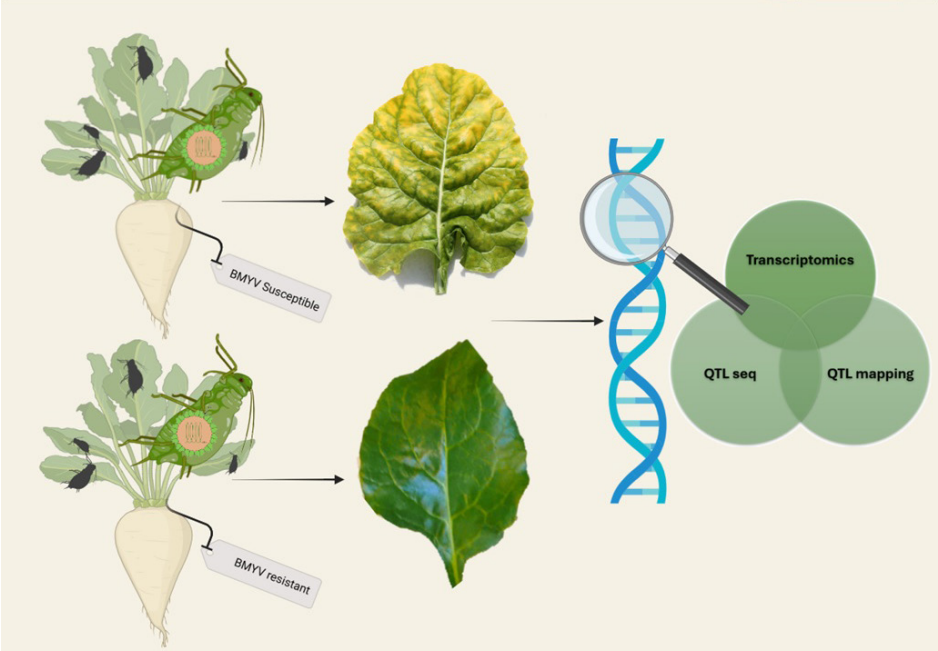
Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2025:2
Faculty of Natural Resources and Agricultural Sciences

Virus yellows of sugar beet – exploring pathogen diversity and host resistance

Groundwork for resistance breeding

Vinitha Puthanveed



Virus yellows of sugar beet – exploring pathogen diversity and host resistance

Groundwork for resistance breeding

Vinitha Puthanveed
Faculty of Natural Resources and Agricultural Sciences
Department of Plant Biology
Uppsala

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS
Uppsala 2025

Acta Universitatis Agriculturae Sueciae
2025:2

Cover: Exploring beet responses to beet mild yellowing virus using transcriptomics and quantitative genetic techniques. Created in BioRender.com by Vinitha Puthanveed.

ISSN 1652-6880
ISBN (print version) 978-91-8046-437-6
ISBN (electronic version) 978-91-8046-487-1
https://doi.org/10.54612/a.aru8g37tkj

Swedish University of Agricultural Sciences, Department of Plant Biology, Uppsala, Sweden

Print: SLU Grafisk service, Uppsala 2025

Virus yellows of sugar beet – exploring pathogen diversity and host resistance: Groundwork for resistance breeding

Abstract

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is a crop primarily cultivated in temperate regions of the world for its high content of sucrose stored in the tap root, which is processed to obtain sugar. The yielding capacity of the crop is threatened by several biotic stresses with virus yellows (VY) being among the viral diseases causing significant yield losses. In Europe, VY is caused by beet mild yellowing virus (BMYV), beet chlorosis virus (BChV) and beet yellows virus (BYV). The main vectors for these viruses are the green peach aphid and the black bean aphid. Insect-transmitted diseases are on rise due to climate change and pose a large threat. We explored the diversity of viruses causing VY in Swedish sugar beet and the incidence of turnip yellows virus (TuYV) in oilseed rape with samples collected from different parts of Sweden in the year 2019. The analyses revealed mixed infections of BMYV and BChV in most of the sugar beet samples and two samples had a triple infection together with TuYV. The survey in oilseed rape revealed a high TuYV incidence with an average infection rate of 75% in the counties of Skåne, Kalmar and Östergötland. With the ban of neonicotinoid chemicals used for managing aphid vectors, there is a greater need for developing resistant or tolerant cultivars as a sustainable alternative. In this study, our aim was also to gain more insights into BMYV – host interactions using transcriptomics and a BMYV-resistant genotype of wild beet in comparison to a susceptible genotype of sugar beet. Virus quantification by RT-qPCR revealed that the wild beet was partially resistant. In the susceptible genotype, a large number of genes were differentially expressed as a response to BMYV infection, while the transcriptomic response of resistant plants was weaker. In the resistant genotype, the differentially expressed genes included seven significantly upregulated genes, which encoded proteins involved in protein processing in the endoplasmic reticulum (ER). This could be one of the mechanisms contributing to the ability of the wild beet genotype to manage ER stress induced by BMYV infection and to reduce the virus level. To identify genomic regions associated with symptom development and BMYV titre, QTLseq and QTL mapping were carried out using F2, S1 and S2 populations of wild beet × sugar beet. These

studies showed that not a single gene was responsible for the responses to BMYV and reduced virus titre, but rather that these traits are governed by multiple loci with minor effects. One significant QTL was identified on chromosome 1 that was linked to reduced virus titre in leaves and explaining 16.7% variation in the trait. Overall, these results would lay a strong foundation for resistance breeding against VY disease in sugar beet.

