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Virology

Milder Autumns May Increase Risk for Infection of Crops with Turnip Yellows Virus

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Abstract

Climate change has increased the risk for infection of crops with insect-transmitted viruses. Mild autumns provide prolonged active periods to insects, which may spread viruses to winter crops. In autumn 2018, green peach aphids (*Myzus persicae*) were found in suction traps in southern Sweden that presented infection risk for winter oilseed rape (OSR; *Brassica napus*) with turnip yellows virus (TuYV). A survey was carried out in spring 2019 with random leaf samples from 46 OSR fields in southern and central Sweden using DAS-ELISA, and TuYV was detected in all fields except one. In the counties of Skåne, Kalmar, and Östergötland, the average incidence of TuYV-infected plants was 75%, and the incidence reached 100% for nine fields. Sequence analyses of the coat protein gene revealed a close relationship between TuYV isolates from Sweden and other parts of the world. High-throughput sequencing for one of the OSR samples confirmed the presence of TuYV and revealed coinfection with TuYV-associated RNA. Molecular analyses of seven sugar beet (*Beta vulgaris*) plants with yellowing, collected in 2019, revealed that two of them were infected by TuYV, together with two other poleroviruses: beet mild yellowing virus and beet chlorosis virus. The presence of TuYV in sugar beet suggests a spillover from other hosts. Poleroviruses are prone to recombination, and mixed infection with three poleroviruses in the same plant poses a risk for the emergence of new polerovirus genotypes.

Keywords: aphids, climate change, disease incidence, high-throughput sequencing, insect vectors, oilseed rape, poleroviruses, *Solemoviridae*, sugar beet, turnip yellows virus-associated RNA

Turnip yellows virus (TuYV; genus *Polerovirus*, family *Solemoviridae*) is one of the most common viruses infecting oilseed rape (OSR, *Brassica napus*). Infections of TuYV in OSR are largely symptomless and have therefore been overlooked for a long time and not considered an important problem in agriculture (Newbert 2016). Symptoms, if present, consist mainly of leaf discoloration and dwarfing (Stevens et al. 2008). However, some TuYV infections in OSR may result in lower yields and extensive economic losses (Congdon et al. 2020). This occurs mainly when plants are infected just after crop emergence, and it has serious effects on many components related to yield (Jay et al. 1999). Infections with TuYV have been found to reduce the number of pods per plant, seeds per pod, and oil content in seeds, and the quality of meal and oil produced is also affected (Coleman 2013; Hardwick et al. 1994; Jones et al. 2007).

The impact of TuYV on the yield depends on factors such as incidence of virus infection and crop variety (Walsh et al. 1989). In Australia, a plot experiment on OSR with 96% TuYV infection resulted in a yield loss of 46% (Jones et al. 2007). In Europe, TuYV is a constant problem and poses challenges to crop production because of frequent infections resulting in considerable yield losses. OSR plots with TuYV incidence of 90 to 100% had yield reductions between 12 and 34% when compared with plots that were virus free (Graichen and Schliephake 1999).

TuYV was previously known as the non-beet-infecting strain of beet western yellows virus (BWYV). TuYV was first reported and identified in the United Kingdom as the European strain of BWYV (Duffus and Russell 1970). Later, the International Committee on Taxonomy of Viruses (ICTV) divided the BWYV strains found in Europe into separate species based on host range and differences in genome sequences. These were TuYV, which infects OSR, and beet mild yellowing virus (BMYV) infecting sugar beet but not OSR (Mayo 2002). The host range of TuYV comprises summer and winter crops, as well as weeds, belonging to the families *Brassicaceae*, *Chenopodiaceae*, *Asteraceae*, and *Amaranthaceae* (Stevens et al. 2008). The effect of TuYV on host plants of other families apart from *Brassicaceae* has not been studied extensively, but these plants are known to be potential reservoirs of the virus. TuYV is transmitted by aphid vectors in a persistent, non-propagative, and circulative manner (Schliephake et al. 2000; Stevens et al. 1995). The most efficient vector is the green peach aphid (*Myzus persicae*), with more than 90% transmission efficiency, and other known vector species include *Macrosiphum euphorbiae*, *Aphis gossypii*, and *Brevicoryne brassicae* (Schliephake et al. 2000; Stevens et al. 1995).

The genome of TuYV consists of a monopartite, linear, single-stranded RNA molecule, which is encapsidated in an icosahedral shell (Hipper et al. 2014). Up to 10 open reading frames (ORFs) have been identified in polerovirus genomes (Sõmera et al. 2021; Stevens et al. 2005). Like other poleroviruses, TuYV uses complex gene expression strategies to express multiple proteins from a single RNA molecule (Beuve et al. 2008; Smirnova et al. 2015; Veidt et al. 1988). ORF0 encodes the P0 protein, which is an RNA-silencing suppressor that has a role in the process of post-transcriptional gene silencing to overcome host resistance, as well as in pathogenicity and determination of host range (Bortolamiol et al. 2007; Clavel

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et al. 2021). ORF1 encodes the viral genome-linked protein (VPg). ORF2 is expressed as a P1-P2 fusion protein functioning as an RNA-dependent RNA-polymerase (Sõmera et al. 2021). ORF3 and ORF3a encode the major coat protein (CP) and the P3a protein, respectively (Brault et al. 2003; Smirnova et al. 2015). ORF4 encodes a movement protein (MP) that has a phloem-specific movement function and a role in the systemic virus spread (Stevens et al. 2005; Ziegler-Graff et al. 1996). The P3-P5 readthrough protein is involved in virus accumulation, phloem retention, and systemic movement in the plant, as well as persistence in the vector (Brault et al. 1995, 2005; Peter et al. 2009; Rodriguez-Medina et al. 2015).

Additional RNA molecules have been found to be associated with TuYV and other poleroviruses. These subviral agents can replicate without a virus but depend on a helper virus for movement, encapsidation, or transmission by vectors. Recently, TuYV-associated RNAs (TuYVaRNAs) have been reported from the United Kingdom, Germany, and Australia (Filardo et al. 2021; Fowkes et al. 2021; Gaafar and Ziebell 2019; Gaafar et al. 2020). So far, they have not been extensively studied for their role in pathogenicity.

