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Characterisation of strawberry mild yellow edge virus isolates detected for the first time in Poland

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Abstract: Strawberry mild yellow edge virus (SMYEV) was detected in 116 samples out of 423 collected from strawberry plants grown in commercial and experimental plantations in seven provinces of Poland. The number of samples infected with strawberry mottle virus (SMoV) accounted for 84.6% of the 26 SMYEV-positive samples selected for sequence analysis. The nucleotide sequence similarity of the coat protein (CP) gene of 26 selected SMYEV isolates ranged from 84.8% to 100%, and 81.4–99.5% identity was found between these isolates and 48 SMYEV strains from different countries. The CP region's phylogenetic analysis showed that most isolates from Poland clustered within group I (type D74). In contrast, Talis and 3233CL isolates represented group III (type MY18), and the San isolate was clustered in group V (type ABY1-01). Recombination analysis of the CP gene sequences detected two possible recombination events. One was noticed in the Argentinian strain 53, which formed group III with isolates from Chile, and Polish isolates Talis and 3233CL. Another was identified in the Chinese strain sy02 sequence with evidence of the same recombination event in Canadian strains, and the Polish isolate San (V group). Leaf epinasty, mottling, and yellowing of the young leaves and dieback of the older leaves were observed on Fragaria vesca 'Alpine' and 'EMC' indicator plants grafted with leaves of strawberry plants co-infected with SMYEV and SMoV. A single infection with SMYEV induced milder symptoms based on these indicators.

Keywords: *Fragaria*; SMYEV; SMoV; SCrV; SVBV; sequence analysis; phylogeny; recombination; diversity

More than 30 viruses have been detected in strawberries. Infection with one of them is usually latent or induces mild symptoms, but coinfection with two or more viruses often leads to severe symptoms (Martin & Tzanetakis 2006). Strawberry mild yellow edge virus (SMYEV) is one of the most common viruses infecting *Fragaria* sp. (Converse et al. 1987). Its natural hosts are also *Rubus rosifolius*, and *Chenopodium* spp. (Verchot-Lubicz & Baulcombe 2011).

SMYEV is classified as a Potexvirus genus in the *Alphaflexiviridae* family (Jelkmann et al. 1990; Thompson & Jelkmann 2004). Its genome consists of a single-stranded, positive-sense RNA genome (+ssRNA) of ~6.0 kb long with a 3' polyA tail (Jelkmann et al. 1992). Occurrence of SMYEV has been reported in North America, Europe, South Africa, Australia, New Zealand (Converse et al. 1987; Thompson & Jelkmann 2004), South America (Conci et al. 2009; Lavandero et al. 2012), Japan, Egypt (Dickinson et al. 2017), Mexico (Silva-Rosales et al. 2013), and Asia (Cho et al. 2011). SMYEV has not been detected in Poland yet. It is naturally transmitted in a semi-persistent manner mainly by *Chaeto-*

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siphon fragaefolii and also by *C. thomasi*, and *C. minor* strawberry aphids (Frazier 1975; Krczal 1979; Converse et al. 1987). SMYEV often causes asymptomatic infection in strawberry plants but produces dwarfing, marginal chlorosis, leaf distortion, and small fruits in some sensitive cultivars (Martin & Tzanetakis 2006). The virus often infects strawberries alongside other aphid-transmitted viruses such as strawberry mottle virus (SMoV), strawberry crinkle virus (SCrV), and strawberry vein banding virus (SVBV). These coinfections can lead to stunted growth, leaf deformation, marginal chlorosis, yellowing, and fruit yield losses of up to 30% (Martin et al. 1998). Some strawberry varieties significantly decline when infected with SMYEV and other viruses (Martin & Tzanetakis 2006). In the past, SMYE disease was diagnosed by grafting onto sensitive indicator plants such as *Fragaria vesca* clones UC-4 and UC-5, and *F. vesca* var. *semperflorens* 'Alpine' (Martin & Tzanetakis 2006). SMYEV can also be detected by enzyme-linked immunosorbent assay (ELISA) test (Jawee & Adams 1995; Quail et al. 1995) and serologically specific electron microscopy (Spiegel et al. 1986). Sequencing of the MY-18 strain (Jelkmann et al. 1992; Lamprecht & Jelkmann 1997) enabled the development of RT-PCR for SMYEV detection (Thompson & Jelkmann 2004). Further studies led to the development of several RT-PCR variants, including IC-RT-PCR (Kadeb-Kreuziger et al. 1995; Conci et al. 2009), multiplex RT-PCR (Thompson et al. 2003) and most recently reverse transcription recombinase polymerase amplification combined with lateral flow strip, SMYEV-RT-RPALF (Zou et al. 2022).

This study aimed to characterise the coat protein gene of strawberry mild yellow edge virus isolates detected in experimental and commercial plantations in seven provinces of Poland.

MATERIAL AND METHODS

Plant material. Strawberry samples were collected from 14 commercial and five experimental plantations in seven provinces of Poland. Eleven plantations were located in Łódzkie (including five experimental fields), three in Kujawsko-Pomorskie, two in Mazowieckie, and Wielkopolskie provinces, and one plantation from Małopolskie, Lubelskie, and Lubuskie provinces. Four hundred twenty-three leaf samples, including 237 from experimental fields and 186 from commercial plantations, were collected for testing on SMYEV presence.