Keywords: BMYV, genetic mapping, QTLs, sugar beet, transcriptomics, TuYV, virus yellows, wild beet

Virusgulsot i sockerbeta – utforskande av patogendiversitet och värdresistens: en grundstomme för resistensförädling

Sammanfattning

Sockerbeta (*Beta vulgaris* ssp. *vulgaris*) är en gröda som huvudsakligen odlas i tempererade regioner av världen för det höga sackarosinnehållet lagrat i pålroten, vilket processas för att erhålla socker. Grödans avkastningskapacitet hotas av flera biotiska stressfaktorer där virusgulsot är en av de virussjukdomar som orsakar betydande skördeförluster. Virusgulsot orsakas i Europa av beet mild yellowing virus (BMYV), beet chlorosis virus (BChV) och beet yellows virus (BYV). De huvudsakliga vektorerna för dessa virus är persikbladlusen och betbladlusen. Insektsöverförda sjukdomar är på uppåtgående på grund av klimatförändringar och utgör ett stort hot. Vi utforskade diversiteten för de virus som orsakar virusgulsot i Sverige och förekomsten av turnip yellows virus (TuYV) i raps med prover som samlats in i olika delar av Sverige under 2019. Analyserna visade att de flesta proverna av sockerbeta samtidigt var infekterade med både BMYV och BChV, och två prover hade en trippelinfektion tillsammans med TuYV. Undersökningen i raps visade på hög förekomst av TuYV med en genomsnittlig infektionsnivå på 75% i Skåne, Kalmar och Östergötlands län. Med förbudet av det kemiska bekämpningsmedlet neonikotinoider för kontroll av bladlusvektorer finns det ett ökat behov av att utveckla resistenta eller toleranta sorter som ett hållbart alternativ. Vårt mål var i denna studie också att erhålla en djupare förståelse av interaktionerna mellan BMYV och dess värd genom att använda transcriptomics och en BMYV-resistent vildväxande betgenotyp jämfört med en mottaglig genotyp av sockerbeta. Viruskvantifiering med RT-qPCR visade att den vildväxande betan var partiellt resistent. Ett stort antal gener var differentiellt uttryckta som svar på BMYV-infektion i den mottagliga genotypen medan den transkriptionella responsen var svagare i resistenta plantor. I den resistenta genotypen inkluderade de differentiellt uttryckta generna sju signifikant uppreglerade gener som kodar för proteiner involverade i protein-processning i endoplasmatiskt retikulum (ER). Detta kan vara

en av de mekanismer som bidrar till den vildväxande betgenotypens förmåga att hantera ER-stress orsakad av BMYV-infektion och att reducera virusnivån. För att identifiera genomregioner kopplade till symtomutveckling och BMYV-titer utfördes QTLseq och QTL-kartläggning med F2-, S1- och S2-populationer av korsningen vildväxande beta x sockerbeta. Dessa studier visade att inte en enstaka gen var ansvarig för BMYV-responsen och sänkt virustiter, utan att dessa egenskaper snarare bestäms av flera loci med låg effekt. På kromosom 1 identifierades ett signifikant QTL som var kopplat till lägre virushalt i blad och som förklarade 16,7% av variationen i egenskapen. Dessa resultat utgör tillsammans en stark grund för resistensförädling mot virusgulsot i sockerbeta.

Nyckelord: BMYV, genetisk kartläggning, QTL, sockerbeta, transcriptomics, TuYV, vildväxande beta

Preface

The five years of this PhD project are filled with lot of memories. I had a wonderful opportunity to work in a project at SLU in close collaboration with an industrial partner, DLF Beet seeds AB. As a researcher, I always had an urge to do an industrial applied research, and it was accomplished through the SLU GroGrund funding platform that supports research involving public-private partnership. This thesis is a big example of a teamwork of several individuals from SLU as well the industry who worked dedicatedly towards a common goal of finding resistance against virus yellows in sugar beet. All these years have been full of wonderful experiences, from my frequent travel to Skåne for doing my experiments at DLF Beet seeds AB, learning new bioinformatic skills, data analysis, attending international conferences and publishing in reputed journals. Each step of this journey has enabled me to accomplish my dream of doing applied research, learn new skills and motivated me to bring the best of myself to this scientific world. I hope that you will find enriching information as you read through this doctoral thesis.

Warm regards,
Vinitha

Dedication

To the god almighty “श्री कृष्णार्पणमस्तु” “ॐ सर्वशक्त्यैकरूप श्री मूकाम्बिकायै नमो नमः”

To my lovely parents, for always letting me pursue my dreams and for always teaching me “विद्याधनं सर्व धनं प्रधानम्” (meaning that gaining knowledge is the supreme of all wealths)

To my dearest husband, for being the wind beneath my wings to make my dreams come true

To my dearest daughter, who cooperated beautifully inside womb as well as coming to this world to make my dreams come to reality

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Puthanveed, V**., Singh, K., Poimenopoulou, E., Pettersson, J., Siddique, A. B., & Kvarnheden, A. (2023). Milder autumns may increase risk for infection of crops with turnip yellows virus. Phytopathology, 113(9), 1788-1798. https://doi.org/10.1094/PHYTO-11-22-0446-V

II. **Puthanveed, V**., Sajeevan, R. S., Siddique, A. B., Alexandersson, E., Joshi, P., Lennefors, B. L., & Kvarnheden, A. (2024) Transcriptomic responses of beet to infection by beet mild yellowing virus. (manuscript)

III. **Puthanveed, V.**, Lennefors, B. L., Siddique, A. B., Snell, P., Westerbergh, A., & Kvarnheden, A. QTLseq and QTL mapping reveal genomic regions for beet mild yellowing virus resistance and susceptibility in wild beet × sugar beet populations. (manuscript)

The contribution of Vinitha Puthanveed to the papers included in this thesis was as follows:

I. Highly involved in performing the sugar beet part of the experiments, contributed to submitting all the viral sequences in NCBI, analysed the data, wrote the original draft of manuscript together with the input of the co-authors.

II. Planned and executed the experiments with co-authors, collected the samples, involved in part of the sample processing, carried out large part of the data analysis, wrote the manuscript together with the input of co-authors.

III. Participated in the planning and designing of the experiment, highly involved in disease scoring and virus inoculations, analysed the data and wrote the manuscript together with the input of co-authors.