The major OSR-growing areas of mainland Europe, such as Germany, Poland, and France, have been seeing TuYV incidences of ≥90% consistently throughout multiple years (Newbert 2016). In the United Kingdom, the first widespread incidence of TuYV was reported by Smith and Hinckes (1985), with an incidence of over 97%. A more recent survey (Asare-Bediako et al. 2020), which was carried out in three different regions of England (Lincolnshire, Warwickshire, and Yorkshire) in three consecutive crop seasons (2007 to 2010), revealed high incidences of TuYV infections in OSR for the regions of Lincolnshire (≤100%), Warwickshire (≤88%), and Yorkshire (1 to 74%). Infections with TuYV have been reported not only across Europe (Fowkes et al. 2021; Gaafar et al. 2020; Stevens et al. 2008) but also from countries in other parts of the world, such as Australia (Coutts et al. 2006), Iran (Shahraeen et al. 2003), China (Wang et al. 2015), Saudi Arabia (LT844559 accession number; *unpublished*), South Africa (New et al. 2016), Egypt, and Morocco (Abraham et al. 2008).

In the future, TuYV could become an extensive problem also in the previously less affected colder regions of northern Europe, not only in OSR, but also in other potential hosts due to global warming allowing more favorable conditions for aphid vectors (Roos et al. 2011). The wide host range of TuYV, consisting of both summer and winter crops, as well as weeds, could expand the potential virus reservoirs, facilitating a "green bridge" for both the pathogen and the vectors (Freeman and Aftab 2011). A mixture of virus genotypes in the same host could trigger an environment conducive to recombination, resulting in the emergence of severe and more virulent strains of viruses (Monci et al. 2002). With rapid change in the agricultural practices and virus reservoir plant populations, there is the risk for emergence of new viruses by recombination during mixed virus infections and that new viral genotypes may outcompete the present ones when switching to a new host (Elena et al. 2011).

In autumn 2018, a high number of aphids, including *M. persicae*, were caught in suction traps in southern Sweden (Aldén et al. 2019), indicating an increased risk for infection with TuYV in OSR. Therefore, the current study was initiated to establish the incidence of TuYV infections in OSR in southern and central Sweden. Sequence and phylogenetic analyses were carried out to determine the relationship between Swedish TuYV isolates and other isolates worldwide. In addition, field samples of sugar beet (*Beta vulgaris*) were studied during the same year from the southern regions of Sweden to reveal the diversity of poleroviruses present in the crop. High-throughput sequencing (HTS) for one of the OSR samples was also carried out to determine the complete genome for a TuYV isolate and to search for potential polerovirus-associated RNAs. These studies would be highly beneficial for developing sustainable crop management practices and control of polerovirus infections in OSR and sugar beet crops in Sweden.

Materials and Methods

Sampling sites and survey

In spring 2019 (March to April), a survey was carried out to look at the prevalence of TuYV in winter OSR in Sweden. Random leaf samples (Fig. 1) were collected by the Swedish Board of Agriculture from 46 fields of OSR (20 or 90 samples/field) from six counties in southern and central Sweden (Fig. 2; Table 1). In addition, in October 2019, leaves showing chlorotic symptoms were collected from a total of 25 sugar beet plants in four fields in the county of Skåne, southern Sweden (Fig. 1; Table 2). The sugar beet material was received from Nordic Beet Research and DLF Beet Seed, Landskrona, Sweden. Six samples that tested positive in enzyme-linked immunosorbent assay (ELISA) for polerovirus infection (sample numbers 2, 3, 17, 19, 22, and 24) were selected for further molecular analysis together with sample number 1 (Table 2; Supplementary Fig. S1). Positive and negative reference material for BMYV and beet chlorosis virus (BChV) was obtained from DLF Beet Seed.

Detection of TuYV in OSR and poleroviruses in sugar beet

For screening of OSR, a double antibody sandwich (DAS)-ELISA kit for TuYV (Loewe Biochemica GmbH) was used with positive and negative controls of the kit. Plant leaves were homogenized in sample extraction buffer (pH 7.4), and the DAS-ELISA was carried out according to the manufacturer's recommendations. Samples were considered positive if the absorbance measured at 405 nm was at least two times higher than the value for the negative controls. A triple antibody sandwich (TAS)-ELISA test for BWYV (Deutsche Sammlung von Mikroorganismen und Zellkulturen) was used for the analyses of sugar beet field samples following the manufacturer's protocol. Sugar beet leaves were homogenized using a Pollähne press to produce liquid extract. The leaf extract was diluted 10 times with the extraction buffer. The specific IgG antibody BWYV (AS-0049) was diluted with coating buffer (1:500). A monoclonal antibody (Mab) BWYV (AS-0049/1) and rabbit anti-rat IgG-AP (RAM-AP) were diluted with extraction buffer (1:500 and 1:1,000, respectively). Samples with an absorbance at 405 nm of 0.1 were considered to be positive after comparing with the negative controls. Both the ELISA tests for TuYV and BWYV also detect closely related poleroviruses, including BMYV and BChV.

RNA extraction, RT-PCR analysis, cloning, and sequencing

Plant samples (100 mg) were homogenized in liquid nitrogen with pestle and mortar. Total RNA was isolated using the Spectrum Plant Total RNA Kit (Sigma-Aldrich) according to the manufacturer's protocol. cDNA synthesis was performed using random hexamer primers and RevertAid Reverse Transcriptase (Thermo Fisher Scientific) according to the manufacturer's protocol. Universal primers targeting the CP gene region of viruses belonging to the genera *Luteovirus* and *Polerovirus* were used for the PCR analysis (Abraham et al. 2006). The expected amplicon length was 635 bp. For PCR, 50% Phusion High-fidelity PCR master mix with HF buffer (Thermo Fisher Scientific) was used in a total reaction volume of 50 µl. The conditions for PCR were as follows: initial denaturation at 98°C for 30 s, followed by 35 cycles of 98°C for

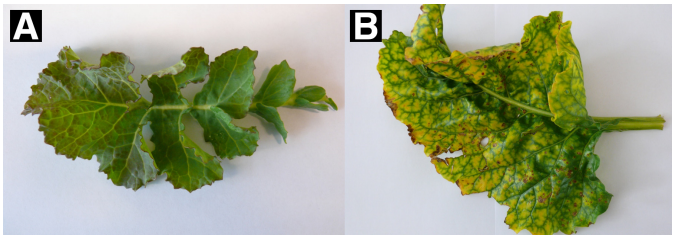


Fig. 1. Leaves of **A,** rapeseed and **B,** sugar beet with symptoms of polerovirus infection.

10 s, 55°C for 30 s, and 72°C for 20 s, and then final extension at 72°C for 5 min. Amplification products were either directly purified by GeneJET PCR Purification Kit (Thermo Fisher Scientific) or extracted from a gel using GeneJET Gel Extraction Kit (Thermo Fisher Scientific) according to the manufacturer's manual. The purified PCR products were cloned into the vector pJET1.2/blunt using Clone JET PCR Cloning Kit (Thermo Fisher Scientific) and competent cells of *Escherichia coli* DH5α (Invitrogen) according to the manufacturers' protocols.

