth reduction evident in the first year of cultivation (Walkey and Cooper 1972). The virus produces the most pronounced symptoms in the temperature range of 22 to 30°C, almost none at 21°C, and is masked completely at 16°C or below. Cold, wet weather not only reduces the severity of the disease but also makes it more difficult for aphids, which migrate mainly on warm, sunny days, to transmit it. The time between inoculation and visible symptoms varies from 9 to 35 days, depending on the air temperature during this period (Chupp and Sherf 1960).

This study aimed to identify TuMV in rhubarb crops in Poland and to perform a phylogenetic analysis of the virus isolates detected.

Fig. 1. Rhubarb leaves infected by TuMV

Materials and Methods

Plant material

Between 2020 and 2022, six commercial rhubarb plantations in south-eastern Poland were monitored for viruses. In two fields, red lesions with necrotic rings were observed on the leaves of some plants (Fig. 1). Although the incidence of viral disease was relatively low during the survey, most of the potentially infected plants showed severe symptoms, as well as abnormal plant growth and loss of vigor. Samples for virus identification were taken from 10 symptomatic and four apparently healthy plants.

ELISA test

ELISA-based serological assays were conducted according to the manufacturer's instructions. For sample preparation, 200 mg of leaves was ground using a pestle and mortar with 4 ml of a universal extraction buffer PBS-TPO [0.01 M phosphate buffered saline (PBS) with 0.05% Tween-20, 2% polyvinylpyrrolidone, 0.02% bovine serum albumin, pH 7.4] at an approximate ratio of 1:20 (w:v). In the first step, a direct immunoenzymatic test (Plate-Trapped Antibody Enzyme-Linked Immunosorbent Assay, PTA-ELISA) for Potyvirus (Bioreba AG, Switzerland) was used to identify the pathogen in the leaf extracts. This was followed by an indirect immunoenzymatic assay (Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay, DAS – ELISA) for TuMV (Loewe, Germany). The analysis was carried out for 10 samples in triplicate. Leaf samples from four symptomless plants were included as negative controls. Lyophilized leaves of TuMV-infected plants (Loewe, Germany) were included as positive controls. Results were registered on a Multiskan FC plate reader (Thermo Scientific, USA) at 405 nm. Samples were considered positive when their absorbance values were at least three times that of the negative control (Crowther 1995).

RNA isolation, RT-PCR, sequencing

Total nucleic acids were isolated from 200 mg of 10 infected rhubarb plants by adsorption on silica gel (SC) (Boom *et al.* 1990). The quality and concentration of the isolated RNA were checked spectrophotometrically (NanoDrop 2000, Thermo Scientific, Waltham, MA, USA). Reverse transcription and amplification of cDNA was performed in a one-step system using the Transcriptor One-Step RT-PCR kit (Roche Diagnostics GmbH, Mannheim, Germany) and TuMV1 -TuMV2 specific primers (Parmar *et al.* 2017). The final volume of the reaction mixture was 10 µl. It

consisted of reaction buffer (2 µl), 10 mM primers (0.4 µl each), nuclease-free water (5.5 µl), and a solution of isolated nucleic acids (1.5 µl). The reverse transcription and amplification reactions were configured as follows: reverse transcription for 30 minutes at 50°C, followed by initial denaturation for 3 minutes at 94°C, and a further 30 cycles at 94°C for 10 s, 55°C for 30 s and 68°C for 1 minute. A final elongation of the product was carried out at 72°C for 5 minutes. PCR products were separated on a 1% agarose gel with 100 pz DNA size markers (Fermentas, Lithuania) and visualized under UV light. The amplified viral cDNA fragments were sequenced at Genomed (Warsaw, Poland). The read TuMV sequences were further analyzed using BLASTN. The obtained sequences were nearly identical, and representative sequences of two virus isolates (R1, R2) were deposited in GenBank (accession nos. MG882689 and MG882690). Multiple sequence alignment (MSA) of nucleotide and amino acid sequences was generated between TuMV sequences obtained for the R1 and R2 isolates and other TuMV isolates downloaded from the NCBI database. MSA was performed using the CLUSTALW software (Thompson *et al.* 1994). Sequences of the gene encoding the TuMV coat protein obtained in this experiment (MG882689 and MG882690) and 43 sequences of TuMV isolates from different hosts and different locations that had previously been deposited in the GenBank were used for phylogenetic analysis (Tab. 1). The program MEGA version 11.0 (Tamura *et al.* 2021) was used for phylogenetic analyses. A phylogenetic tree was constructed using the neighbor-joining (NJ) method with 1000 bootstrap replicates (Tamura *et al.* 2021). The sequence of the homologous region of narcissus yellow stripe virus 1 was used as an outgroup for this analysis.