ELISA. DAS-ELISA was applied for the screening tests for SMYEV presence in 86 samples from experimental field no. 1 and 17 from commercial plantation in Łódzkie province using SMYEV test kit (Agdia Inc., USA), according to the manufacturer's instructions.

Nucleic acid extraction and RT-PCR. Total nucleic acids were extracted from approximately 0.3 g of leaf tissue using the silica capture (Boom et al. 1990). Samples were collected from 320 strawberry plants, including 151 from experimental fields (Nos 2–5) in Łódzkie province and 169 samples from commercial plantations located in Łódzkie, Kujawsko-Pomorskie, Mazowieckie, Wielkopolskie, Małopolskie, Lubelskie, and Lubuskie provinces. One-step reverse transcription-polymerase chain reaction (RT-PCR) was performed using SMYEV-specific primers SYEupstcp1a 5' CCGCTGCAGTTGTAGGGTA 3' and SYEPolyTb 5' TTTTTTTTTTTTTTTAA-GGAAAAAGAAAAACAAAC 3' (Thompson & Jelkmann 2004) following the manufacturer's protocol (EURx Sp. z o. o., Poland), to amplify the entire coat protein (CP) gene and flanking region. Reverse transcription was carried out at 50 °C for 30 min. PCR cycling conditions included an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 1 min, with a final elongation at 72 °C for 5 min. RT-PCR products were analysed by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, and visualised under UV light.

The samples positive for SMYEV were also tested for strawberry mottle virus (SMoV), strawberry vein banding virus (SVBV), and strawberry crinkle virus (SCrV) using primers Smdetncr4a/Sm2ncr1b (Thompson et al. 2003) specific for SMoV, SVBV-deta/SVBVdetb (Thompson et al. 2003) for SVBV, and primers MKC-F/MKC-R (Klerks et al. 2004) for detection of SCrV.

DNA sequencing, analysis of nucleotide sequences and phylogenetic analysis. RT-PCR products for 26 selected samples (Table 1) were sequenced with the primer pair SYEupstcp1a/SYE-PolyTb in both directions. The obtained sequences were assembled and analysed using DNAStar's Lasergene software (version 7.0), then compared with sequences available in GenBank using the program BLAST (http://ncbi.nlm.nih.gov/BLAST/). Multiple nucleotide alignments of detected isolates with se-

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Table 1. Isolates of strawberry mild yellow edge virus included in this study

Host plant species, cultivar	Isolate	GenBank accession No.	Geographic origin
Fragaria × *ananassa,* Granat	Grnt4	MT080940	Poland, Łódzkie, EF2*, this study
Fragaria × *ananassa,* clone	Clone37	MT080941	Poland, Łódzkie, EF3, this study
Fragaria × *ananassa*, clone	Clone2	MT080942	Poland, Łódzkie, EF4, this study
Fragaria × *ananassa,* Grandarosa	Grand4	MT080943	Poland, Łódzkie, EF1, this study
Fragaria × *ananassa*, unknown	3233CL	MT080944	Poland, Lubuskie, CP**, this study
Fragaria × *ananassa*, San Andreas	San	OR044009	Poland, Łódzkie, CP, this study
Fragaria × *ananassa*, Malwina	Mal	OR044010	Poland, Łódzkie, CP, this study
ragaria × *ananassa*, clone	HP1501	OR044011	Poland, Mazowieckie, CP, this study
Fragaria × *ananassa*, Darselect	Dar	OR044012	Poland, Łódzkie, CP, this study
Fragaria × *ananassa*, Azja	AzWil	OR044013	Poland, Mazowieckie, CP, this study
Fragaria × *ananassa*, Azja	Azja	OR044014	Poland, Mazowieckie, CP, this study
Fragaria × *ananassa*, Albion	Alb	OR044015	Poland, Łódzkie, CP, this study
Fragaria × *ananassa*, Grandarosa	Grand3	OR044016	Poland, Łódzkie, EF1, this study
Fragaria × *ananassa*, Grandarosa	Grand5	OR044017	Poland, Łódzkie, EF1, this study
Fragaria × *ananassa,* clone	Clone15	OR044018	Poland, Łódzkie, EF3, this study
Fragaria × *ananassa*, clone	HP1548	OR044019	Poland, Łódzkie, CP, this study
Fragaria × *ananassa*, Azja	AzKar	PV535680	Poland, Łódzkie, CP, this study
Fragaria × *ananassa*, clone	Clone3	PV535681	Poland, Łódzkie, CP, this study
Fragaria × *ananassa*, clone	Clone6	PV535682	Poland, Łódzkie, CP, this study
Fragaria × *ananassa*, Dominika	Domin	PV535683	Poland, Łódzkie, EF1, this study
Fragaria × *ananassa*, Grandarosa	Grand2	PV535684	Poland, Łódzkie, EF1, this study
Fragaria × *ananassa*, Grandarosa	Grand7	PV535685	Poland, Łódzkie, EF4, this study
Fragaria × *ananassa,* Marmolada	Marm	PV535686	Poland, Łódzkie, EF5, this study
Fragaria × *ananassa*	NN141114	PV535687	Poland, Małopolskie, CP, this study
Fragaria × *ananassa*, Olimp	Olimp	PV535688	Poland, Mazowieckie, CP, this study
Fragaria × *ananassa*, Talisman	Talis	PV535689	Poland, Łódzkie, CP, this study
Fragaria × *ananassa*	D/L.14	AJ577348	Germany
Fragaria × *ananassa*	D/V.180	AJ577354	Germany
Fragaria × *ananassa*	D/M.110	AJ577352	Germany
Fragaria × *ananassa*	D/K.159	AJ577353	Germany
Fragaria × *ananassa*	D/L.19	AJ577349	Germany
Fragaria × *ananassa*	D/L.9	AJ577344	Germany
Fragaria × *ananassa*	D/L.13	AJ577347	Germany
Fragaria × *ananassa*	D74	AJ577359	Germany
Fragaria × *ananassa*	314CP2cav	AJ577351	Italy
Fragaria vesca, clone UC5	IndukaA	AJ577356	Czech Republic
Fragaria vesca, clone UC5	IndukaB	AJ577357	Czech Republic
Fragaria × *ananassa*	69N	AJ577350	Belgium
Fragaria chiloensis	1CH	AJ577337	Chile
Fragaria chiloensis	2CH	AJ577338	Chile
Fragaria chiloensis	3CH	AJ577339	Chile
Fragaria chiloensis	4CH	AJ577340	Chile
Fragaria × *ananassa*	36-2-4-6	KP284158	Argentina