For each isolate, two or three clones with the expected insert size were sequenced by Sanger sequencing at Macrogen Europe. Clones with unique sequences were sequenced in both directions using pJET1.2 forward and reverse primers. The polerovirus CP gene sequences were deposited in the GenBank database under the accession numbers OP719286-OP719310.

HTS

HTS was performed using RNA from a TuYV-positive OSR sample collected from Karpalund, county of Skåne. Ribosomal RNA was removed from the total RNA extract using Ribo-Minus Plant Kit (Thermo Fisher Scientific). For input to HTS, 100 ng of rRNA-depleted RNA was used. Library preparation was done with the Illumina stranded mRNA kit without poly-A selection. Illumina sequencing was carried out by the SNP&SEQ Technology Platform in Uppsala with one lane NovaSeq SP and a read length of PE150 bp, producing at least 325 M read pairs per sample.

Bioinformatics analysis

The HTS data for the TuYV-infected OSR sample from Karpalund were managed through an established bioinformatics workflow called the 'nf-metavir' pipeline (pipeline for a metavironomics https://github.com/amrei-bp/nf-metavir) on the UPPMAX HPC server (https://www.uppmax.uu.se/). In brief, raw Illumina paired-end sequencing reads were demultiplexed, and fastq files were assigned to the sample. Sample reads were filtered with Fastp (0.23.2) for quality-checking, trimming of adapter sequences, low-quality scores at the tails (QS less than 15 were filtered out), and removing duplicate reads (Chen et al. 2018). Filtered reads were classified using Kraken2 (2.1.2, a k-mer based approach) to assign taxonomy using a lowest common ancestor algorithm against the latest NCBI nucleotide database (nt), and the report file was visualized with Krona (2.7) plots (Ondov et al. 2011; Wood et al. 2019). All the filtered reads classified to virus family *Solemoviridae* were extracted and processed in two ways. First, extracted reads of *Solemoviridae* were assembled using SPAdes (3.15.3) and Megahit (1.2.9) de novo genome assembly (Li et al. 2015; Prjibelski et al. 2020), followed by assembly quality assessment using QUAST (5.0.2) on assembly scaffolds (Mikheenko et al. 2018), Kraken2 taxonomic classification of the assembly contigs against the nucleotide database, and alignments of contigs against reference genomes by bwa (0.7.17, option *mem*) and bowtie2 (2.3.5.1). Second, extracted reads of *Solemoviridae* were mapped against TuYV, TuYVaRNA, and TuYVaRNA2 reference genomes (closely related RefSeq assembly accessions: OK030774.1, MK450521.1, MN497827.1, respectively) with bwa and bowtie2 aligners, and mapping results were visualized on IGV (2.8.13) (https://software.broadinstitute.org/software/igv/2.8.x) to assess the alignment (Langmead and Salzberg 2012; Li 2013; Li et al. 2009). The consensus was exported from the bwa and bowtie2 alignments of *Solemoviridae* reads separately using samtools mpileup (1.14) and ivar (1.3.1). For each nucleotide position with a minimal coverage of 8 reads, the base with a frequency of more than 51% was called (Grubaugh et al. 2019; Maurier et al. 2019). To improve base calling for the consensus sequences of TuYVaRNA and TuYVaRNA2, all their classified reads (filtered reads classified as TuYVaRNA or TuYVaRNA2 in GenBank, respectively) were mapped against their

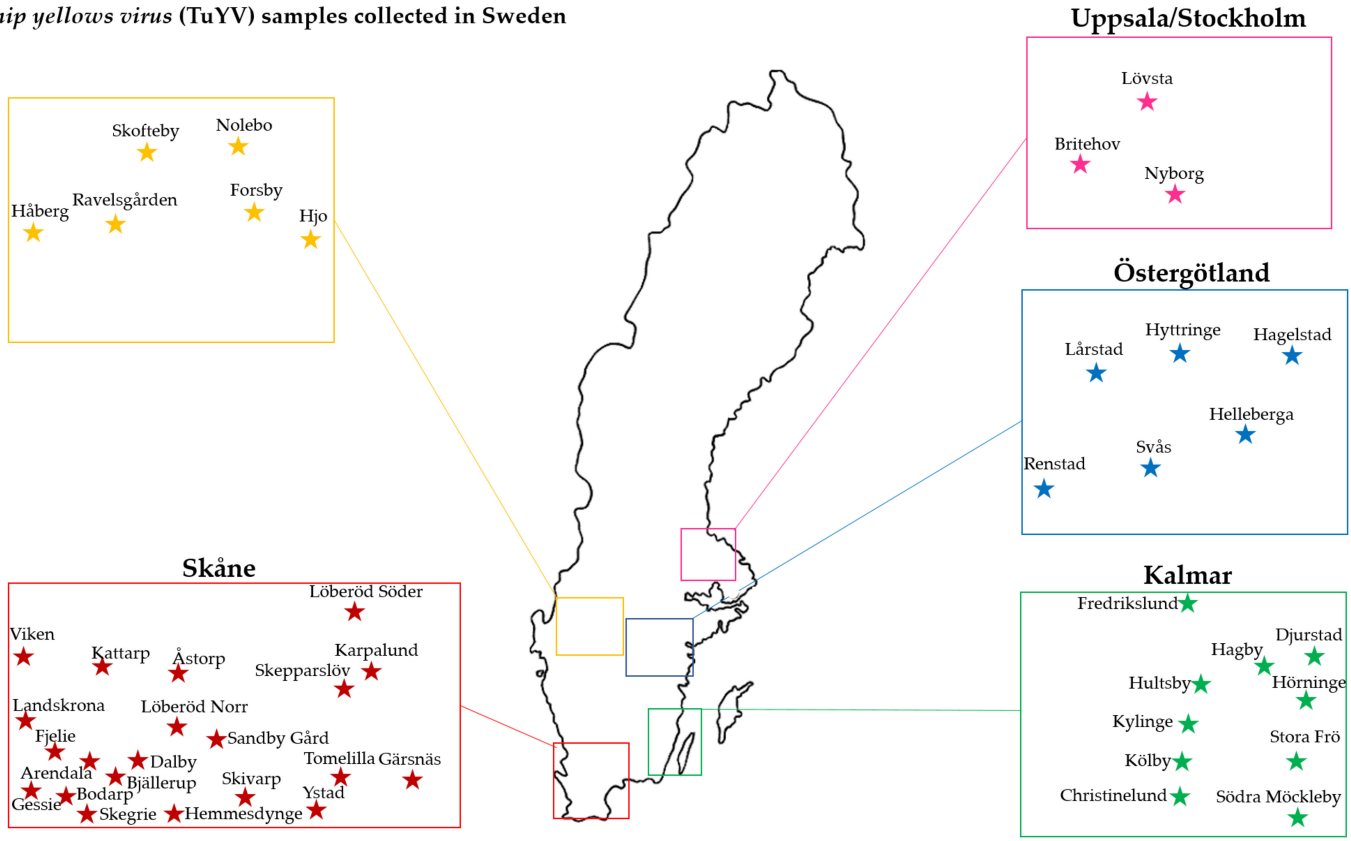


Fig. 2. Sites for sampling of oilseed rape in the southern and central parts of Sweden.

respective genomes using bwa. The assessed consensus sequences for TuYV, TuYVaRNA and TuYVaRNA2 were deposited in the GenBank database under the accession numbers OP719311, OP719312, and OP719313, respectively.