Results and Discussion

To date, only a few publications on viral diseases of rhubarb are available in the literature. Most of the papers were written in the 1960s and 1970s. The most recent data concern the detection of TuMV in Alaska (Robertson and Lanson 2005) and ArMV in Poland (Komorowska *et al.* 2018). As part of routine inspections of rhubarb crops, plants with symptoms suggestive of the viruses were sampled.

Table 1. TuMV isolates used for phylogenetic analysis

Isolate	Original host	Host type	Country	Accession nos.
MYA2	*Raphanus sativus*	B	Burma	LC537564
CH6	*Raphanus sativus*	BR	Japan	AB252103
AD911J	*Raphanus sativus*	BR	Japan	LC639373
YAD022J	*Raphanus sativus*	BR	Japan	LC639661
USA6	*Raphanus sativus*	BR	USA	AB701741
IND27	*Raphanus sativus*	BR	India	LC537552
UKR35	*Raphanus sativus*	BR	Ukraine	LC537589
CHN301	*Raphanus sativus*	B(R)	China	LC537506
CHZJ26A	*Brassica campestris*	B(R)	China	AB252106
CHN212	*Brassica juncea*	B(R)	China	LC537499
VIET58	*Brassica juncea*	BR	Vietnam	AB747288
MYA36	*Brassica juncea*	B	Burma	LC537570
NLD2	*Brassica oleracea*	B	Netherlands	AB701727
CZE11	*Brassica oleracea*	B	Czech Republic	LC537541
KEN1	*Brassica oleracea*	B	Kenya	AB093605
GRC25	*Brassica oleracea*	B	Greece	AP017829
GRC2	*Brassica oleracea*	B	Greece	AB188998
T52	*Brasica napus*	B	Czech Republic	OQ675603
T47	*Brasica napus*	B	Czech Republic	OQ675601
DSMZ PV-1360	*Brassica napus*	B	Germany	OP150415
PV376-Br	*Brasica napus*	B	Germany	AB076528
CDN1	*Brasica napus*	B	Canada	AB093610
I2	*Brasica napus*	B	Russia	KC297103

Table 1. TuMV isolates used for phylogenetic analysis – continuation

Isolate	Original host	Host type	Country	Accession nos.
TAJZ6-05	*Brassica napus*	B	China	EF490536
UK1	*Brassica napus*	B	Great Britain	NC002509
GBR7	*Rheum rhabarbarum*	B	Great Britain	AB701709
R1	*Rheum rhabarbarum*	B	Poland	MG882689
R2	*Rheum rhabarbarum*	B	Poland	MG882690
-	*Rheum rhabarbarum*	B	USA	AY744930
DEU5	*Lactuca sativa*	B	Germany	AB701702
DEU5	*Lactuca sativa*	B	Germany	AB188979
UT	*Utricularia* sp.	B	Germany	AB701736
AU1	*Hirschfeldia incana*	B	Australia	AB989628
CZE2	*Armoracia rusticana*	B	Czech Republic	LC537537
NZ12	*Nasturtium officinale*	B	New Zealand	AB989645
PV389	*Tulipa gesnerana*	B	USA	AB701738
OM	*Orchis militaris*	(B)	Germany	AB701690
OMA	*Orchis militaris*	(B)	Germany	AB701691
OS	*Orchis simia*	(B)	Germany	AB701693
ORM	*Orchis morio*	(B)	Germany	AB701692
AUST23	*Rapistrum raphanistrum*	B(R)	Australia	AB989639
PV0104	*Lactuca sativa*	BR	Italy	AB093603
TIGD	*Tigridia* sp.	(B)	Germany	AB701735
ITA7	*Rapistrum raphanistrum*	BR	Italy	AB093600
Tu-2R1	*Japanese radish*	BR	Japan	AB105135