*(EF 1–5) – experimental field nos. 1–5; **CP – commercial plantation

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Table 1. to be continued...

Host plant species, cultivar	Isolate	GenBank accession No.	Geographic origin
Fragaria × *ananassa*	15	KP284154	Argentina
Fragaria × *ananassa*	16.II	KP284155	Argentina
Fragaria × *ananassa*	264	KP284160	Argentina
Fragaria × *ananassa*	13-13-4	KP284153	Argentina
Fragaria × *ananassa*	16.04.2005	KP284156	Argentina
Fragaria × *ananassa*	53	KP284159	Argentina
Fragaria × *ananassa*	36-1-3	KP284157	Argentina
Fragaria × *ananassa*	13.02.2005	KP284152	Argentina
Fragaria × *ananassa*	16.I	KP284162	Argentina
Fragaria × *ananassa*	Berra-2	KX150372	Argentina
Fragaria × *ananassa*	20	KP284161	Argentina
Fragaria × *ananassa*	9Redland	AJ577345	Australia
Fragaria × *ananassa*	1182-53F	AJ577355	USA
Fragaria × *ananassa*	MY18	D12517	USA
Fragaria × *ananassa*	WSU1988	AJ577358	USA
Fragaria × *ananassa*	MH	MK040457	South Korea
Fragaria × *ananassa*	KNS1	EU284709	South Korea
Fragaria × *ananassa*	SH	MG418838	South Korea
Fragaria × *ananassa*	sy01	AY955375	China
Fragaria × *ananassa*	sy02	EU107084	China
Fragaria × *ananassa*	sy03	EU107085	China
Fragaria × *ananassa*	sy04	EU107086	China
Fragaria × *ananassa*	BJZJ	MT076195	China
Fragaria × *ananassa*	ZJZP	MT076196	China
Fragaria × *ananassa*	FJ1	OK562580	China
Fragaria × *ananassa*	NS26	KR559736	Canada
Fragaria × *ananassa*	AB5-2	KR707814	Canada
Fragaria × *ananassa*	AB41-01	KR350470	Canada
Fragaria × *ananassa*	NB1165	KR559735	Canada
Fragaria × *ananassa*	QC3v1	MN173712 LC515235	Canada
Fragaria × *ananassa*	T1	–	Japan

*(EF 1–5) – experimental field nos. 1–5; **CP – commercial plantation

lected sequences of SMYEV strains were conducted using the program ClustalW (Thompson et al. 1994).

Phylogenetic relationships of the analysed isolates were established using the neighbour joining method with the Tamura 3-parameter model and MEGA software (version 11.0.13) (Tamura et al. 2021). The statistical significance was estimated using a bootstrap test with 1 000 replicates. The sequences of the CP gene of the detected isolates and SMYEV strains from different hosts and geographical regions (Table 1) were used to construct the phylogenetic tree.

Recombination analysis. Recombination analysis was conducted using nine detection methods (RDP, GENECONV, BootScan, Maxchi, Chimaera, SiSscan, 3Seq, LARD, and Phylpro) included in the Recombination Detection Program 4 (RDP4) software (Martin et al. 2015). Only putative recombination events were accepted when detected by four or more different recombination detection methods with a *P*-value cut-off of 0.05.

Leaf grafting onto indicator plants. Selected from 12 strawberry plants, positively tested samples were indexed by grafting onto petioles of in-

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Table 2. Pairwise genetic distances and percentage identities between aligned coat protein gene sequences of SMYEV isolates. Values above the diagonal represent % identity, and values below represent evolutionary distance