List of tables

List of figures

Abbreviations

STK	Serine/threonine kinase
TNL	Toll/interleukin-1 receptor-nucleotide-binding leucine-rich repeat (TNL)
CMS	Cytoplasmic male sterility
QTL	Quantitative trait loci
PAMP	Pathogen associated molecular pattern
PTI	PAMP triggered immunity
ETI	Effector triggered immunity
PRR	Pattern recognition receptor
RLKs	Receptor like kinases
MAPKs	Mitogen activated protein kinases
ROS	Reactive oxygen species
HR	Hypersensitive response
CP	Coat protein
MP	Movement protein
NB-LRR	Nucelotide-binding leucine rich repeat
ER	Endoplasmic reticulum
ERAD	ER associated degradation
VY	Virus yellows
BMYV	Beet mild yellowing virus
BChV	Beet chlorosis virus
BYV	Beet yellows virus
BWYV	Beet western yellows virus
BtMV	Beet mosaic virus
ORF	Open reading frame
ELISA	Enzyme linked immunosorbent assay

MPR	Mature plant resistance
MAS	Marker assisted selection
NGS	Next generation sequencing
SNP	Single nucleotide polymorphism
QTLseq	Quantitative trait loci sequencing
GWAS	Genome-wide association studies
OSR	Oilseed rape
DEG	Differential gene expression analysis
BiP	Luminal binding protein
GO	Gene Ontology
LOD	Logarithm of odds
H-K	Haley-Knott regression
LMM	Linear mixed model
LOCO	Leave one chromosome out model

1. Introduction

1.1 Sugar beet crop

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is a biennial tuber crop cultivated mainly in temperate regions of the world (Draycott, 2008; Cooke and Scott, 2012). This crop is found to be highly adaptable and can be grown under other climatic conditions as well, like in parts of South America and Asia, and recently found to be growing well in North Africa (Hossain et al., 2017). Already 2000 years ago, different beet varieties were primarily grown as a garden vegetable (Draycott, 2008). During the 1700's, it was further cultivated as a fodder crop and, onwards, produced mainly for sucrose (Biancardi et al., 2012). The history of sugar beet goes back to the mid-18th century. In 1747, sugar crystals from beet juice was first discovered by Andreas Sigismund Marggraf who was a German chemist (Pathak et al., 2022). Franz Karl Achard, who was a student of Marggraf, further developed the process to extract sugar on a bigger scale (Cooke and Scott, 2012). Hence, becoming a pioneer in sugar beet industrial production, in 1801, he selected a local cultivar with white conical tubers called white Silesian sugar beet, which accounted for 6 - 7 % of sugar content for sugar production in Halberstadt (presently Saxony Anhalt, Germany) (Coons, 1955; Bosemark, 1979; Langer and Hill et al., 1991). This is believed to be the progenitor for all the varieties presently cultivated worldwide (Langer and Hill, 1991).

Currently, sugar beet has become one of the major crops for sugar production, apart from sugar cane, accounting for 20% of the sugar production in the world (Food and Agricultural Organization of United Nations, 2024). The major ten producers of sugar beet in the world are

Russia, France, Germany, USA, Turkey, Poland, China, Egypt, Ukraine, and the UK according to the FAOSTAT, 2022 (Figure 1). Currently, sugar beet is cultivated across 50 countries in the world, where the US and Europe are the global leaders in the production of beet sugar (Rana et al., 2022).

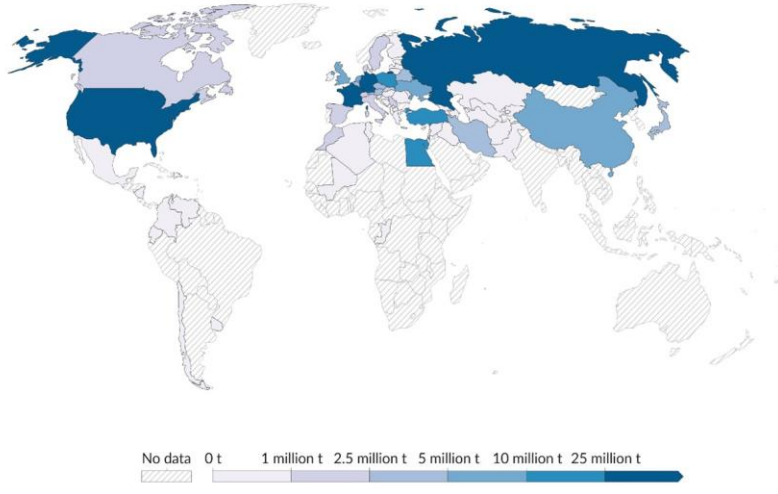


Figure 1 Distribution of sugar beet production in the world, Food and Agriculture Organization of the United Nations (2023). (OurWorldInData.org/agricultural-production)

Sugar beet has a tap root system that stores the sugar produced by the leaves after photosynthesis. The sucrose content can range between 13 and 22% depending on cultivar. Economically, sugar beet is of high importance since the tubers are a major source of sugar for consumption by humans, and there are by-products like the pulp for animal feed and the molasses for bioethanol production (Yu et al., 2020).

1.1.1 Sugar beet gene pool

Sugar beet is a diploid species with 18 chromosomes (2n=18). It is a cross-pollinated crop and biennial in nature. In the vegetative year, it stores carbohydrates in its tap root as a resource for the reproductive phase in the second year (Biancardi, 2005). Sugar beet belongs to the family of Amaranthaceae, order Caryophyllales and it has a C3 photosynthetic system

(Schwichtenberg et al., 2016; Zicari et al., 2019). Other crop plants of this family that are of high economic importance includes spinach, which is consumed as leafy vegetable, and the pseudo cereal quinoa. Ornamentals, like Celosia and Alternanthera, and weeds, like redroot pigweed and alligator weed, also belong to this family. The other cultivated forms of *B. vulgaris* include field beet/fodder beet or the mangelwurzel, containing a lower concentration of sucrose, that is used for livestock, chard (*B. vulgaris* ssp. *vulgaris*, Cicla-Group) and beet root (*B. vulgaris* ssp. *vulgaris*, Conditiva-Group) (Galewski and McGrath, 2020).