Sequence and phylogenetic analyses

To identify accessions with the highest nucleotide identity, GenBank was searched using BLASTn (Altschul et al. 1990). Pairwise nucleotide identities among the Swedish isolates and available sequences in GenBank were determined by the Sequence Demarcation Tool (Muhire et al. 2014). For phylogenetic analyses, selected sequences of poleroviruses or polerovirus-associated RNAs were aligned using MEGA version X (Kumar et al. 2018). The maximum-likelihood method in MEGA version X (Kumar et al. 2018) was used for constructing unrooted phylogenetic trees. Genetic distances were calculated using the Kimura 2 parameter as a substitution model. Bootstrap analysis with 1,000 replications was used to validate the branches of the phylogenetic trees.

Results

High incidence of TuYV in OSR

In spring 2019, a survey was carried out with random leaf samples from 46 OSR fields (20 or 90 samples/field) in southern and central Sweden using DAS-ELISA (Fig. 2). The survey revealed that TuYV was very commonly occurring in Sweden (Table 1), with TuYV being detected in all the fields across six counties, except for a single field in Hjo, Västra Götaland. In the counties of Skåne,

TABLE 2. Sugar beet samples from Skåne county, Sweden, selected for polerovirus analyses using RT-PCR and sequencing

Plant sample number	Location
1	Vadensjö
2, 3	Dalby
17, 19, 22	Alnarp
24	Kongsmarken

TABLE 1. Incidence of turnip yellows virus in fields of southern and central Sweden

Region	Sampling location[a]	Date of sampling[b]	Positive samples	Virus incidence (%)[c]
Uppsala	Britehov, Enköping	04/02/19	8	40
	Lövsta, Uppsala		5	25
Stockholm	Nyborg, Upplands-Bro		4	20
Kalmar	Södra Möckleby, Degerhamn	03/11/19	18	90
	Stora Frö, Mörbylånga		20	100
	Hörninge, Borgholm*	03/18/19	20	100
	Djurstad, Borgholm		79[d]	88
	Hagby, Borgholm		9	45
	Christinelund, Vassmolösa	03/19/19	18	90
	Kölby, Ljungbyholm		12	60
	Fredrikslund, Hagby		20	100
	Kylinge, Kalmar		16	80
	Hultsby, Rockneby		19	95
Skåne	Viken	03/11/19	76[d]	84
	Kattarp*		3	15
	Åstorp		3	15
	Landskrona		19	95
	Löberöd Norr (Sassner)	03/01/19	13	65
	Löberöd Söder (Lönshult)		13	65
	Fjelie	03/12/19	18	90
	Arendala	04/01/19	14	70
	Bjällerup		18	90
	Dalby	03/11/19	11	55
	Gessie	03/10/19	15	75
	Skegrie (Brynell)		17	85
	Bodarp		20	100
	Hemmesdynge		20	100
	Skivarp		20	100
	Ystad (Charlottenlund)		20	100
	Sandby Gård*	03/12/19	18	90
	Gärsnäs		14	70
	Tomelilla*		17	85
	Karpalund*	04/08/19	19	95
	Skepparslöv		19	95
Västra Götaland	Håberg, Grästorp	04/01/19	2	10
	Ravelsgården, Järpås		3	15
	Skofteby, Lidköping*	04/09/19	3	15
	Nolebo, Lundsbrunn*		6	30
	Forsby		3	15
	Hjo	04/10/19	0	0
Östergötland	Renstad, Ödeshög	04/08/19	14	70
	Helleberga, Linköping		20	100
	Svås, Mjölby		17	85
	Hyttringe, Motala*		20	100
	Lårstad, Motala*		18	90
	Hagelstad, Norsholm	04/15/19	14	70

[a] * indicates virus infection of samples confirmed by RT-PCR.
[b] Date pertain to all sampling locations until the next date is listed.
[c] Virus incidence in percentage was calculated by the number of positive samples in enzyme-linked immunosorbent assay (ELISA) over the total number of tested samples (20 random field samples were collected at all locations except two sites).
[d] The total number of samples collected and tested was 90.

Kalmar, and Östergötland, the average incidence of TuYV-infected plants was 75% and reached 100% for nine fields. The incidence of TuYV was lower in the counties of Västra Götaland (0 to 30%), Stockholm (20%), and Uppsala (25 to 40%). These results show that the incidence of TuYV was highest in the counties of southern (Skåne) and southeastern (Kalmar and Östergötland) Sweden compared with southwestern (Västra Götaland) or central Sweden (Stockholm and Uppsala).

Nine OSR samples testing positive in ELISA were selected for RT-PCR testing and sequence analyses (Table 1). An RT-PCR fragment of the expected length (0.6 kb) was obtained for all nine samples, and sequence analyses confirmed that the fragment corresponded to the CP gene of TuYV. Sequence comparisons revealed that the Swedish isolates of TuYV from OSR all shared very high sequence identities at 99.5 to 100% (Fig. 3). The isolates from Hörninge and Kalmar showed highest nucleotide identity of 99.5 to 100% to OSR isolate 3b from the United Kingdom (L39970), whereas isolates from Nolebo, Tomelilla, Karpalund, Kattarp, Sandby gård, Hyttringe, and Skafteby showed the highest nucleotide identity to two U.K. isolates from pea (OK030774 and OK030792). This also agrees with the observation in the phylogenetic tree where the TuYV isolates from Hörninge and Lårstad grouped closest to OSR isolate 3b from the United Kingdom and the other Swedish TuYV isolates from OSR grouped in a clade with the two pea isolates of TuYV from the United Kingdom with a bootstrap value of 84 (Fig. 4).