Percent identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
1		87.1	99.3	98.5	98.5	98.2	98.4	98.4	98.2	98.4	98.2	98.4	98.4	99.6	98.4	99.6	98.4	86.7	85.7	98.2	98.2	98.4	98.8	97.8	97.8	99.7	1	AzWil
2	14.3		87.1	86.6	86.6	86.0	87.0	87.0	86.8	87.0	86.8	87.0	87.0	86.7	87.0	86.8	87.0	98.2	86.0	86.8	86.8	87.0	86.4	86.7	87.0	86.8	2	3233CL
3	0.7	14.3		98.9	98.9	98.6	98.5	98.5	98.4	98.5	98.4	98.5	98.5	99.2	98.5	99.2	98.5	86.7	85.6	98.4	98.4	98.5	98.9	98.2	98.2	99.3	3	ALB
4	1.5	15.0	1.1		100.0	98.4	98.2	98.2	98.1	98.2	98.1	98.2	98.2	98.6	98.2	98.9	98.2	86.1	85.3	98.1	98.1	98.2	98.6	98.4	97.7	98.5	4	Azja
5	1.5	15.0	1.1	0.0		98.4	98.2	98.2	98.1	98.2	98.1	98.2	98.2	98.6	98.2	98.9	98.2	86.1	85.3	98.1	98.1	98.2	98.6	98.4	97.7	98.5	5	AzKar
6	1.5	15.4	1.1	1.4	1.4		97.9	97.9	97.8	97.9	97.8	97.9	97.9	98.5	97.9	98.6	97.9	85.6	85.0	97.8	97.8	97.9	98.6	97.9	97.7	98.2	6	DAR
7	1.7	14.5	1.5	1.8	1.8	1.8		100.0	99.9	100.0	99.6	100.0	100.0	98.5	100.0	98.8	100.0	86.6	84.9	99.6	99.6	100.0	98.6	97.7	97.3	98.4	7	Clone2
8	1.7	14.5	1.5	1.8	1.8	1.8	0.0		99.9	100.0	99.6	100.0	100.0	98.5	100.0	98.8	100.0	86.6	84.9	99.6	99.6	100.0	98.6	97.7	97.3	98.4	8	Clone15
9	1.7	14.5	1.5	1.8	1.8	1.8	0.0	0.0		99.9	99.5	99.9	99.9	98.4	99.9	98.6	99.9	86.4	84.9	99.5	99.5	99.9	98.5	97.5	97.3	98.2	9	Clone37
10	1.7	14.5	1.5	1.8	1.8	1.8	0.0	0.0	0.0		99.6	100.0	100.0	98.5	100.0	98.8	100.0	86.6	84.9	99.6	99.6	100.0	98.6	97.7	97.3	98.4	10	Grand5
11	1.8	14.7	1.7	2.0	2.0	2.0	0.4	0.4	0.4	0.4		99.6	99.6	98.4	99.6	98.6	99.6	86.4	84.9	99.5	99.5	99.6	98.5	97.5	97.1	98.2	11	Domin
12	1.7	14.5	1.5	1.8	1.8	1.8	0.0	0.0	0.0	0.0	0.4		100.0	98.5	100.0	98.8	100.0	86.6	84.9	99.6	99.6	100.0	98.6	97.7	97.3	98.4	12	Grand2
13	1.7	14.5	1.5	1.8	1.8	1.8	0.0	0.0	0.0	0.0	0.4	0.0		98.5	100.0	98.8	100.0	86.6	84.9	99.6	99.6	100.0	98.6	97.7	97.3	98.4	13	Grand3
14	0.4	14.8	0.8	1.4	1.4	1.2	1.5	1.5	1.5	1.5	1.7	1.5	1.5		98.5	99.7	98.5	86.3	85.3	98.4	98.4	98.5	98.9	97.9	97.7	99.6	14	MAL
15	1.7	14.5	1.5	1.8	1.8	1.8	0.0	0.0	0.0	0.0	0.4	0.0	0.0	1.5		98.8	100.0	86.6	84.9	99.6	99.6	100.0	98.6	97.7	97.3	98.4	15	GRNT4
16	0.4	14.7	0.8	1.1	1.1	1.1	1.2	1.2	1.2	1.2	1.4	1.2	1.2	0.3	1.2		98.8	86.4	85.3	98.6	98.6	98.8	99.2	98.2	97.9	99.6	16	HP1501
17	1.7	14.5	1.5	1.8	1.8	1.8	0.0	0.0	0.0	0.0	0.4	0.0	0.0	1.5	0.0	1.2		86.6	84.9	99.6	99.6	100.0	98.6	97.7	97.3	98.4	17	HP1548
18	14.9	1.8	14.9	15.5	15.5	15.9	15.0	15.0	15.0	15.0	15.2	15.0	15.0	15.4	15.0	15.2	15.0		86.4	86.4	86.4	86.6	86.0	86.3	86.6	86.4	18	Talis
19	15.8	15.5	15.9	16.3	16.3	16.3	16.8	16.8	16.7	16.8	16.8	16.8	16.8	16.3	16.8	16.3	16.8	14.9		84.8	84.8	84.9	84.8	85.5	85.2	85.6	19	SAN
20	1.8	14.7	1.7	2.0	2.0	2.0	0.4	0.4	0.4	0.4	0.6	0.4	0.4	1.7	0.4	1.4	0.4	15.2	17.0		100.0	99.6	98.5	97.5	97.1	98.5	20	GRAN4 CP
21	1.8	14.7	1.7	2.0	2.0	2.0	0.4	0.4	0.4	0.4	0.6	0.4	0.4	1.7	0.4	1.4	0.4	15.2	17.0	0.0		99.6	98.5	97.5	97.1	98.5	21	Grand7
22	1.7	14.5	1.5	1.8	1.8	1.8	0.0	0.0	0.0	0.0	0.4	0.0	0.0	1.5	0.0	1.2	0.0	15.0	16.8	0.4	0.4		98.6	97.7	97.3	98.4	22	klon3
23	1.1	15.0	1.0	1.3	1.3	1.0	1.3	1.3	1.3	1.3	1.4	1.3	1.3	1.0	1.3	0.7	1.3	15.6	16.8	1.4	1.4	1.3		98.2	97.7	98.8	23	klon6
24	2.2	14.8	1.8	1.7	1.7	1.8	2.4	2.4	2.4	2.4	2.5	2.4	2.4	2.1	2.4	1.8	2.4	15.4	16.1	2.5	2.5	2.4	1.7		97.8	97.8	24	Marm odwr
25	2.2	14.5	1.8	2.4	2.4	2.1	2.8	2.8	2.7	2.8	3.0	2.8	2.8	2.4	2.8	2.1	2.8	15.0	16.4	3.0	3.0	2.8	2.2	2.2		97.8	25	NN1411
26	0.3	14.7	0.7	1.5	1.5	1.5	1.7	1.7	1.7	1.7	1.8	1.7	1.7	0.4	1.7	0.4	1.7	15.2	15.9	1.5	1.5	1.7	1.1	2.2	2.2		26	Olimp
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		