Several wild relatives of sugar beet have been acknowledged for a long time and utilized in research for various economic benefits. The *B. vulgaris* ssp. *maritima* (sea beet) is the ancestor of the species cultivated worldwide and found on shores (Biancardi et al., 2010; Biancardi et al., 2012). Sugar beet is interbreedable with sea beet and has been found to be a precious source of resistance against several biotic and abiotic stresses (Schneider et al., 2007). The beets that are found in the Mediterranean are the ones that were first selected and used later for breeding (Draycott, 2008). European coastal areas (Mediterranean and east Atlantic coastlines) are the regions where the wild relatives are predominantly found (Monteiro et al., 2013). The species namely *B. vulgaris* ssp. *adanensis*, *B. macrocarpa*, *B. patula* are found to be in the primary gene pool. The secondary gene pool concerning the relatedness to *B. vulgaris* ssp. *vulgaris* consists of furthermore species belonging to the section Coronillae (*B. nana*, *B. macrorhiza*, *B. carollifora*, and *B. lomatogona*). The wild relatives under the genus *Patellifolia* belong to the tertiary gene pool. They could be an important source for novel traits for broadening the genetic pool against varying biotic and abiotic stresses. There have been different traits successfully introgressed from wild beet species through numerous breeding programme for disease resistance (Coons, 1975; Biancardi, 2005). For example, resistance against *Heterodera schachtii* called as the beet cyst nematode was derived from *B. webbiana*, *B. patellaris* and *B. procumbens* (Cai et al., 1997). Identification of key genes underlying various traits require fine tuning and improvement of the molecular resources, for which genetic and physical maps are essential for a better understanding of the genomic regions responsible for the various traits (Schneider et al., 2007)

1.1.2 Sugar beet genome

During the last many years, several types of research have been done to dissect molecularly the regions underlying several traits in sugar beet. Dohm et al. (2012, 2014) generated a genetic map and physical map of sugar beet with high resolution and density comprehensively giving information about all the nine chromosomes. The genome size of sugar beet is estimated to be 714-758 Mbp. It is also found to share an ancient, triplicated genome with other plants belonging to eudicots (Dohm et al., 2014). Dohm et al. (2014) developed the reference genome, where chromosomes constitute 85% of the genome sequence containing 567 Mbp. A large proportion consists of repetitive sequences, covering 63% of the assembly. Retrotransposons were the most abundant repeat fraction.

The annotation based on sequence homology predicted 27,421 regions coding for proteins. Out of the expected regions, 91% contained start and stop codons, and 73.6% of the genes were observed to be within the chromosomally allotted scaffolds. In average, there were 5.2 genes/100 kb. The average gene length was found to be 5252 bp with introns, with the coding sequence consisting of 1159 bp. There were on average 4.9 exons in a gene. Out of the 17,151 (63%) reference beet genes functionally annotated depending on sequence homology obtained from orthologues and database searches, it was observed that the genes conferring resistance to diseases were comparatively fewer, especially for serine/threonine kinase (STK) domain classes. The toll/interleukin-1 receptor-nucleotide-binding leucine-rich repeat (TNL) class of genes was previously found to be absent in the sugar beet genome according to two studies done by Hunger et al. (2003) and Tian et al. (2004). In contrast, the functional annotation by Dohm et al. (2014) revealed the presence of a TNL class gene in the genome of sugar beet and spinach as a feature of the family Amaranthaceae. This depicts that defence and stress related genes could be one of the vital targets during the process of evolution, resulting in both expansion (adaptation to pathogen pressure or environmental stress) or loss of (reduced pathogen pressure or high maintenance of genes or domestication) some gene families. Compared to the RefBeet genome by Dohm et al. (2014), the newer genome assembly EL10 is more contiguous (McGrath et al., 2023). This reduces the uncertainty regarding genetic marker distribution and improves positions, which can assist the usage of more marker-assisted technology focusing on

any region of the genome. This genome facilitates inter-cultivar comparison between accessions that vary for different traits of interest. EL10.1 is utilized for anchoring other assemblies, e.g., the one used for the identification of beet curly top virus resistance (Galewski et al., 2022; Majumdar et al., 2022). All this information about the genome could be utilized in the future for mapping traits, identifying genes, and cross-referencing with the sequences of other species. When it comes to trait discoveries, one of the major focuses in sugar beet breeding would be to breed for resistance against pest and diseases.

1.1.3 Sugar beet breeding

The main purpose of sugar beet breeding programs is to improve the sugar content in beets. Sugar yield primarily depend on the length of the vegetative growing period of the crop and also on external environmental stress conditions, depending on where the crop is cultivated. Traits from wild sea beet are incorporated into today's breeding programs in sugar beet in different parts of the world to improve the genetic diversity and serve as an important source of resistance or tolerance to various biotic and abiotic stresses (Biancardi et al., 2012). The other traits in sugar beet breeding that are important, apart from breeding for enhanced sugar yield, include resistance to bolting, resistance or tolerance to various pests and pathogens and tolerance to abiotic stresses like drought, salinity and heat. Sugar beet is a biennial plant, but it is commercially grown as an annual crop. Sugar beet plants require exposure to a cold period known as vernalisation to change from vegetative to reproductive phase. Tapping the maximum yield potential greatly depends on the length of the growing season and this is achieved by planting the crop early in spring to prolong it (Draycott, 2008). The reproductive phase of the crop is used for breeding purposes and seed production. External environmental factors like temperature and day length influence the induction of flowering in beets and these factors can be adjusted to shorten the breeding cycle of the crop. One of the important milestone achievements is the transition from multigerm to monogerm plants, and currently, all the beet seeds used for farming in developed countries are monogerm (Savitsky, 1950; Biancardi, 2005; Richardson, 2010).

All commercially available cultivars of sugar beet are 3-way hybrids, which are produced using the male sterility system (McGrath and Panella,

2018). The two kinds of male sterility used in breeding of sugar beet are genetic or nuclear male sterility (NMS), and cytoplasmic male sterility (CMS) (Biancardi, 2005; Draycott, 2006). NMS is controlled by one or more nuclear genes and utilized for cross-pollination by plant breeders. CMS is maternally inherited and governed by both nuclear and cytoplasmic factors. In this type of male sterility, plant breeders have complete control over pollination, which is exploited to produce 3-way hybrids. The initial cross in hybrid production is between the CMS line and O-type pollinator. The O-type line is a genetically divergent genotype with the same nuclear sterility genes as the CMS line but in a normal cytoplasm. The resulting hybrid from this cross is F1MS. F1MS is male sterile and used again as the mother plant in the second cross with the pollinator. The newly produced offspring from this cross is known as the hybrid. Nowadays, the traditional breeding methods in sugar beet are accelerated using modern biotechnological and molecular techniques, such as the use of molecular markers for identifying haploid regenerants from tissue culture that contain sterile cytoplasm, which helps in advancing the breeding process for hybrid production (Karakotov et al., 2021).