Mixed infection of TuYV and two other poleroviruses in sugar beet

In autumn 2019, leaves of 24 sugar beet plants with strong yellowing symptoms were collected from four fields in the county of Skåne, Sweden (Table 2). Tests with TAS-ELISA and RT-PCR for the CP gene confirmed polerovirus infection in seven of the samples. For virus identification, the amplification products were cloned, and three clones were sequenced for each sample. Sequence analyses

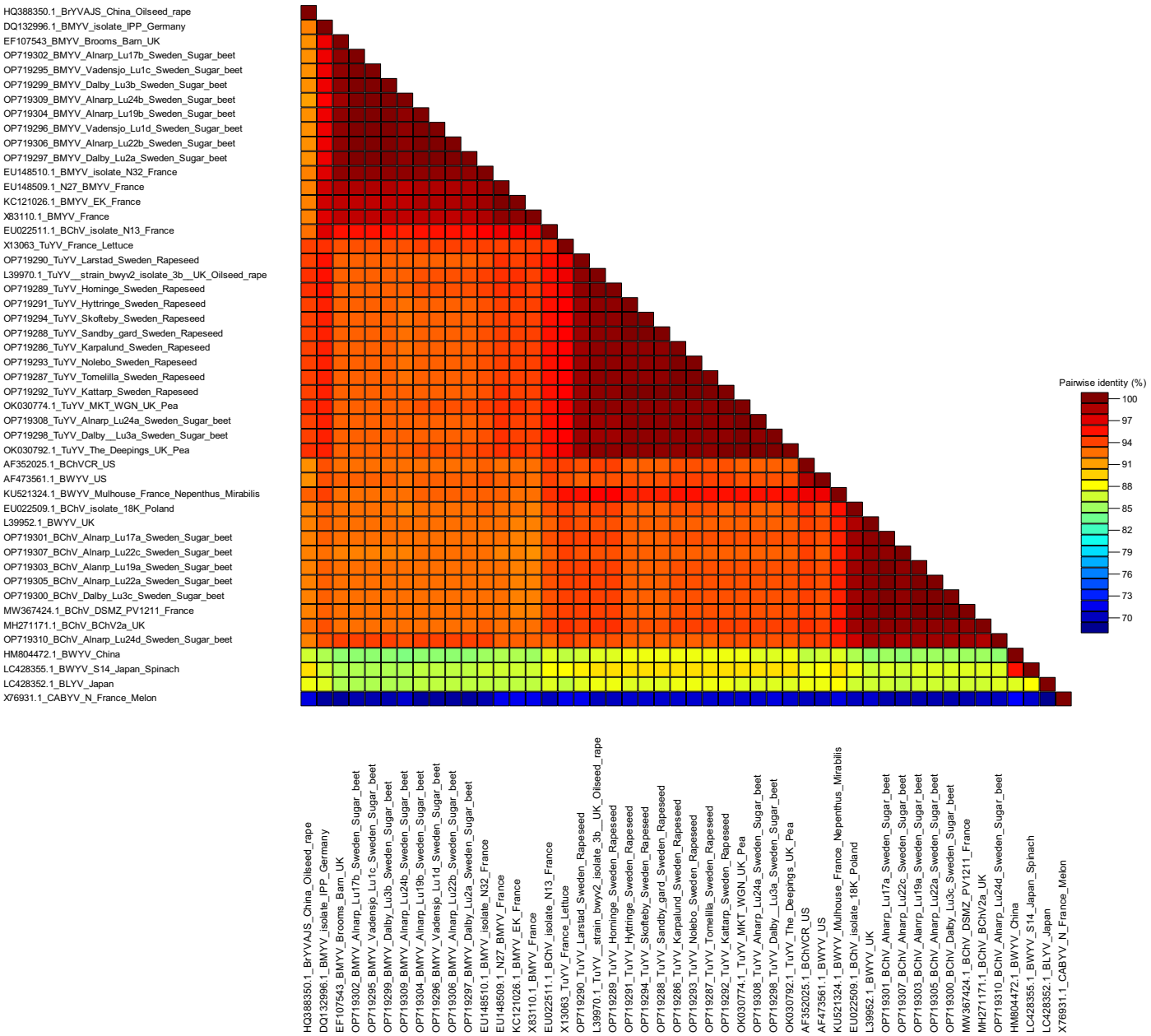
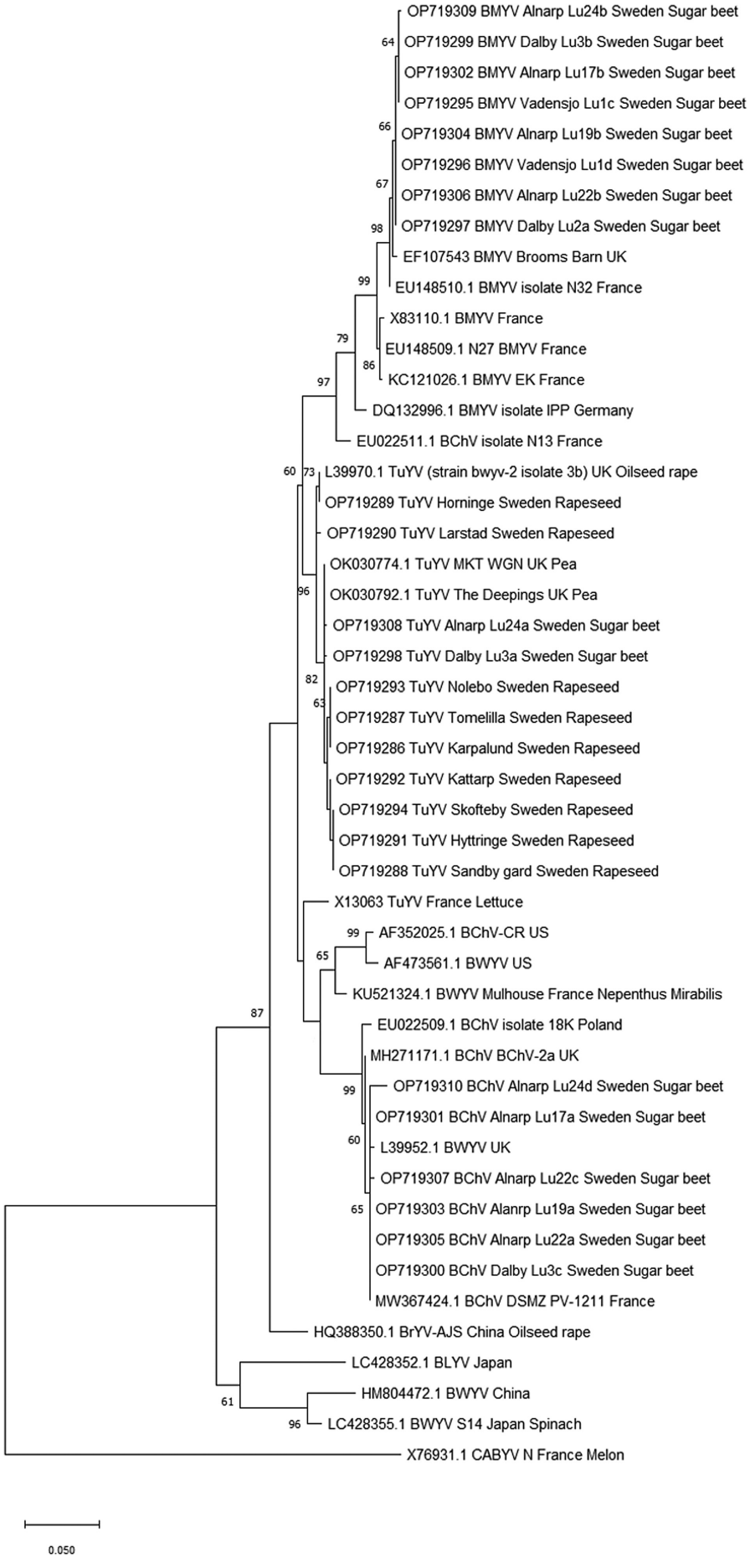