dicator plants *Fragaria vesca* 'EMC' and *F. vesca* var. *semperflorens* 'Alpine' sensitive for SMYEV (Converse et al. 1987). The indicators were kept in a greenhouse under controlled conditions, and their survey was carried out two weeks later and continued for one month after grafting.

RESULTS

Symptoms. Marginal chlorosis was observed only in two plants of 'Grandarosa' cultivar labelled Grand3 and Grand7 from an experimental plantation No. 1 in Łódzkie province, and one plant of an unknown cultivar from Lubuskie province infected with isolate 3233CL (Figure 1). No disease symptoms were observed on the remaining plants from which samples were collected for testing.

Detection. DAS-ELISA detected SMYEV in 69 out of 86 leaf samples (69/86, 80%) collected from strawberry plants grown in experimental field No. 1, as well as in three out of 17 samples (3/17, 18%) originating from a commercial plantation in the Łódzkie province. The presence of SMYEV was subsequently confirmed in all 72 ELISA-positive samples by RT-PCR, through amplification of a specific ~930 bp fragment. Additionally, SMYEV was also detected by RT-PCR in 26 out of 151 samples collected from experimental fields Nos. 2–5 in Łódzkie province. The RT-PCR result was also positive for 18 samples out of 169 tested from commercial plantations. SMYEV was detected in 10 out of 62 tested samples from the Łódzkie province, seven out of 56 tested from the Mazowieckie province, two out of 29 tested from the Lubuskie Voivodeship, and two out of 22 tested from the Małopolskie province.

Out of all 116 SMYEV isolates detected, for sequence analysis were selected 26 isolates. These isolates originated from experimental fields and commercial plantations in different regions of the country (Table 1). In the plants infected by SMYEV isolates Grnt4, Clone37, Clone2, Grand4, 3233CL, San, HP1501, Dar,

Figure 1. Symptoms on a strawberry of unknown cultivar infected with the 3233CL isolate of SMYEV

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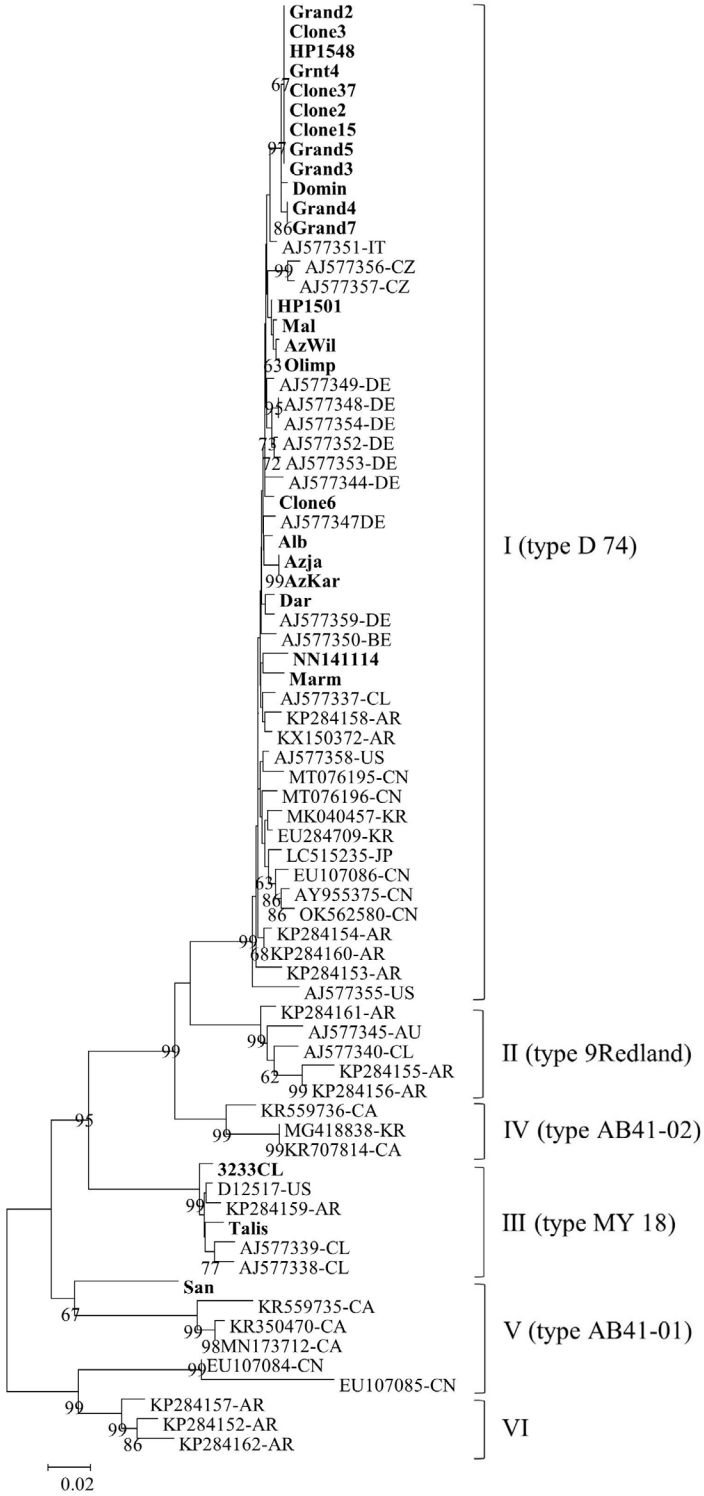