1.2 Plant-pathogen interactions and immunity

In natural ecosystems, plants encounter a myriad of abiotic and biotic stresses that impede their growth or lead to metabolic dysfunction (Atkinson and Urwin, 2012; Suzuki et al., 2014; Ben Rejeb et al., 2014). As a result, plants develop complex and dynamic interactions with organisms such as insects and microbes. Among the microorganisms, whether they are pathogenic or not depends upon the balance between the mechanisms used by both sides during the encounter. Resistance to pathogens is of two kinds: host and non-host resistance (Gill et al., 2015). The first is where a particular plant genotype of a susceptible species can exhibit resistance. This type of resistance is observed when R genes, multiple quantitative trait loci (QTLs) for resistance or recessive resistance genes are introgressed into a cultivar/accession of a susceptible genotype, and they are found to be less durable. In contrast, non-host resistance is exhibited by the entire plant species against the virus and can act against all races of the pathogen. Non-host resistance is more durable and complex since it includes multiple pathways for defence. Tolerance is also seen in plants where the plant-

pathogen interaction results in the accumulation of pathogens without causing significant loss of vigour or fitness to their host plant, thereby mitigating the impact of the infection irrespective of the pathogen load. There is a multitude of mechanisms in plants either for resisting or tolerating pathogens and pests. When the interaction between the pathogen and the plant results in a disease, it is called a compatible interaction, and if it results in no disease then the interaction is called incompatible.

1.2.1 Plant-defence mechanisms

During plant-pathogen interactions, plants have a plethora of surveillance mechanisms to identify the invaders and trigger the defences to arrest the pathogen infection (Freeman and Beattie, 2008). The first barrier of defence seen in natural resistance in plants against pathogens is with the help of physical barriers like rigid cell walls, trichomes, wax layers and cuticular lipids or by producing secondary metabolites and degrading enzymes (Muthamilarasan and Prasad, 2013; Wan et al., 2021; Cavaco et al., 2021; Kumar et al., 2023). Once they overcome this barrier, the two layers of immune responses usually seen are called pathogen-associated molecular pattern (PAMP) triggered immunity (PTI) and effector-triggered immunity (ETI) (Boller and Felix, 2009). PTI is elicited once the PAMPs (e.g., bacterial flagellins, cell wall components or elongation factors) are recognized at the cell membrane by pattern recognition receptors (PRRs) (Zipfel, 2008; Dodds and Rathjen, 2010). PTI involves the interaction of pathogens with receptor-like kinases (RLKs) on the cell surface that are proteins playing vital roles in immune responses, cell differentiation and plant growth. PTI and structures of the epidermis constitute the “foremost defence” resulting in basal or non-host resistance. Some of the PAMP-induced immune responses observed are activation of mitogen-activated protein kinases (MAPKs), production of reactive oxygen species (ROS) and reactive nitrogen species like nitric oxide (NO), ion influx and rise in the generation of defence hormones. This cascade of events leads to the synthesis of pathogenesis-related protein (PR proteins), cell wall changes like callose deposition at plasmodesmata and production of antimicrobial compounds (Newman et al., 2013). Pathogens that get adapted to specific hosts find ways to circumvent PTI in different ways. Pathogens have evolved effector proteins in order to weaken the PTI-mediated defence, thereby allowing the pathogen to grow inside the plant host. In order to counteract

these effector proteins, plants have R proteins which consist of three domains, namely nucleotide-binding site (NBS), leucine-rich repeat domain (LRR) and N terminal Toll Interleukin1 receptor (TIR) or coiled-coil (CC) domain. When the R proteins recognize the effector molecules, it leads to ETI (Dodds and Rathjen, 2010). ETI results in programmed cell death at the site of infection causing necrotic spots also known as hypersensitive response (HR) (Muthamilarasan and Prasad, 2013; Wang et al., 2014). It has been now shown that the intracellular receptors and the cell surface are co-dependent on each other to activate the defence responses (Ngou et al., 2021). PTI-induced production of protein kinases and NADPH oxidases is seen to be increased by the intracellular receptors. The elicitation of surface receptors leads to HR triggered by ETI. Thus, there is an interdependence between ETI and PTI.

Among all the pathogenic organisms attacking plants, phytoviruses are among the microbes that rely exclusively on the plant hosts for the completion of their life cycle due to the limited functions encoded by the viral genome. Viruses being obligate parasites, use the host machinery for several purposes such as for their genome replication, expressing viral genes and establishing infection (Calil and Fontes, 2017). Plant viruses require vector organisms, like insects, mites, nematodes, fungi and protists, or mechanical wounds in order to enter host cells. Plant virus particles (virions) consist of nucleic acids surrounded by coat protein forming a capsid and sometimes a lipid envelope. Once entering plant cells, they disassemble to release their genome and commence their infectious cycle. This infectious phase of their life cycle includes replication of their genome, expression of viral genes, cell-to-cell movement, long-distance transport of virus particles or their genomes and transmission to new hosts via vectors. Resistance is attained either naturally or by genetic engineering by preserving plant fitness and not allowing virus accumulation or systemic virus movement in plants (Majumdar et al., 2023). On the other hand, tolerance is accomplished by controlling the over-accumulation of the virus or by reducing the concentration of virulence proteins thereby limiting the damage to the host (Paudel and Sanfaçon, 2018; Jeger, 2023).

