

## ORIGINAL ARTICLE

## Detection and genetic variability of newly identified dasheen mosaic virus in Poland

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### Abstract

Dasheen mosaic virus (DsMV) is one of the most important viral pathogens of aroids and can cause major economic losses for ornamental crops. Here, we present the detection and molecular characterisation of DsMV isolates originating from *Monstera adansonii* plants in Poland. Moreover, the genetic variability of DsMV isolates was analyzed based on the coat protein gene (CP) of the Polish and other DsMV isolates described to date. The presence of DsMV was confirmed by transmission electron microscopy (TEM) and reverse transcription polymerase chain reaction (RT-PCR) with specific, diagnostic primers in three out of ten examined plants. To obtain full-length sequences of CP, two pairs of primers were designed and used in the RT-PCR. The specificity of obtained products was confirmed by Sanger sequencing. The obtained sequences of CP were compared with 44 other DsMV sequences retrieved from the GenBank. Analyses revealed that DsMV population is very diverse. The variability of DsMV isolates was confirmed by low sequence identity and pervasive recombination events. The phylogenetic analysis was performed based on 37 non-recombinant CP sequences. The maximum-likelihood reconstruction revealed that the Polish isolates are distinct and grouped separately from other DsMV isolates. Due to the high genetic diversity, detecting the virus could be difficult. Nonetheless disease management relies strongly on a fast and accurate identification of the causal agent. To our knowledge this is the first report of DsMV in Poland.

**Keywords:** dasheen mosaic virus (DsMV), genetic variability, *Monstera adansonii*, RT-PCR

## Introduction

*Monstera adansonii* Schott., a member of the family *Araceae*, is a common ornamental plant, cultivated in many households worldwide. Unfortunately, *M. adansonii* plants can be infected by bacteria, fungi and viruses, causing changes in the leaves' phenotype, which significantly reduces their market value. In recent years, numerous viruses infecting aroids have been identified, such as: dasheen mosaic virus (DsMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV) and cucumber mosaic virus (CMV) (Zettler *et al.* 1970; Mokra and Gotzova 1994; Miura *et al.* 2013).

*Dasheen mosaic virus* (DsMV), belonging to the genus *Potyvirus*, family *Potyviridae*, was first detected in dasheen (taro; *Colocasia esculenta* (L.) Schott) in 1970 (Zettler *et al.* 1970) and since then has been

detected rapidly throughout the world (Babu *et al.* 2011; Wang *et al.* 2017; Escalante *et al.* 2021; Qin *et al.* 2021). The incidences of DsMV infection were recorded in many countries: Nicaragua, Ethiopia, the USA, Czech Republic, Bosnia and Herzegovina, Taiwan, Vietnam, China, India, Papua New Guinea, Samoa, Solomon Islands, French Polynesia and New Caledonia (Mokra and Gotzova 1994; Revill *et al.* 2005; Babu *et al.* 2011; Grausgruber-Groger *et al.* 2016; Kidanemariam *et al.* 2018; Escalante *et al.* 2021; Qin *et al.* 2021). Nowadays, DsMV is known as one of the most important aroid pathogens, infecting over 16 genera of edible (*Colocasia*, *Xanthosoma*, *Cyrtosperma*) and ornamental (*Aglaonema*, *Caladium*, *Dieffenbachia*, *Philodendron* and *Zantedeschia*) plants in the family *Araceae* (Nelson

2008). Nevertheless, the virus still poses the greatest threat to taro, an important crop in many tropical and subtropical countries (Chair *et al.* 2016). Depending on the host plant and season, the virus causes various disease symptoms in infected plants (Reville *et al.* 2005). The most common symptoms include: mottle, mosaic, chlorosis, stunting and leaf distortion (Wang *et al.* 2017; Escalante *et al.* 2021). DsMV is transmitted by several widely distributed aphid species, including *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover, in a non-persistent manner. The virus is also spread by mechanical inoculation and during vegetative reproduction with infected propagative material (Nelson 2008). Filamentous and flexible virus particles are 750 nm in length and contain positive sense single stranded (+ss) RNA genome. The virus genome (~10 kb) includes 5'- and 3'-untranslated regions (UTR), and one open reading frame (ORF), encoding a single polyprotein, which is cleaved into 10 proteins: protease (P1), helper component proteinase (HC-Pro), P3, 6 K1, cylindrical inclusion protein (CI), 6K2, viral protein genome-linked (VPg), serine-like cysteine protease (NIa-Pro), RNA-dependent RNA polymerase (NIb-RdRp), coat protein (CP) and additional protein PIPO-P3 encoded by small ORF embedded within P3 cistron (expressing via ribosomal frameshifting or transcriptional slippage at a highly conserved G(1-2)A(6-7) motif at the 5' end of PIPO). The presence of this protein was described for many potyviruses and is essential for virus intercellular movement (Chung *et al.* 2008). At the 5' end VPg is covalently attached, whereas 3' end is terminated with polyA tail (Chung *et al.* 2008; Kamala *et al.* 2015).