Figure 2. Unrooted neighbour-joining phylogenetic tree showing a relationship among strawberry mild yellow edge isolates based on the coat protein gene sequences. The names of the isolates detected in this study are indicated in bold

*IT – Italy, DE – Germany, CZ – Czech Republic, BL – Belgium, CL – Chile, AR – Argentina, AU – Australia, KR – South Korea, CN – China, CA – Canada, US – United States of America, JP – Japan

AzWil, Azja, Grand3, Grand5, Clone15, HP1548, Talis, AzKar, Grand2, Grand7, Clone3, Clone6, NN141114, and Marm, SMoV was also detected. Only four samples: Mal, Alb, Domin, and Olimp tested negative for SMoV. Strawberry crinkle virus (SCrV) and strawberry vein banding virus (SVBV) were not detected in 26 SMYEV-infected samples.

Sequence analysis. RT-PCR amplification products, including the coat protein (CP) gene, of 11 SMYEV isolates from experimental fields and 15 from commercial plantations were sequenced. The raw sequences were edited and aligned to 729 nt in length. This genome fragment contained the coat protein gene encoding a protein consisting of 242 amino acids.

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Table 3. Recombination events detected in the coat protein gene sequence of SMYEV isolates

Recombinant	Major parent	Minor parent	Breakpoints		Method* (*P*-value)
			start	end	
Sy02	sy03 (group V)	D/L.9 (group I)	610	663	G (4.994×10^{-4}) M (6.454×10^{-8}) C (1.228×10^{-7}) 3S (3.423×10^{-19}) R (2.429×10^{-1})
53	16-1 (group VI)	69N (group I)	1	165	G (3.843×10^{-2}) M (2.451×10^{-3}) C (1.607×10^{-2})

* C – Chimaera, G – Geneconv, M – Maxchi, R – RDP, 3S – 3Seq

The genetic analysis of this region revealed substantial diversity among the Polish SMYEV isolates, with nucleotide sequence similarities ranging from 84.8% to 100% (Table 2). The San, Talis, and 3233CL isolates were the most genetically diverse, exhibiting the lowest nucleotide sequence similarity of the CP gene compared to the remaining 23 SMYEV isolates detected in Poland (84.8–86.4%, 86.0–86.4%, and 86.0–87.1%, respectively).

The sequence similarity of the Polish isolates and sequences of 48 SMYEV strains from different geographical regions published in GenBank was 81.4–99.5%.

Percent identity. Coat protein gene sequences of detected isolates were deposited in the GenBank database on April 18, 2020, August 21, 2023, and April 25, 2025, under accession numbers MT080940–MT080944, OR044009–OR044019, and PV535680–PV535689, respectively.

Phylogenetic analysis. The phylogenetic analysis of the CP gene from 26 isolates detected in this study and 48 SMYEV strains from various geographical regions revealed the presence of six distinct groups. Isolates from Poland were represented in three of these groups. Notably, most Polish isolates clustered within group I (type D74), which predominantly includes strains from Europe (Figure 2).

The Talis and 3233CL isolates were grouped within group III (type MY18), alongside strains MY-18 from the USA, 2CH and 3CH from Chile, and 53 from Argentina. The San isolate and three Canadian strains represented group V (type ABY1-01) as proposed by Bhagwat et al. (2016). Interestingly, the San isolate formed a distinct subgroup within this clade.

Recombination analysis. Recombination analysis of the coat protein gene sequences of the SMYEV isolates detected two possible recombina-