Plants deploy several defence strategies against viruses to restrict their replication and movement. These consist of defence mechanisms like gene

silencing, translational repression, protein-degradation pathways, hormone-mediated defence and immune receptor signalling (Calil and Fontes, 2017). Plant viruses are found to use PTI to limit viral infection (Kørner et al., 2013). Regarding viruses, the concept of PTI is more complex than for bacteria and fungi. The double-stranded RNA (dsRNA) acts as a PAMP of the viral pathogen and the antiviral defence mechanism of RNA silencing is analogous to PTI (Ding, 2010). As per the virus-plant interaction model of Mandadi and Scholthof (2013), the viral suppressors of RNA silencing like the coat protein (CP) or movement protein (MP) are considered effectors. R proteins (NB-LRR) recognise and counteract these effectors to trigger ETI. Viral infections can disrupt hormonal pathways and hence induce defence responses moderated by phytohormones (Alazem and Lin, 2015). Host plants are also found to utilize the ubiquitin-proteosome system (UPS) and modify the host proteins involved to defend against viruses (Alcaide-Loridan and Jupin, 2012; Verchot, 2016). Alongside the 26S proteasome, ubiquitin modification of the endoplasmic reticulum (ER) associated cellular as well as viral proteins is vital for infection of the host. Viral proteins are seen to be degraded by ER-associated degradation pathway (ERAD), thereby reducing ER stress and limiting cell death in plants (Verchot, 2016). Autophagy is another protein degradation pathway, whereby viral proteins are transported to vacuoles for degradation and hence preventing the over-accumulation of viral proteins in host plants (Üstün et al., 2017). Mutations in host factors essential for viral infection could lead to developing resistance and mutations in negative regulators of plant defence lead to triggering defence signalling. Host factors (recessive resistance genes) that are naturally present in plants can be identified (e.g., the eukaryotic translation initiation factor (eIF) 4F or its isoforms) and exploited for resistance breeding against viruses (Revers and Nicaise, 2014).

1.2.2 Sugar beet pests and diseases

There are several pathogens and pests that attack the sugar beet crop (Stevanato et al., 2019). The major viral diseases include rhizomania caused by beet necrotic yellow vein virus and transmitted by the plasmodiophorid *Polymyxa betae*; virus yellows (VY) caused mainly by poleroviruses (BMYV, BChV, BWYV) and a closterovirus (BYV) transmitted by aphids; beet curly top caused by beet curly top virus vectored by the beet leafhopper *Circulifer tenellus* (Tamada, 2016; Creamer, 2020; Hossain et al., 2021). The

fungal pathogens causing seedling diseases include *Pythium* spp. and *Aphanomyces cochlioides* (Farhaoui et al., 2023). Foliar disease caused by fungus *Cercospora beticola*, known as Cercospora leaf spot, is also predominant in sugar beet (Tan et al., 2023). Nematodes are also found to cause significant damage in sugar beet, including beet weariness disease caused by sugar beet cyst nematode (*Heterodera schachtii*) and galled roots caused by root knot nematode (*Meloidogyne* spp.) (Chowdhury et al., 2022). Aphids, beet flies, thrips, pygmy mangold beetle, centipedes and silver Y are among the other pests causing feeding damages, and some of these transmit disease as well (Baitha et al., 2022).

1.3 Virus yellows in sugar beet

Virus yellows is a complex disease associated with multiple viruses: beet mild yellowing virus (BMYV), beet chlorosis virus (BChV), beet yellows virus (BYV), beet western yellows virus (BWYV) and beet mosaic virus (BtMV)(Kaya and Yılmaz, 2016; Hossain et al., 2021; Dewar and Qi, 2021). BYV belongs to the family of *Closteroviridae* and genus of *Closterovirus*, while BMYV, BChV and BWYV belong to the family of *Solemoviridae* and are part of the genus *Polerovirus*. Poleroviruses were previously under the family *Luteoviridae*, but in 2021, this genus was moved to the family *Solemoviridae* (Walker et al., 2021). BtMV is very rarely observed and belongs to the genus *Potyvirus* in the family *Potyviridae*. Previously, the TuYV isolates were named as BWYV and were first reported from the United Kingdom (Duffus and Russell, 1970; Stevens et al., 2005a). They are predominantly found to infect plants of the family Brassicaceae, like oilseed rape and weeds as well as legumes like pea. However, in the study conducted by Newbert (2016), one of the isolates of TuYV, called Cau74-R, named after Cau74 from cauliflower, was detected in sugar beet in the UK. Another study have also reported that an isolate suspected to be TuYV was infecting sugar beet in Czechoslovakia (Pálak, 1979). This suggests that TuYV probably has been able to infect sugar beet for many years, but that this has not been studied much in detail.

Figure 2 Image of VY-affected sugar beet along with weeds (Landskrona, Sweden).

1.3.1 Host range, symptoms and impact of disease

The host range for BMYV, BChV, BWYV and BYV varies greatly but are also found to share some host plants (Yoshida and Tamada, 2019). Among all, BWYV has the widest host range with over 150 species in about 23 families (Duffus and Russel, 1970). BMYV has been reported to have a more limited host range (23 species in 8 families) (Duffus, 1973). BYV has been reported to infect plants of 120 species across 15 dicot plant families (Agranovsky and Lesemann, 2011). The alternative host plants serve as virus sources for the VY disease and can also influence the amount of viruliferous aphids. The susceptible plants, like infected overwintering weeds and autumn-sown plants like spinach, can affect the spread of the disease (Björling and Möllerström, 1974). Weeds like *Capsella bursa-pastoris*, *Chenopodium bonushenricus*, and *Senecio vulgaris*, which are perennial in nature, harbour viruses throughout the year acting as virus sources known as a “green bridge” for many years in succession (Björling and Möllerström, 1974; Stevens et al., 2005a).

The green peach aphid (*Myzus persicae*) is the predominant and most efficient vector for viruses causing VY disease in sugar beets (Schliephake et al., 2000; Stevens et al., 2005a; Kozlowska-Makulska et al., 2009; Dewar & Qi, 2021). The other aphid species apart from *M. persicae*, that can transmit BYV, is *Aphis fabae* (Limburg et al., 1977). In the case of poleroviruses, *Macrosiphum euphoria* is also reported to transmit viruses with 89-98% efficiency (Kozlowska-Makulska et al., 2009). BYV is transmitted by aphids in a semi-persistent manner and their virions are

transported via the phloem. They are detected in mesophyll cells, epidermal cells and also plasmodesmata (Dolja and Koonin, 2013). The mode of transmission of poleroviruses is persistent and they are limited to only the phloem cells (Gray and Gildow, 2003; Boissinot et al., 2017). BtMV has a non-persistent way of transmission by aphid vectors (Gallet et al., 2018). The vector transmission efficiency can vary in case of co-infections of viruses causing VY in sugar beet compared to single infections. Co-infection of BChV and BYV in sugar beet, compared to plants with a single infection, resulted in a reduction of BChV transmission by 50%, whereas there was no effect on the transmission of BYV (Khechmar et al., 2024).