The CP protein of potyvirus contains three domains: N-domain (17-78 aa), core domain (213-218 aa, starting with KDK/D residues and ending with TER/H residues), C-domain (17-21 aa) (Yan *et al.* 2021). The most variable part of CP is the N-domain, where insertions and deletions occur the most frequently, causing differences in the length of nucleotide sequences (Pappu *et al.* 1994). Moreover, N-domain also contains highly conservative motif DAG, interacting with the PTK motif at C-terminus of Hc-Pro to facilitate binding of Hc-Pro to the CP of virions. At the N-terminus of Hc-Pro is also localized the KITC motif, which interacts with putative receptor in aphids stylets. Thanks to both of these interactions Hc-Pro enable creating a bridge between virion and aphids mouthpart. Therefore mutations within DAG motif can lead to inhibition of aphid transmission (Gadhav *et al.* 2020).

Previous research on DsMV focused on virus identification, distribution and development of detection methods. The phylogenetic analysis carried out by Wang *et al.* (2017) revealed that despite the high level of genetic diversity DsMV isolates grouped and clustered with ten other potyviruses, such as zucchini

yellow mosaic virus (ZYMV), passion fruit woodiness virus (PWV), hardenbergia mosaic virus (HarMV), bean common mosaic virus (BCMV), bean common mosaic necrosis virus (BCMNV), east asian passiflora virus (EAPV), wisteria vein mosaic virus (WVMV), watermelon mosaic virus (WMV), soybean mosaic virus (SMV), and calla lily latent virus (CLLV), forming one subgroup. In addition, recombination analysis showed the presence of recombinant variants and indicated that recombination occurred in the regions of P1, C1 to NIa-Pro and NIb to CP (Wang *et al.* 2017).

DsMV has not been reported in Poland before. However, during 2020–2021 several plants of *M. adansonii* showing symptoms of leaves mosaic and growth reduction were observed in commercial cultivation of ornamental plants. Here, we present the detection and partial molecular characterization of the Polish DsMV isolates. Moreover, the genetic variability of the DsMV population was established using isolates obtained in this study and others retrieved from the GenBank database.

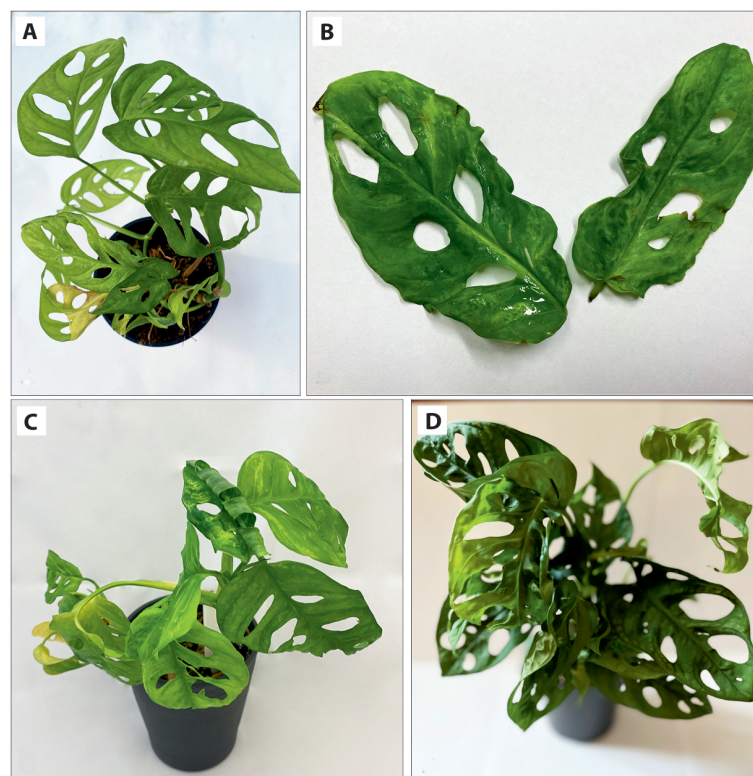
## Materials and Methods

### Transmission electron microscopy (TEM)

In 2020–2021 ten *M. adansonii* plants with virus-like symptoms such as bright green mosaic, mottle, leaves distortion and stunting, were collected (Fig.1). The plants were unrelated and came from different garden centers located in Wielkopolska region. All samples were analyzed using transmission electron microscope (TEM) (HT7700, Hitachi, Japan) to confirm virus infection. Fragments of leaves with visible symptoms were crushed in distilled water and obtained sap was applied to formvar coated copper grids (Polysciences, Warrington, UK). Then the grids were dyed with ammonium molybdate (MA) or phosphotungstic acid (PTA) and dried. The presence of the viral particles was examined by TEM at an accelerating voltage of 100 kV.