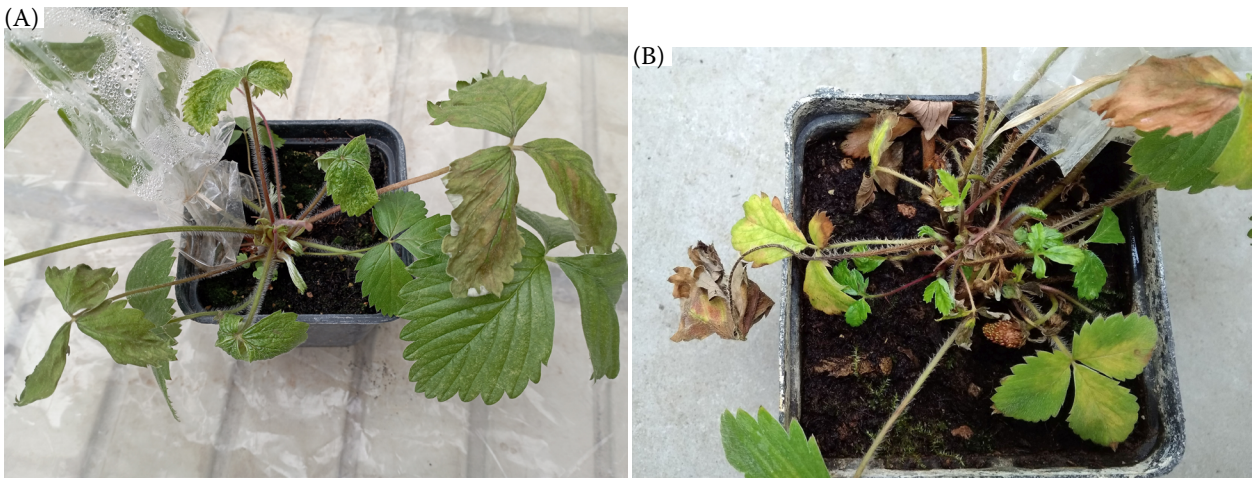

Figure 3. Symptoms induced by the complex of SMoV and Grnt4 isolate of SMYEV on EMC indicator plant: young leaves epinasty and mottling, (A) dieback of maturing leaves, (B) dying of the whole plant

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Figure 4. Vein necrosis of maturing leaves induced by the Mal isolate of SMYEV on 'Alpine' indicator plant

tion events using four of the nine recombination detection programs in the RDP4 software package (Table 3). One was noticed in the Argentinian strain designed 53 and was supported with *P*-values 1.607×10^{-2} to 3.843×10^{-2} depending on the methods. Strain 53 formed group III with 2CH, 3CH from Chile, MY18, and Polish isolates Talis and 3233CL.

Another recombination event was identified in the Chinese strain sy02. This event was supported with *P*-values 1.225×10^{-7} to 6.454×10^{-4}. Sequences with evidence of the same recombination event were Canadian strains AB41-01, NB1165, QC3v1, and Polish isolate San, grouping with the sy02 strain in V group (type-AB41-01) proposed by Bhagwat et al. (2016).

Biological indexing. Two weeks after grafting of leaf fragments of Grnt4, Clone37, Grand4, San, HP1501, Dar, Azja, NN141114, and Marm samples infected with complex of SMYEV and SMoV, leaf epinasty, mottling, yellowing of the tissue along the veins of the young leaves and dieback of the older leaves were observed on *Fragaria vesca* 'Alpine' and 'EMC' indicator plants (Figures 3A, 3B). Another symptoms, including epinasty and chlorotic flecking on young leaves and vein necrosis of maturing leaves, were shown by these indicators grafted with Mal, Domin, and Olimp infected with SMYEV only (Figure 4).

DISCUSSION

SMYEV was detected in 27,4% of tested strawberry plants. Most originated from experimental fields, as 95 out of 237 collected samples were tested positively. The large number of infected strawberry plants grown in the experimental plantations probably resulted from the spread of the virus, which originally came from one source. SMYEV was detected in 21 out of 186 samples (11.3%) collected on commercial plantations. The high prevalence of SMYEV underlines the virus's potential spread within the country. It suggests that the virus might be more widespread than previously thought and highlights the need for rigorous phytosanitary measures to prevent further virus spread. Recently, the threat from SMYEV has been increasing in Poland, which may be related to importing the new strawberry cultivars infected plants and climate warming, which is favourable for developing *Chaetosiphon fragaefolii* vector. Although the presence of *Ch. fragaefolii* aphid was not confirmed in this study, its role in virus transmission is assumed, especially given the climate change and the increasing threat of SMYEV in Poland. It is known that warmer temperatures, humidity and altered precipitation patterns may enhance the virulence and spread of various plant diseases (Lahlali et al. 2024). Moreover, the global trade of strawberry plants facilitates the spread of the virus to new regions, making it a significant concern for strawberry growers worldwide.

Only a few infected plants tested in this study showed marginal chlorosis. This symptom is characteristic of SMYEV, particularly in cases where the infection becomes apparent under specific conditions, such as stress or coinfection with other pathogens (Converse et al. 1987). It is known that while most SMYEV-infected strawberries remain asymptomatic, some isolates can induce distinct symptoms, demonstrating the variability in the pathogenicity of different SMYEV strains. The studies by Thompson et al. (2003) have shown that specific genetic changes in the CP gene can alter the interaction of the virus with the host plant, leading to differences in symptom severity. No correlation was observed between the pathogenicity of the isolates and their genetic variability in these studies, as disease symptoms on strawberry plants were observed in plants infected with isolates classified into group I (Grand 3, Grand7) and group III (3233CL). Virus infection in symptomatic and asymptomatic plants emphasises the importance of molecular diagnostic tools, such as RT-PCR, in detecting the virus since symptoms alone are unreliable indicators of infection.

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SMYEV isolates found in Poland showed genetic diversity of the CP gene with nucleotide sequence similarity of 84.8–100%. Similarly, the sequence identity ranged from 84.0 to 99.6% was revealed for CP of the four Canadian isolates designated AB41-01, AB41-02, NB1165, and NS26 (Bhagwat et al. 2016) while SMYEV isolates found in Argentina shared 81.5–99.6% nt identity (Torrico et al. 2016). The Polish isolates shared 81.4–99.5% sequence similarity of the CP gene nucleotides with 48 SMYEV isolates from different countries. Depending on number of analysed isolates, their geographical origin, and host plant, the previous studies revealed the identity of CP gene nucleotide sequence of SMYEV isolates ranged: 85.4–100% (Thompson & Jelkmann 2004), 81.6–99 % (Torrico et al. 2016), and 79.5–86.6% (Li & Yang 2011).