BYV symptoms are observed clearly in older leaves with a yellowish discolouration followed by reddish necrosis (de Koeijer and van der Werf, 1999). Beet poleroviruses induce symptoms (chlorosis that range from yellow to orange) 4-6 weeks after the infection in the old leaves of the plants. These discolourations later spread to the whole leaf, which thickens and become brittle resulting in premature death of leaves (Lewellen et al., 1999). The yellowing symptom appearing in the leaves causes a reduction in photosynthesis, in turn affecting the ability of the plants to grow, resulting in loss of yield up to 29% (Stevens et al., 2005a). The symptoms induced by BtMV in beets appear initially as speckles of yellow and later turn into mosaic-like structures. The leaves are also malformed due to the infection (Dunning and Byford, 1982). The intensity of the symptoms can vary in case of co-infections. BtMV co-infection with BYV is reported to cause increased symptoms (measured using fresh biomass) of severe stunting in sugar beet plants. In addition, the overall symptom expression of BWYV-infected plants increased when co-infected with BtMV (Wintermantel, 2005). Traditionally, the disease scoring of VY in sugar beets is performed by visually observing the extent of chlorosis in leaves, and the overall appearance of the plants. However, it is a cumbersome process, labour-intensive and prone to human errors (Bock et al., 2022). Nowadays, efforts are being made to use more advanced tools like unmanned aerial vehicle (UAV) and machine and deep learning techniques to replace the traditional way of phenotyping the VY disease (Okole et al., 2024).

In 2005, a study was done looking at VY incidence and spread, covering 10 countries across three continents (Stevens et al., 2005b). At that time, BMYV was seen more in the northern and western regions of Europe. BChV was more predominant in the southern areas of Europe and in Chile. In a

more recent study (2017 - 2019), BMYV and BChV were more prominent in the northern and western regions of Europe (Hossain et al., 2021). In northern Europe, during the 20th century, VY was considered as the worst disease in sugar beet cultivation (Jaggard et al., 1998). Among the poleroviruses infecting sugar beet, BMYV and BChV cause significant yield reduction (23-27%) (Smith and Hallsworth, 1990; Hossain et al., 2021). In comparison to BMYV, BChV infection is reported to result in more varying yield losses from 8-24% (Stevens et al., 2004). The time point of virus infection is reported to be a crucial factor in determining the extent of yield losses caused by VY (Borgolte et al., 2024). This disease made a devastating comeback in 2020 following the ban of neonicotinoids, which were used for managing the aphid vectors (Dewar and Qi, 2021; Stevens and Bowen, 2021). With climate change, the diseases transmitted by insect vectors like aphids are predicted to increase in the future (Roos et al., 2011). Overwintering survival of the aphid vector facilitated by milder winters results in infection of crops sown in early spring and increases disease incidence (Dewar and Qi, 2021). Mixed infections of poleroviruses (BMYV and BChV) have also been reported in sugar beet (Kazlowska-Makulska et al., 2015). Poleroviruses are seen to be very prone to recombination and with mixed infections, there is a higher risk for the emergence of new virus variants or species (Pagán and Holmes, 2010; Kazlowska-Makulska et al., 2015).

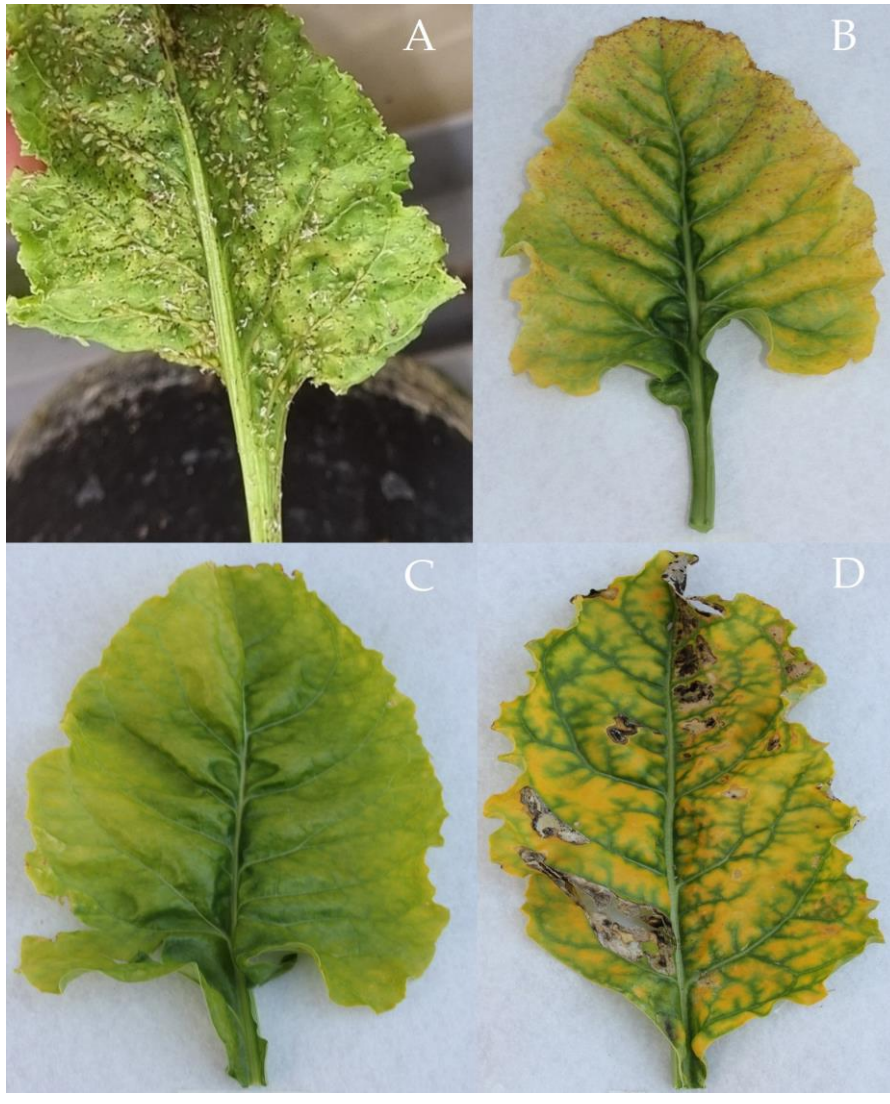


Figure 3 (A) Sugar beet leaf (abaxial side) infested with the aphid *Myzus persicae* (Landskrona, Sweden) (B) BYV symptom in sugar beet leaf (C) BChV symptom in sugar beet leaf (D) BMYV symptom in sugar beet leaf.