### RNA isolation and virus detection

Total RNAs were extracted from each plant sample using the phenol/chloroform procedure (Sambrook and Russell 2001) and the quality and quantity of obtained RNA were measured spectrophotometrically (Nanodrop 2000, Thermo Fisher Scientific, USA). The samples were tested for the presence of DsMV using DMV5708-5731F and DMV6131-6154R primers (Wang *et al.* 2017; Table 1). The reaction mixture consisted of 22  $\mu$ l of sterile water, 25  $\mu$ l of DreamTaq Green PCR Master Mix (2x) (Thermo Fisher Scientific, USA), 1  $\mu$ l of each DMV5708-5731F (10  $\mu$ M  $\cdot$   $\mu$ l<sup>-1</sup>)



**Fig. 1.** *Monstera adansonii* plants with visible symptoms of DsMV on leaves (A – isolate B2, B – isolate B1, C – isolate M1, D – non-infected, symptomatic plant)

**Table 1.** Primers used in reverse transcription polymerase chain reaction (RT-PCR)

Primer	Sequence 5'–3'	Melting temperature [°C]	Product size [bp]	Reference
DMV5708-5731F	CAGGCACATCAATTTTCTAAC	57	447	Wang <i>et al.</i> 2017
DMV6131-6154R	GGCTCCACACCARAAATGTGCACG			
DsMV9412F	GCATCACTTTTCTGACGC	49	439	designed in this study
DsMV9851R	GGGAAACCACTCTGTAGT			
DsMV8555F	GCAGCCATGATAGAAGCA	53	1124	
DsMV9679R	GTGCCTTTCAGTGTCTC			

and DMV6131-6154R ( $10 \mu\text{M} \cdot \mu\text{l}^{-1}$ ) primers,  $1 \mu\text{l}$  of RevertAid Reverse Transcriptase (Thermo Fisher Scientific, USA) and  $1 \mu\text{l}$  of total RNA. The RT-PCR reaction was carried out in thermal profile as previously described (Wang *et al.* 2017). Obtained PCR products were separated in 1% agarose gel with Midori Green dye (NIPPON GENETICS EUROPE, Germany) with HyperLadder100bp marker (Bioline, Great Britain). The PCR products of appropriate size were purified using GeneJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Fisher Scientific, USA), according to the manufacturer's protocol and sequenced in both directions via the Sanger method by an external company (Genomed S.A., Poland).

To obtain full-length *CP* gene sequences of DsMV two sets of primer pairs were designed (Table 1) using

OligoAnalyzer based on the set of DsMV sequences retrieved from GenBank. The RT-PCR reaction was optimized using designed primers in a T-Professional thermocycler with a temperature gradient block (Biometra, Göttingen, Germany). The reaction mixture was prepared as described above and followed the manufacturer's protocol. Primer combinations used to obtain particular *CP* sequences together with optimal annealing temperatures are presented in Table 1. Obtained RT-PCR products were separated in 1% agarose gel with Midori Green dye and GeneRuler Express DNA Ladder (Thermo Fisher Scientific, USA), purified using GeneJET Gel Extraction and DNA Cleanup Micro Kit, according to manufacturer's procedure and sequenced via the Sanger method (Genomed S.A., Poland).

## Phylogenetic and recombination analyses

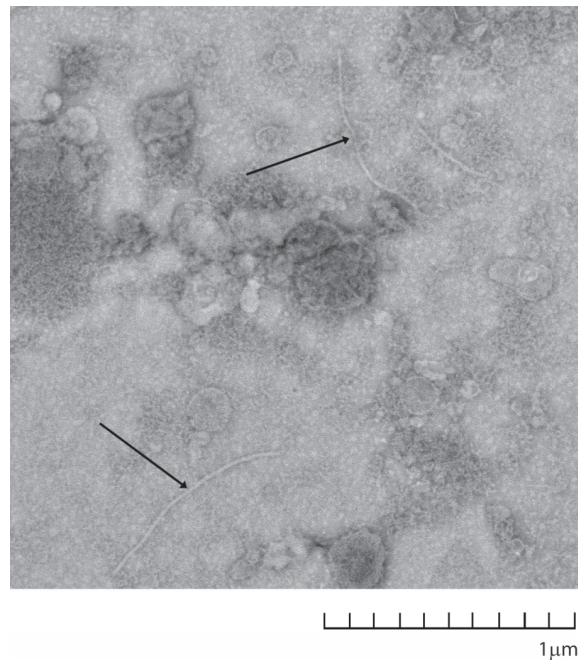
DsMV *CP* sequences of the Polish isolates were edited and compiled in BioEdit (Hall 1999) and deposited in GenBank under the following accession numbers: ON049418 (isolate M1), ON049419 (isolate B1) and ON049420 (isolate B2). Subsequently, obtained sequences were gathered together with 44 other DsMV *CP* sequences available in GenBank. The *CP* sequences were aligned using the MUSCLE algorithm as implemented in MEGA X (Kumar *et al.* 2018). Sequence identity matrices were prepared using BioEdit (Hall 1999) and Sequence Demarcation Tool Version 1.2 (SDTv1.2) (Muhire *et al.* 2014). Firstly, the presence of recombination events between DsMV isolates was examined using seven methods: RDP, GENECONV, Chimaera, MaxChi, BootScan, SiScan, 3 Seq as implemented in the RDP4 program (Martin *et al.* 2015). Default parameters and a *p*-value threshold of 0.05 were used. The recombination events were considered as significant if five or more methods had a *p* < 0.05. Next, selective pressure for individual sites was investigated using the Datamonkey Adaptive Evolution Server (Weaver *et al.* 2018) and four methods: Mixed Effects Model of Evolution (MEME), Fixed Effects Likelihood (FEL), Fast Unconstrained Bayesian Approximation (FUBAR), Single-Likelihood Ancestor Counting (SLAC), based on the ratio of nonsynonymous and synonymous substitutions ( $d_N/d_S$ ). Depending on the  $d_N/d_S$  value selection is considered to be diversifying ( $d_N/d_S > 1$ ), neutral ( $d_N/d_S = 1$ ) or purifying ( $d_N/d_S < 1$ ). The significance value for MEME, FEL and SLAC was set to *p* < 0.05 and for FUBAR according to the Bayesian approach, to posterior probability > 0.9.