Fig. 3. Pairwise identity matrix of polerovirus coat protein gene sequences of Swedish isolates from oilseed rape and sugar beet as well as of reference isolates. Reference isolates are named by the GenBank accession number, virus, geographic origin, and host.

Fig. 4. Maximum likelihood tree of the coat protein gene from Swedish isolates of turnip yellows virus (TuYV), beet mild yellowing virus (BMYV), and beet chlorosis virus (BChV). Reference isolates of TuYV, BMYV, BChV, beet western yellows virus (BWYV), cucurbit aphid-borne yellows virus (CABYV), and Brassica yellows virus (BrYV) were included in the analysis. Reference isolates are named by the GenBank accession number, virus, geographic origin, and host (if not stated, then the host is sugar beet). The values at the nodes are bootstrap values (1,000 iterations) exceeding 60%. The scale shows nucleotide substitutions per site.



revealed mixed infections in the sugar beet samples with up to three poleroviruses. Pairwise nucleotide identities between the Swedish polerovirus sequences from sugar beet and those from GenBank revealed clear distinction of clones as BMYV, BChV, or TuYV (Fig. 3). BMYV was detected in all seven analyzed samples, and BChV was detected in samples 3, 17, 19, 22, and 24. In addition, TuYV was found to be present in samples 3 and 24. The two TuYV clones from sugar beet, Lu3a and Lu24a, shared a nucleotide identity of 99.7%. They both showed a high nucleotide identity of 99.0 to 99.7% to the Swedish TuYV isolates from OSR and an identity of 99.8% to U.K. isolates from pea (OK030774 and OK030792). A phylogenetic analysis confirmed the classification of the CP gene clones from sugar beet as BMYV, BChV, and TuYV with high bootstrap values (Fig. 4). The sugar beet field samples 3 and 24 each had a clone belonging to BMYV, BChV, and TuYV, indicating mixed infection of three poleroviruses in these samples.

Whole genome sequence of a Swedish TuYV isolate

A TuYV-positive OSR sample from Karpalund was selected for HTS and determination of the whole genome sequence of TuYV. From the HTS, approximately 77 million pair-end sample reads were generated for the sample ("Sample_UC-2888-K"). The taxonomic classification tool Kraken2 classified 99% of the total filtered reads, where viruses and *Solemoviridae* represented 1.83 and 1.71% of the reads, respectively (Table 3). The assembled TuYV sequence of 5,661 nt covered the complete genome, except for the terminal ends. Similar to the sequenced CP gene, the assembled genome sequence of the TuYV isolate from Karpalund showed high nucleotide sequence identities at 97.5 and 97.4% to the U.K. pea isolates Chatteris (OK030770) and MKT WGN (OK030774), respectively (Supplementary Fig. S2). A phylogenetic analysis confirmed the close relationship between the Karpalund isolate and European isolates of TuYV from pea (Fig. 5).

First identification of TuYV-associated RNAs in Swedish OSR

Through HTS, TuYVaRNA and TuYVaRNA2 were identified in the Swedish OSR sample from Karpalund. The consensus sequence for the Swedish TuYVaRNA isolate showed the highest nucleotide sequence identities at 92.6% to the German TuYVaRNA isolate Landkreis Meissen_16 from pea (MN497834), followed by 92.2% identity to BWYV-associated RNA (BWYVaRNA) isolate C20A9 from Australia (MT642437), 92.1% to the German TuYVaRNA isolate Salzlandkreis2_16 from pea (MN497832), and 89.2% to isolate BWYVaRNA ST9 from the United States (NC_004045) (Supplementary Fig. S3). The consensus sequence for TuYVaRNA2 showed the highest nucleotide sequence identities at 97.9% to the German TuYVaRNA2 isolates Salzlandkreis2_16 (MN497828) and Salzlandkreis1_17 (MN497827) from pea and a weed, respectively (Supplementary Fig. S3). In the phylogenetic analysis, TuYVaRNA Karpalund grouped basally in a clade of TuYVaRNA and BWYVaRNA isolates (bootstrap value 99), and TuYVaRNA2 Karpalund was in a well-supported clade (bootstrap value 93) together with the German isolates of TuYVaRNA2 (Fig. 6).

TABLE 3. Summary of data for sequenced sample (Karpalund) analyzed in this study, taxonomic classification of reads, and assembly of the reads

High-throughput sequencing results	Karpalund (sample K)
Collection date	04/08/19
Sample type	Leaf
Sequence output	77,997,942
Good quality PE reads	75,762,046
Good quality PE reads (%)	97.13%
Classified (% of raw data)	75,422,635 (99.5%)
Eukaryotes	58,790,003 (77.6%)
Virus	1,386,238 (1.83%)
Fungi	446,253 (0.59%)
Bacteria	251,987 (0.33%)
Family *Solemoviridae* (virus family)	1,292,823 (1.71%)
TuYV	483,189 (0.64%)
TuYV RNA	178,523 (0.24%)
TuYV RNA2	261,215 (0.34%)
Assembled contigs	46
Largest contig length (nt)	3,012
Classified contigs	42
Viral contigs	22