[(B)], [B], [B(R)], [BR] – the host types consistent with the phylogenetic groups

A positive Potyvirus test was obtained for symptomatic plants using an indirect enzyme-linked immunosorbent assay (ELISA). A subsequent direct ELISA test showed that the potyvirus-positive samples were infected with turnip mosaic virus (TuMV). RT-PCR was used to confirm the presence of TuMV. The PCR product of the expected size was amplified from all TuMV-infected plants and then sequenced. No amplification products were obtained from asymptomatic plants. The sequencing results of the PCR products confirmed that all symptomatic plants were infected with TuMV. Sequence reads showed 99.8–100% nucleotide identity. Two sequences (1,077 nt) that matched the TuMV coat protein gene and the 3'-terminal noncoding region were uploaded to the GenBank database (accession no. MG882689 and MG882690). These sequences showed 99.5 and 98.8% nucleotide and amino acid identity, respectively, and high identity with other TuMV isolates from the GenBank database. Phylogenetic analysis performed on the sequences of the coat protein (CP) coding gene of two Polish TuMV isolates from rhubarb and 43 sequences of other isolates from the Gene Bank showed low nucleotide and amino acid diversity of TuMV isolates from different hosts and geographical regions. The range of identities of the analyzed nucleotide sequences was 96.4 to 99.5% and 95.5 to 98.9% for amino acid sequences. The Polish isolates were grouped with the GBR7 isolate from rhubarb (AB701709) found in the UK (98% nucleotide identity). A TuMV isolate from rhubarb detected in Alaska (AY744930) (Robertson and Lanson 2005) is in a separate cluster and shows 96% nucleotide identity with isolates from Poland (Fig. 2). Oshima *et al.* (2002) studied a worldwide collection of about 100 TuMV isolates and showed that the virus has four phylogenetic lineages. The four host types are predominantly consistent with the phylogenetic groups. Isolates of type [(B)] sporadically infect *Brassica* plants, often latently, but not *Raphanus* plants. Type [B] isolates infect most *Brassica* species, giving systemic mosaic symptoms, but do not infect *Raphanus* plants. Type [B(R)] isolates cause systemic mosaicism in most *Brassica* species and sometimes infect *Raphanus* plants in a latent manner. Type [BR] isolates give systemic mosaic symptoms in *Brassica* and *Raphanus* plants. Based on the distribution in the phylogenetic tree, the Polish TuMV isolates