Phylogenetic analysis was carried out using the maximum-likelihood method implemented in MEGA X with 37 non-recombinant full-length sequences of DsMV *CP* gene. The General Time Reversible and discrete Gamma-distribution (GTR+G) nucleotide substitution model was established as the most appropriate. The bootstrap values were counted using the 1000 pseudorandom replicates method. The sequence of konjack mosaic virus (KoMV, accession number: AB219545.1) was used as an outgroup. The phylogenetic tree was edited and visualized in Evolview online platform (Subramanian *et al.* 2019).

## Results

### Transmission electron microscopy (TEM)

During the initial virus identification in TEM, filamentous particles about 750 nm in length in three out of ten examined plants were found (Fig. 2). This type of particles is typical for members of the *Potyvirus* genus.



**Fig. 2.** Filamentous particles of DsMV observed in transmission electron microscopy (TEM). Arrows pointed to viral particles

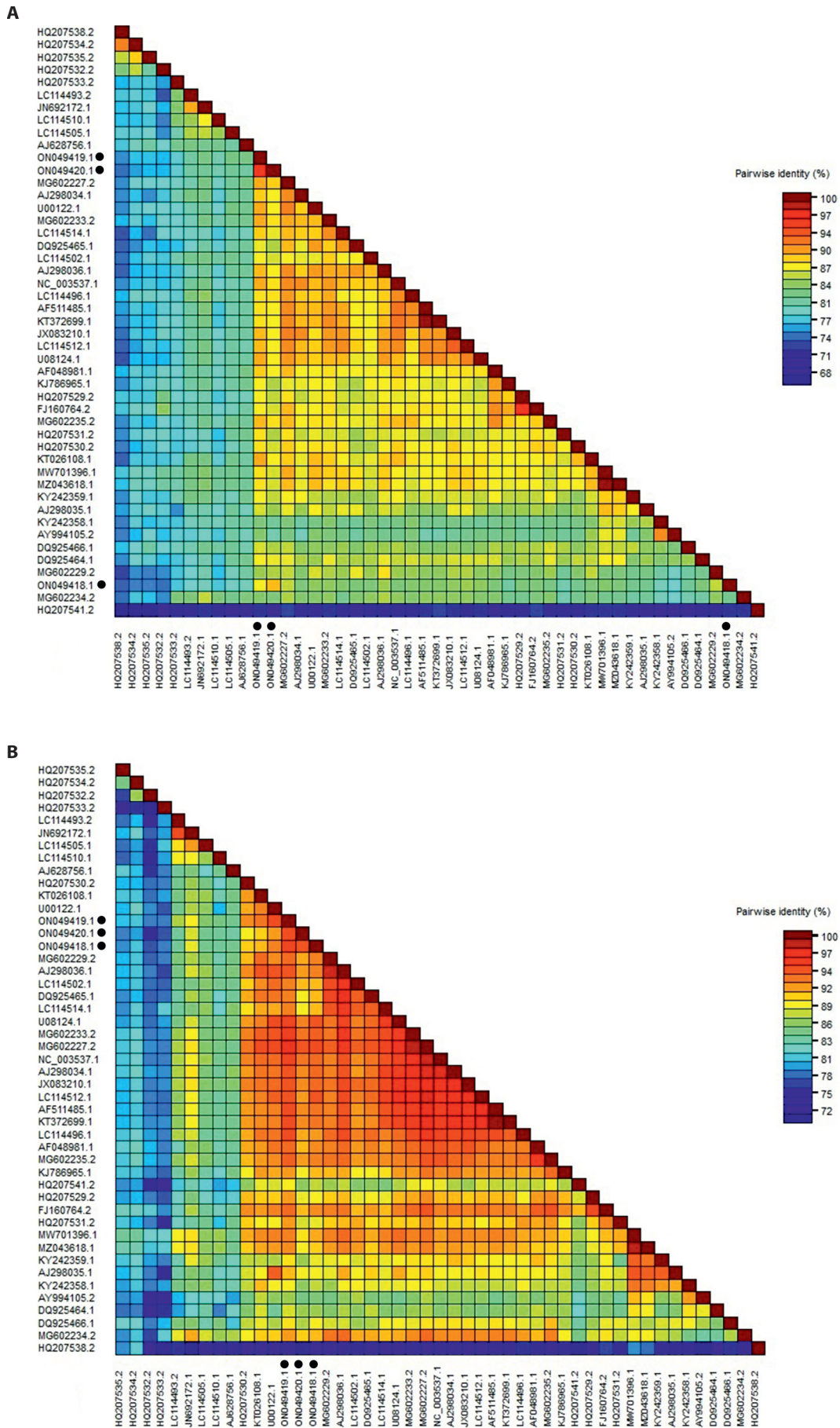
### Reverse transcription polymerase chain reaction (RT-PCR)

The presence of DsMV was confirmed in TEM-positive samples. RT-PCR with primers DMV5708-5731F and DMV6161-6154-R, resulted in products of appropriate size (447 bp). The full-length *CP* sequences of about 942 bp of DsMV were obtained with two pairs of specific primers (DsMV9412F and DsMV9851R; DsMV8555F and DsMV9679R) and used for further analyses (Table 1).