Discussion

Crops can be host to a multitude of viruses, and with the changing climate and the increased urge for sustainable practices for disease management, it is of utmost importance to determine the diversity of viruses present in a crop. The present study focused on two surveys carried out in 2019 using Swedish field samples of OSR and sugar beet. TuYV was found to be very common in Sweden and was, for the first time, detected in Swedish sugar beet. The high incidence of TuYV in 2019 may be related to the increased number of aphids in autumn 2018. In Sweden, TuYV has not been monitored on a regular basis, and the current study revealed a considerably higher incidence in OSR compared with surveys conducted in the county of Skåne during 1999 to 2000 and 2003 to 2005, when the incidence in individual fields generally was found to be below 20% and rarely above 50% (Nilsson 2000; Sigvald 2005). This may suggest that the incidence of TuYV is increasing in Swedish OSR crops. There are two discrete ways by which climate change can influence the relationship between crop plants and pests (Roos et al. 2011). First, it affects the biology of the insects, including reproduction, spread, and survival. Second, it influences the agricultural practices, which in turn cause changes in the availability of host plants for the insects transmitting the viruses. The cultivation of winter crops has been on the rise in Sweden, and winter crops receive the aphid vectors carrying the virus quite early in autumn, causing spread of infections. With the rise in temperature in temperate countries, including Sweden, disease epidemics caused by viruses transmitted by aphids have been predicted to be more severe in the future (Jones 2009; Roos et al. 2011). In the current study, the incidence of TuYV was found to be higher in the warmer southern counties of Sweden compared with the central counties of Sweden, which indicates that climate-induced increases in temperature may lead to more active virus vectors and a higher incidence of virus infections in crops. To some extent, the increase in TuYV incidence could be a result of the negative effects of the neonicotinoid ban in the EU (Lundin 2021). Following the ban, the cropping area of winter OSR in Sweden expanded by approximately 40%, making it around 100,000 ha, whereas spring OSR declined by 90% to 4,000 ha (Lundin 2021). This is in contrast with other countries, such as the United Kingdom and Germany, where there has been a decline in the cropping area of winter and spring OSR attributed to increased insect pests (Dewar 2017; Scott and Bilsborrow 2019; Zheng et al. 2020). For management of virus infections, future OSR production in Sweden should include the use of TuYV-resistant cultivars and other integrated pest management practices (Hackenberg et al. 2020; Lundin 2021).

The phylogenetic study and pairwise nucleotide sequence identities of Swedish TuYV OSR isolates, as well as those of TuYVaRNAs, revealed that they were closely related to isolates of pea from Germany and the United Kingdom (Fowkes et al. 2021; Gaafar and Ziebell 2019; Gaafar et al. 2020). Also, in Australia, there are outbreaks of TuYV infections both in legumes and OSR (Filardo et al. 2021). These results indicate that TuYV and TuYVaRNAs easily move between OSR, pea, and other legumes.

In addition, the current study revealed the presence of TuYV in Swedish sugar beet in mixed infections together with two other poleroviruses, BMYV and BChV. The CP gene sequences of the two Swedish TuYV isolates from sugar beet were found to share

high nucleotide identities with TuYV isolates from OSR and pea, suggesting that the infection of sugar beet came from OSR or possibly legumes. So far, there have been only limited reports of TuYV infections of sugar beet in the United Kingdom (Newbert 2016) and possibly in former Czechoslovakia (Pálak 1979; Stevens et al. 2005). The host range of TuYV has not been thought to include sugar beet, but it is possible that some isolates of TuYV have become adapted to sugar beet or that TuYV is able to infect sugar beet in the presence of sugar beet-infecting poleroviruses, such as BMYV or BChV. The occurrence of mixed infections of poleroviruses in sugar beet also increases the risk for the emergence of new virus variants after recombination (Kozlowska-Makulska et al. 2015; Yoshida and Tamada 2019). Recombination is an important driving force behind virus evolution and variations (García-Arenal et al. 2003; Gibbs et al. 2010). In the study by Newbert (2016), among 179 sequenced TuYV genomes, 89 isolates had recombination sites within their genome at nucleotide positions 3,488 (ORFs P3a and P3) and 4,823 (ORF P5). These kinds of recombinations could eventually also lead to an altered host range for viruses.

During the HTS analysis, two TuYVaRNAs were identified in a Swedish OSR sample. Poleroviruses have been found to have associated RNAs, which are single-stranded RNAs with a size around 2.8 to 3 kb, containing two major ORFs (Gaafar and Ziebell 2019). These RNAs replicate autonomously and depend on the helper virus for vector transmission. This is possible by encapsidation of the associated RNAs within the CP of the helper virus to form hybrid virions that can be transmitted by aphids. The associated RNAs are also dependent on helper viruses for systemic movement within the host plants (Chin et al. 1993; Falk and Duffus 1984; Passmore et al. 1993; Sanger et al. 1994). Some of the polerovirus-associated RNAs reported are BWYV ST9 aRNA (Chin et al. 1993), carrot red leaf virus-associated RNA (Adams et al. 2014; Tang et al. 2009), tobacco vein distorting virus associated RNA (Tan et al. 2021), and pepper vein yellows virus-associated RNA (Schravesande et al. 2021). Coinfection with BWYV ST9-aRNA has been reported to elevate the BWYV titer and escalate pathogenicity (Falk and Duffus 1984; Falk et al. 1989; Passmore et al. 1993). Potentially, the TuYVaRNAs could increase the severity of disease, and more studies