The Canadian SMYEV isolate AB5-2 shared 86% and 90% nucleotide sequence identities to MY-18 and D74 strains, respectively (Ma et al. 2015). In further study, 83.5–89.9% nt sequence similarity was reported between these two reference strains and the four isolates designated as AB41-01, AB41-02, NB1165, and NS26 found in eastern Canada (Bhagwat et al. 2016). SMYEV isolates from four provinces of eastern Canada revealed 81.62–99.86% nucleotide identity within the CP gene, indicating a high degree of genetic diversity (Xiang et al. 2020). Based on the results of the evolutionary analysis, the authors suggested that these strains may have originated outside of Canada. Still, genetic mutations have enabled their adaptation to the regional conditions.

The unique positioning of Talis, 3233CL and San isolates suggests the introduction of the virus into Poland from another geographical region or the evolution of different lineages within the country. This diversity could have significant implications for strawberry cultivation in Poland, as different SMYEV strains may vary in their virulence, transmission efficiency, and impact on crop yield.

Based on the genetic diversity of the coat protein gene of SMYEV strains from different geographic regions, Thompson and Jelkmann (2004) suggested their division into three distinct strain groups: I (type D74), II (type 9Redland) and III (type MY18). Identifying new genetically distinct isolates was the basis of constructing other phylogenetic trees for SMYEV strains. Torrico et al. (2016) proposed two groups (A and B) with discrimination of SMYEV isolates in two subgroups within each of them. Bhagwat et al. (2016) proposed a new division designating five groups, including the previous three groups (I, II and III) and two new groups: IV – type AB41-02 with Canadian strains and V – type AB41-01 with Canadian and Chinese strains grouped separately in two subclades. In turn, Xiang et al. (2020) divided the strains to three groups with five subgroups: three in group I and two in group III. Phylogenetic analysis carried out by Torrico et al. (2016) did not include newly detected isolates from Canada, and vice versa, isolates from Argentina were not present on the phylogenetic tree proposed by Bhagwat et al. (2016).

Based on the analysis conducted during this study, six phylogenetic groups would be distinguished when constructing the phylogenetic tree. Subgroup IV with three isolates from Argentina labelled 36-1-3, 13-2-5, 1nd 16-1 (Torrico et al. 2016), would correspond to group VI on the phylogenetic tree resulting from the sequence analysis carried out as part of this study.

Most of the isolates detected in Poland belonged to group I (type D74), alongside European isolates included in the phylogenetic analysis. This suggests that most SMYEV isolates in Poland are closely related to strains found in other European countries, indicating a possible common origin or shared evolutionary pressures. However, the genetic variation of the Talis and 3233CL isolates positioned them in group III (type MY 18), alongside isolates from Chile, the USA, and Argentina, while the San isolate, along with Canadian isolates, was classified in group V (type AB41-01). These isolates originated from foreign cultivars, and there is a possibility that they were introduced to Poland with nursery stock. Conversely, the isolates from Chile, Argentina, the USA, China, and Japan also belonged to type D74 alongside European isolates. The identification of SMYEV isolates classified into different phylogenetic groups (types) indicates a lack of correlation between the genetic diversity of virus isolates and their geographical origin, which may result from the spreading of the SMYEV isolates and/or the independent evolution of the virus in different regions. Genetic diversity of the SMYEV strains could have implications for disease severity and transmission efficiency of the virus. Understanding phylogenetic relationships is crucial for tracking the spread of the virus, which is why it is important to continue monitoring and genetic characterisation of the newly detected SMYEV strains.

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Although the recombination analysis of the coat protein gene sequences revealed two recombinants – the sy02 isolate from China, classified into group V, and the 53 isolate from Argentina, classified into group III – Polish isolates 3233CL and Talis also represented type MY 18 (group III) and the San isolate – type AB41-01 (group V). Recombination of Potexvirus species was previously reported in the C-terminal region of the CP gene, the polymerase gene, and the triple gene block regions (Sherpa et al. 2007; Hasiów Jaroszewska et al. 2010). Recombination analysis of the whole genome or its longer fragments, including genes other than CP, could identify potentially more recombination events, indicating the diversity of SMYEV isolates resulting from the evolutionary process of intraspecific recombination. The intraspecific recombination is an evolutionary process common in single-stranded positive-sense RNA plant viruses (Chare & Holmes 2006), resulting in an increase in viral pathogenicity, host range, and also the capacity to break resistance in crop varieties (García Arenal & McDonald 2003).

Epinasty and chlorotic flecking on young leaves and vein necrosis of maturing leaves were shown by *Fragaria vesca* 'Alpine' and 'EMC' indicators grafted with samples of strawberry single infected with SMYEV. These symptoms were previously described as characteristic for SMYEV (Converse et al. 1987; Martin & Tzanetakis 2006).

CONCLUSION

In summary, our results demonstrate the presence of strawberry mild yellow edge virus in Poland for the first time and provide valuable insights into the genetic diversity of the detected SMYEV isolates. The virus detection in both commercial and experimental fields highlights the need for stringent phytosanitary measures to prevent further spread. This includes monitoring for the presence of *C. fragaefolii*, implementing virus-free certification programs for planting material, and continued surveillance of strawberry plantations.

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