### Phylogenetic and recombination analyses

Comparison of obtained sequences with 44 DsMV *CP* sequences retrieved from GenBank revealed that sequence identity in analyzed population ranged from 61.3 to 99.5% and from 66.2 to 99% for nucleotide and amino acid sequences, respectively (Fig. 3). Sequence diversity of the Polish isolates varied from 4.5 to 12.7% for nucleotide and from 4.5 to 5.5% for amino acids sequences. Analyzed DsMV *CP* sequences were diverse in length from 855 to 1008 nt, resulting up to 51 aa differences in protein sequence size.

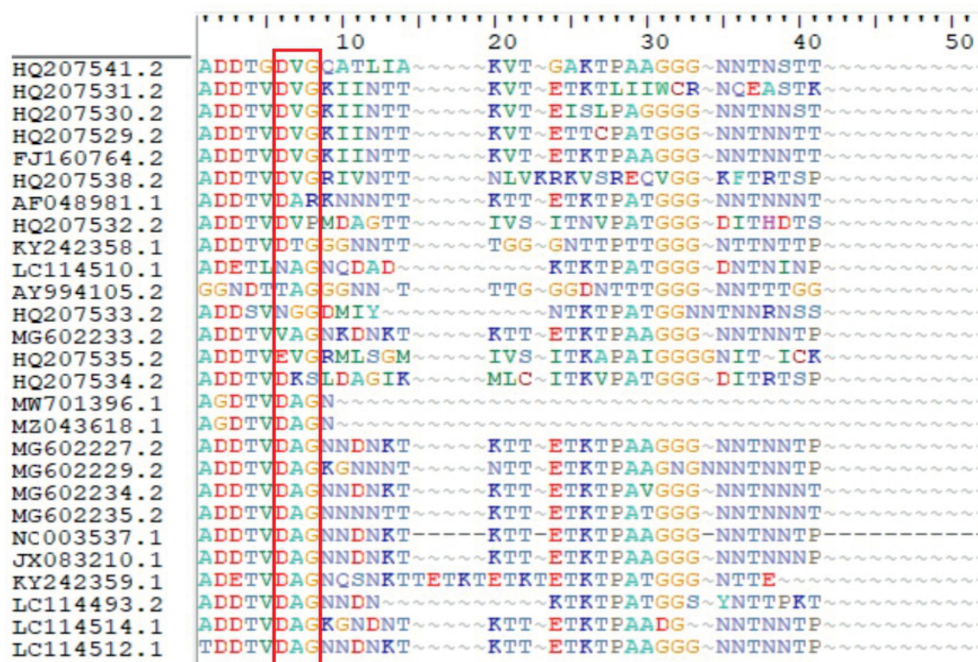
Most variations were localized within the asparagine rich N-terminal of *CP*, which also includes the carbohydrate *pfkB* type kinase domain. The DAG motif in the N-terminus of amino acids sequence of *CP* remained unchanged in 32 of the analyzed sequences. However, in 15 of the analyzed amino acids sequences the following DAG motif variations were observed: DAR, VAG, DTG, DKS, DVP, TAG, EVG, NGG, NAG, DVG. The most frequent were alanine substitution by



**Fig. 3.** Two dimensional visualization of nucleotide (A) and amino acids (B) CP gene sequence identity of 47 DsMV isolates examined in this study. The matrices were performed using SDTV1.2. Polish isolates marked by black dots

valine, leading to the formation of DVG variation instead of DAG motif, which were detected in six isolates from India (Fig. 4).