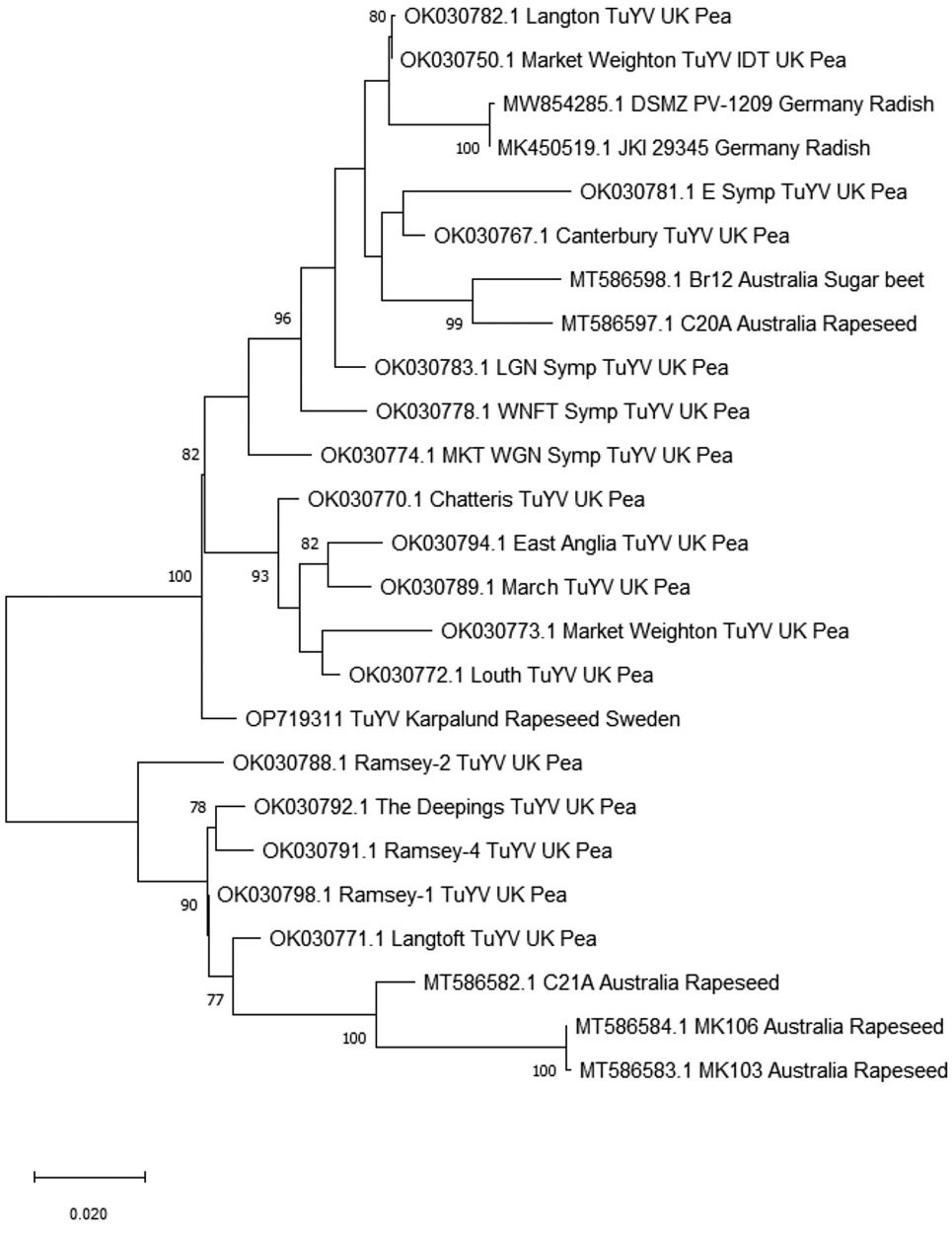


Fig. 5. Maximum likelihood tree with the whole genome of the Swedish isolate of turnip yellows virus (TuYV) from oilseed rape in Karpalund. Reference isolates of TuYV from different regions of the world were included in the analysis, and they are named by GenBank accession number, virus, geographic origin, and host. The values at the nodes are bootstrap values (1,000 iterations) exceeding 60%. The scale shows nucleotide substitutions per site.

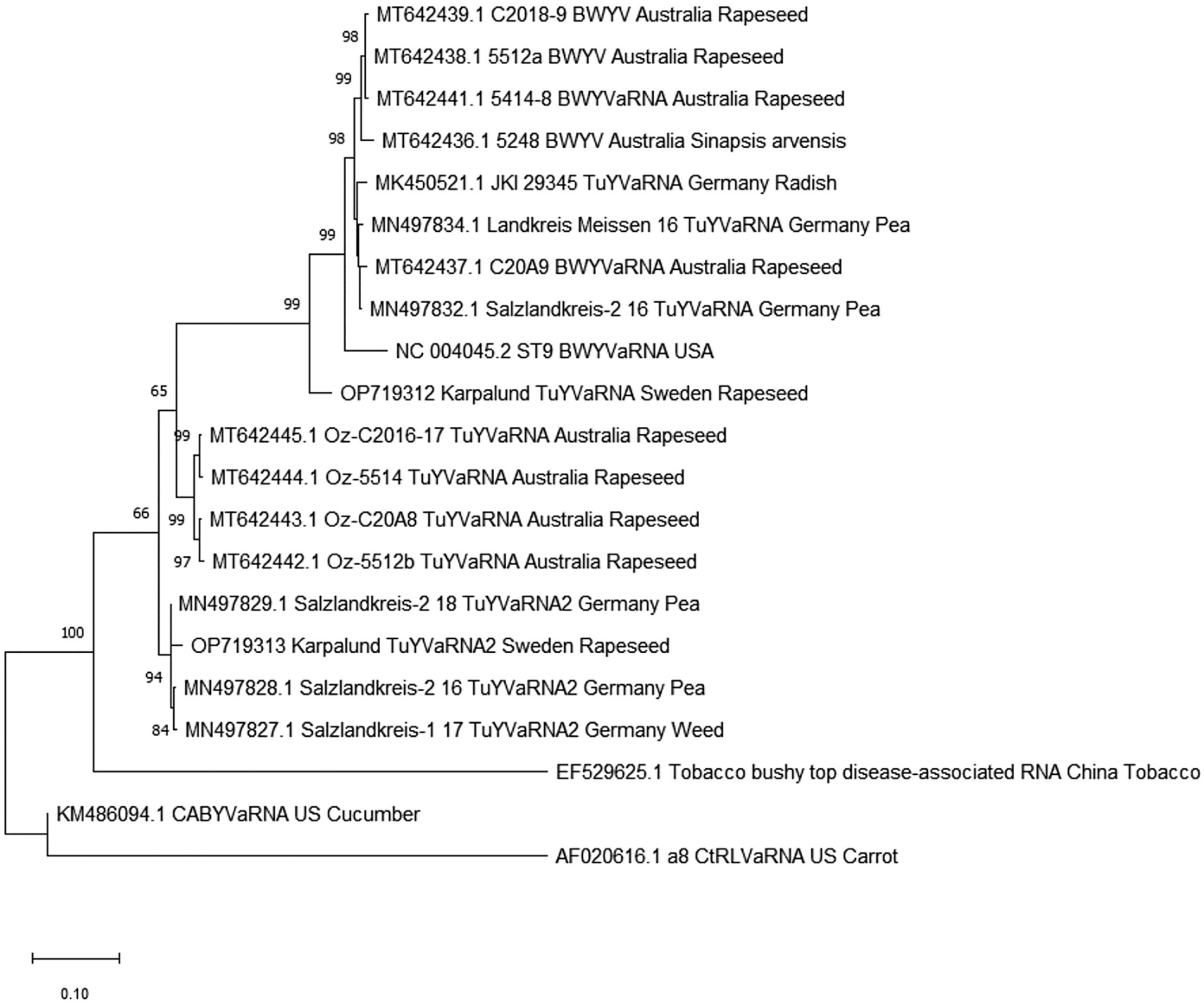


Fig. 6. Maximum likelihood tree with the sequences of the Swedish isolates of turnip yellows virus-associated RNA and turnip yellows virus-associated RNA2 from oilseed rape in Karpalund. Reference isolates of polerovirus-associated RNAs were included in the analysis, and they are named by GenBank accession number, virus, geographic origin, and host. The values at the nodes are bootstrap values (1,000 iterations) exceeding 60%. The scale shows nucleotide substitutions per site.

are required to study the incidence of TuYVaRNAs and their effects on crop plants.

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