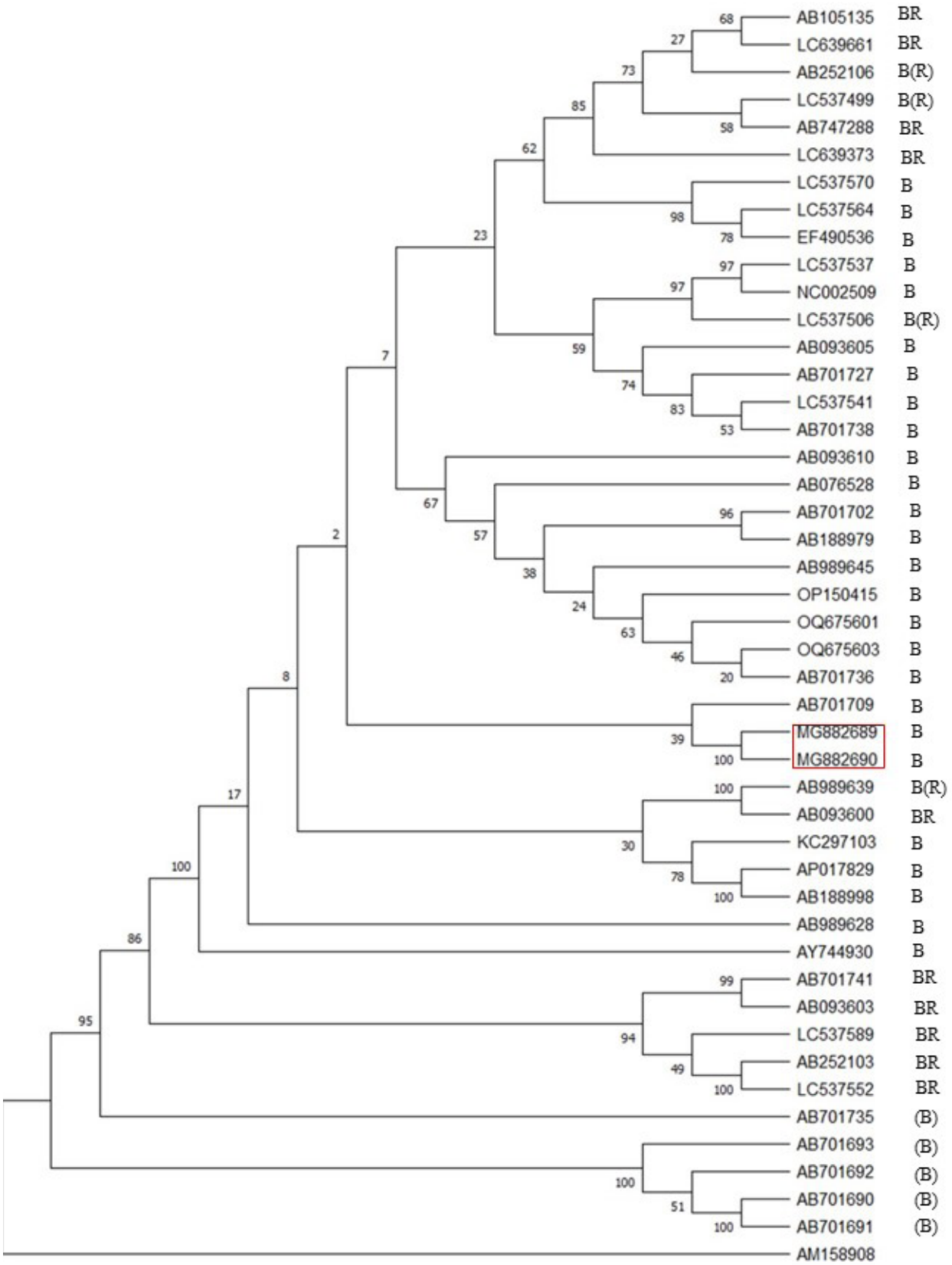

Fig. 2. A phylogenetic tree was constructed using the sequence of the gene encoding the TuMV coat protein of Polish rhubarb isolates (highlighted by a red box) and selected isolates from the GenBank. The sequence of narcissus yellow stripe virus 1 was used as an outgroup

and the other isolates from rhubarb belong to type [B], the most variable of the four main TuMV clusters. It includes isolates from cultivated and wild species of *Brassicaceae*, as well as from plants belonging to other families, mainly collected in Europe (Tomimura *et al.* 2004). Phylogeographic analysis of the entire potyvirus genus showed that the species of the genus, like TuMV, originated in western Eurasia and/or North Africa and probably evolved from a virus of monocotyledonous plants. All species of the two earliest diverging potyvirus lineages were first isolated from monocotyledonous plants that were originally domesticated in the same region (Simmonds 1976).

The presence of TuMV in rhubarb crops in Poland may not only affect the quality and quantity of the crop but also threaten other crops due to its possible spread by aphids. Knowledge of the genetic diversity of TuMV can be valuable for developing and improving methods to detect the broadest possible spectrum of virus isolates.

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