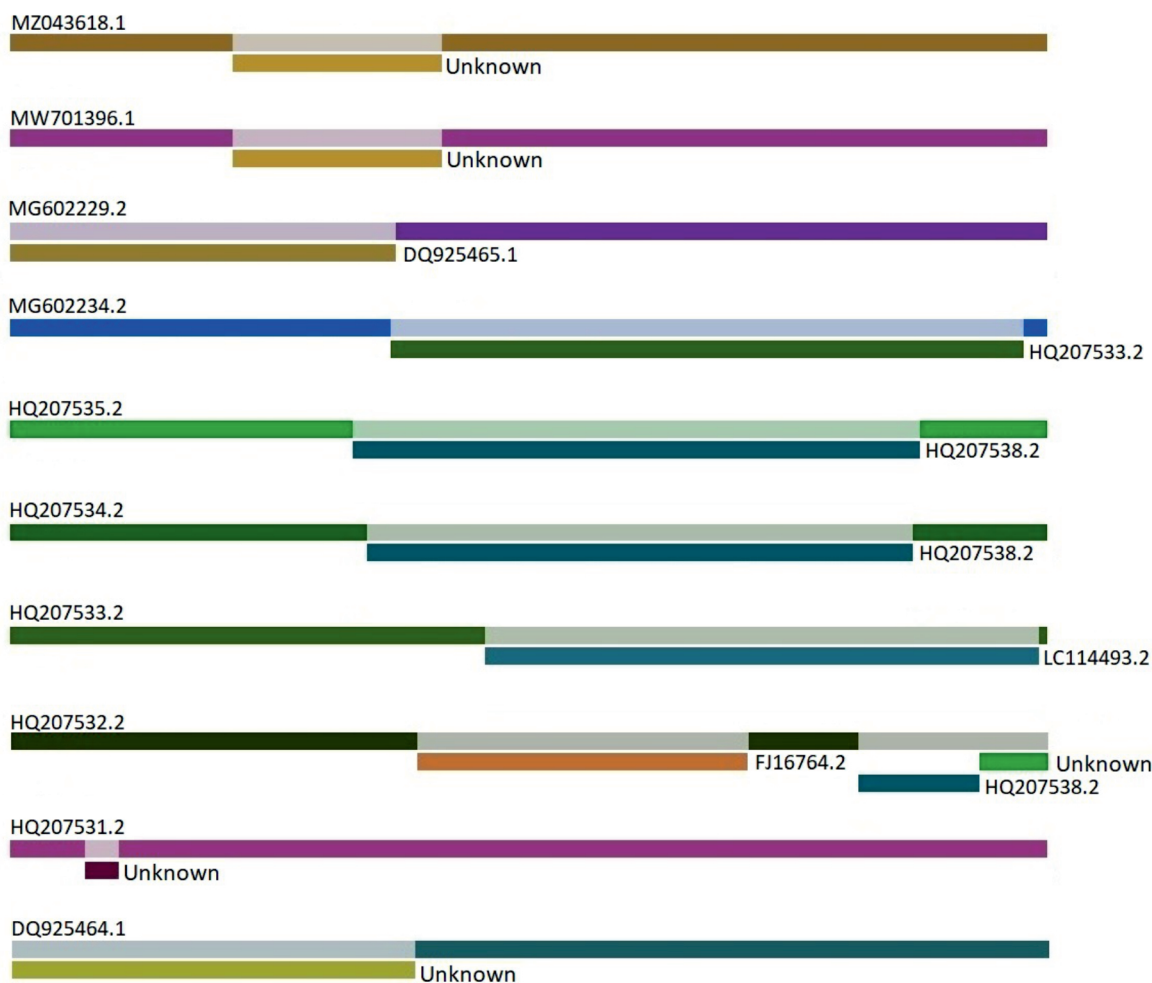
In the analyzed DsMV population the presence of 10 recombinant variants was confirmed (Table 2, Fig. 5). Identified recombinants originated from India



**Fig. 4.** Variations of DAG motif in N-domain of CP, occurred in analyzed population of DsMV, showed in alignment in BioEdit

**Table 2.** Recombinants detected by at least five methods in RDP4 program, using 44 nucleotide sequences of DsMV CP

Isolate name (accession number)	Region of origin/Host	Position with gaps/ without gaps [nt]	Parental isolate (accession number)	
			major	minor
BF39 (MZ043618.1)	China/ <i>Typhonomi giganteum</i> Engl.	236-459/ 44-213	TEN (U08124.1)	unknown
BF1 (MW701396.1)	China/ <i>Typhonomi giganteum</i> Engl.	236-459/ 44-213	TEN (U08124.1)	unknown
Et26 (MG602229.2)	Ethiopia/ <i>Colocasia esculenta</i>	1-412/ 1-253	unknown	DsMV-VN/Ce2 (DQ925465.1)
Tz34 (MG602234.2)	Tanzania/ <i>Xanthosoma</i> sp.	405-1078/ 246-919	Et26 (MG602229.2)	DsMV-Amp6 (HQ207533.2)
DsMV-Amp8 (HQ207535.2)	India/ <i>Amorphophallus paeoniifolius</i>	365-969/ 203-807	DsMV-Amp5 (HQ207532.2)	DsMV-Amp11 (HQ207538.2)
DsMV-Amp7 (HQ207534.2)	India/ <i>Amorphophallus paeoniifolius</i>	365-969/ 203-807	DsMV-Amp5 (HQ207532.2)	DsMV-Amp11 (HQ207538.2)
DsMV-Amp6 (HQ207533.2)	India/ <i>Amorphophallus paeoniifolius</i>	506-1097/ 344-932	unknown	Ds01 (LC114493.2)
		434-786/ 272-624	unknown	DsMV-Amp1 (FJ160764)
DsMV-Amp5 (HQ207532.2)	India/ <i>Amorphophallus paeoniifolius</i>	904-1032/ 742-870	DsMV-Amp2 (HQ207529.2)	DsMV-Amp11 (HQ207538.2)
		1033-1105/ 871-939	DsMV- Amp7 (HQ207534.2)	unknown
DsMV-Amp4 (HQ207531.2)	India/ <i>Amorphophallus paeoniifolius</i>	81-121/ 63-100	DsMV-Ch (AF048981.1)	DK (AJ298035.1)
DsMV-VN/Ce1 (DQ925464)	Vietnam/ <i>Colocasia esculenta</i>	1-430/ 1-337	Ds20 (LC224512.1)	unknown



**Fig. 5.** Recombinants detected by at least five methods in RDP4 program, using 44 nucleotide sequences of DsMV CP, visualized in RDP4 program, with possible recombination sites and minor parent name

(DsMV-Amp8, DsMV-Amp7, DsMV-Amp6, DsMV-Amp5, DsMV-Amp4), China (BF39, BF1), Vietnam (DsMV-VN/Ce1), Ethiopia (Et26) and Tanzania (Tz34). Therefore, the recombinant variants were excluded from further analyses.

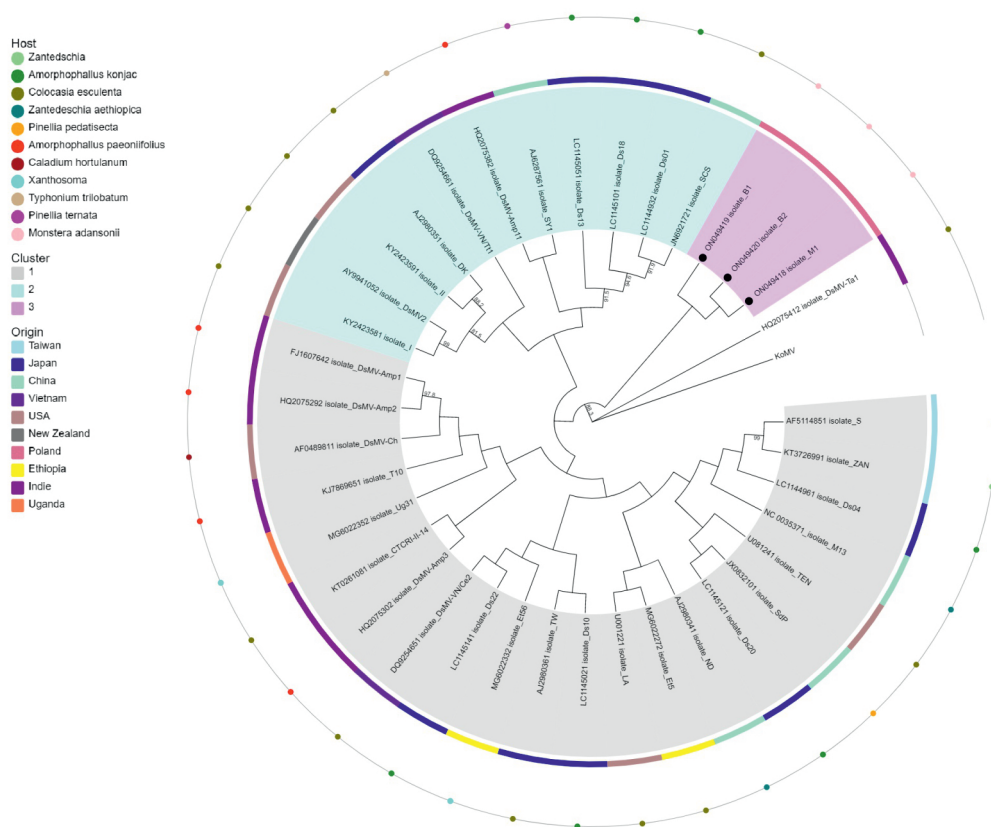
The selection pressure analysis showed 271 negatively selected sites, from which 203 codons were confirmed by three methods (FUBAR, FEL, SLAC), 19 codons by two methods (FUBAR and FEL) and 49 only by one method (48 by FUBAR and 1 by FEL). No evidence of positive selection pressure was confirmed by more than one method.

The phylogenetic analysis revealed the presence of two groups of isolates originating from various countries in Asia, Australia, Africa and North America, and a third containing only the Polish isolates. Isolates derived from *Amorphophalus konjack* Koch., *A. paeoniifolius* (Dennst.) Nicolson., *C. esculenta* occurred in both first and second group. Group 1 contained also isolates obtained from *Zantedeschia* sp. Spreng., *Z. aethiopica* (L.) Spreng., *Pinellia pedatisecta* Schott., *Xanthosoma* sp. Schott, *Caladium hortulanum*

Bridsey., which did not occur in group 2, containing additionally isolates derived from *Typhonium trilobatum* (L.) Schott. and *Pinellia ternata* (Thunb.) Makino. Isolate DsMV Ta-1 (HQ207541.1) originating from India and isolated from *C. esculenta* created a separate cluster, however other Indian isolates derived from *C. esculenta* grouped in the first and second clusters. Isolates derived from *M. adansonii*, created distinct group (Fig. 6).

## Discussion

The *Araceae* family is the third largest family of monocots, containing over 3.6 thousand species, ten of them (*Calla palustris* L., four species of the *Arum* genus, four species of *Lemna* genus and *Spirodela polyrrhiza* L. Schleid.) naturally occurring in Poland (Croat and Ortiz 2020; Govaerts et al. 2022). Moreover, many aroids are ornamental plants cultivated worldwide. In 2019, the European Union flower and ornamental plants



**Fig. 6.** Phylogenetic tree created in MEGA X, using GTR+G nucleotide substitution model. Information about host, region of origin and clusters were placed in the tree and explained in legend. Polish isolates, obtained in this study, are marked by black dots

market was valued at 20.618 billion euros. The country well-known from its large contribution to ornamental plants production is the Netherlands (market value: 6.717 billion euros) but other European countries including Poland (market value: 148 million euros) are also involved in global market trade (EUROSTAT 2020). Pathogens, causing visible leaf symptoms, such as DsMV can seriously threaten efficient production of ornamental plants, triggering significant economic losses. DsMV also infects edible aroids, such as taro cultivated in Africa, Asia, Oceania and Americas affecting corms quality and quantity, with production losses ranging from 25% up to 50% (Reyes *et al.* 2006; Rashmi *et al.* 2018; Otekunrin *et al.* 2021).

Here, we report the first detection of DsMV infecting *M. adansonii* plants in Poland. Infected plants displayed mosaic and leaf malformation as well as growth reduction. In our study only in three examined plants DsMV infection was confirmed. The observed phenotype changes of the remaining plants could be caused by physiological disorders determined by nutrient deficiencies or unfavorable growing conditions. Our research confirmed previous reports regarding high diversity of DsMV population (Pappu *et al.* 1994). The analysis of CP sequences of the Polish and other isolates described to date revealed that CP size varied from 855 to 1008 nt, inducing up to 51 aa in protein

sequence size. Those differences are caused by insertions and deletions occurring mainly in N-domain of CP. The N-domain also included many point synonymous and non-synonymous mutations, even within the highly conservative motif DAG, which changes can lead to inhibition of aphid transmission (Gadhav *et al.* 2020). In some cases nucleotide and amino acid sequence variability is below the species differentiation criteria for the genus *Potyvirus* (76 and 82% for nucleotide and amino acids, respectively; Adams *et al.* 2005). Spathiphyllum chlorotic vein banding virus (SCVbV), closely related to DsMV was detected and proposed as distinct potyvirus species (Padmavathi *et al.* 2011). The opposite situation was described for vanilla mosaic virus (VanMV), which was firstly considered as a distinct species but further research allowed for its classification as a DsMV strain that exclusively infects *Vanilla fragrans* Ames. (Wang and Pearson 1992; Farreyrol *et al.* 2006). It indicates that further studies are required regarding DsMV differentiation and adaptation to different hosts.

Our results confirmed that DsMV population is being shaped by recombination (Wang *et al.* 2017; Kidanemariam *et al.* 2021). Recombination is an important evolutionary factor involved in plant virus populations genetic variation and evolution. It has been previously shown that the C1 and CP regions



are recombinant hotspots in potyviruses (Wang *et al.* 2017). The recombination events between DsMV isolates originating from China and the USA might be correlated with increasing global trade of plant material. The recombination in DsMV population may lead to high levels of genetic diversity and emergence of new variants. The recombination has also been associated with the adaptation to new hosts, the emergence of new viral variants or even viruses and increases in virulence and pathogenesis (Pérez-Losada *et al.* 2015; Moury and Desbiez 2020). The selection pressure analysis of DSMV CP gene revealed an excess of synonymous over nonsynonymous diversity, indicating a relatively moderate negative selection. Many codons in the CP sequence are under negative selection also those localized in the beginning of the N-domain containing DAG motif region, which is responsible for aphids transmission. These results are consistent with those obtained by Kidanemariam *et al.* (2021). Strong purifying selection is often observed for functional proteins where the majority of amino acids are largely invariable due to strong structural and functional constraints. Moreover, it is expected that purifying selection concern codons, which changing may have effect on virus properties and may result in elimination of mutated genetic variants from the virus population. CP is involved in virion formation and vector transmission therefore it can be more variable and may assist these viruses in infecting a range of host plants in different agro-ecological zones (Nigam *et al.* 2019).

Phylogenetic analysis of the CP gene carried out using 37 non-recombinant DsMV sequences revealed that the Polish isolates originating from *M. adansonii* are distinct and form a separate clade. Previous studies conducted by Wang *et al.* (2017) and Kidanemariam *et al.* (2021) indicated that DsMV isolates grouped into two clades and groupings were not correlated with the geographical origin however, there was some correlation with the host. Further studies using larger data sets are required to analyze the host-associate traits.

In summary, the presence of DsMV has been confirmed for the first time in Poland. Due to the common presence of the virus vector: *M. persicae* and *A. gossypii* (Wojciechowski *et al.* 2015) and effective mechanical transmission during vegetative reproduction, the virus may pose a serious threat to the production of ornamental plants in Poland. This finding is important for producers and national agricultural research services. However, the high level of genetic variability of the DsMV population can have an impact on the development of efficient and reliable diagnostic tools. Therefore, further research is required regarding both occurrence, host range and evolutionary dynamics of DsMV in Poland.

## Acknowledgements

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