1.3.2 Polerovirus genome and gene expression strategies

The genome organization and its gene expression strategies contribute to the adaptability of the virus to new environments, vectors and host plants

(Mayo and Ziegler-Graff., 1996). The viruses belonging to the genus *Polerovirus* share the same genome structure. The physical properties include a non-enveloped spherical virion with icosahedral symmetry (26-34 nm in diameter, 180 monomers of capsid proteins) and the genetic material is single-stranded +sense RNA (Stevens et al., 2005a; Sõmera et al., 2021; LaTourrette et al., 2021). Aphids transmit poleroviruses in a persistent, circulative, non-propagative manner and they are all phloem limited (Schliephake et al., 2000, Gray and Gildow, 2003; Stevens et al., 2005a). The functions of the genes and the expression strategy for one species will apply to viruses of other species within the genus (Stevens et al., 2005a). The genome of poleroviruses (5.6 – 6.2 kb) has at least seven open reading frames (ORFs) and among these there are six ORFs whose functions are known (Stevens et al., 2005a; Delfosse et al., 2021). Out of the six, three ORFs found in the proximal 5′ end are translated directly from the genomic RNA (gRNA). The 5′ end of the genome is protected by a cap formed by the viral genome-linked protein (VPg). The remaining downstream ORFs are translated from sub-genomic RNA (sgRNA).

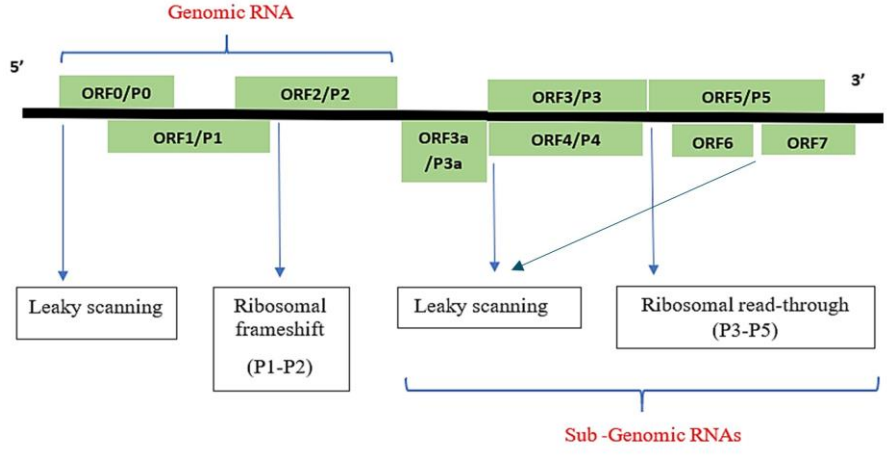


Figure 4 Overview of genome organization and gene expression strategies of poleroviruses.

ORF0 encodes the protein called P0, which is an RNA silencing suppressor and has a role in the process of post-transcriptional gene silencing to overcome host resistance (Pazhouhandeh et al., 2006; Kozlowska-Makulska et al., 2010). ORF1 encodes the VPg, which is released from P1

by proteolysis (Osman et al., 2006). ORF2 encodes an RNA-dependent RNA-polymerase (RdRp), which is expressed as a P1-P2 fusion protein (Hipper et al., 2013; Li et al., 2007). ORF3 and ORF3a encode major coat protein (CP) and P3a, respectively (Kaplan et al., 2007; Smirnova et al., 2015). ORF4 produces a movement protein (MP) (Lee et al., 2002), and the P3-P5 fusion protein from ORF3 and ORF5 is called the minor coat protein (CPm) (Brault et al., 2000; Peter et al., 2009). ORFs 6 and 7 encode P6 and P7, respectively (Patton et al., 2020). Poleroviruses have four significant strategies to express multiple proteins from a genome of a single RNA molecule (Stevens et al., 2005a; LaTourrette et al., 2021). The first strategy is the initiation bypass by leaky scanning of ribosomes from the short sequence at the AUG codon of ORF0 to initiate the translation at the ORF1 start codon. The second type of gene expression strategy is ribosomal frameshift from ORF1 in order for ORF2 to be translated. The last two strategies are the production of sgRNAs (3′ proximal cluster ORFs 3, 4 and 5) and finally proteolytic processing. ORF3a is located in the inter-genic region between ORF2 and ORF3, expressed by sgRNA1 and translated by non-AUG initiation (Smirnova et al., 2015). The translation of P3a, CP and MP occurs by leaky scanning. The ORF5 is expressed as an effect of translational read through by suppressing the amber-stop codon in ORF3. Hence, P5 is part of the minor fusion protein called P3-P5 or the CP read-through (RT) fusion protein. The various functions of the translated proteins are listed in Table 1. The 5′ end of the ORF that encodes P0 and the 3′ end of the read-through domain are found to be more variable among the poleroviruses (LaTourrette et al., 2021). These genome regions could be utilized for distinction between these viruses (Hauser et al., 2000a).

Table 1 Functions of proteins encoded by poleroviruses

ORF	Translated protein	Fusion protein and Read-through protein	Function	Reference
ORF0	P0		Role in virus accumulation	Mayo and Ziegler-Graff, 1996 Sadowy et al., 2001

ORF	Translated protein	Fusion protein and Read-through protein	Function	Reference
			Acts as suppressor in the process of post-transcriptional gene silencing to overcome host resistance	Kozlowska-Makulska et al., 2010 Delfosse et al., 2014 Cascardo et al., 2015
			Role in plant-aphid interaction	Patton et al., 2020
ORF1	P1	P1-P2	Contains two trans-membrane domains in the amino-terminus that have a role in replication complex formation	Hipper et al., 2013
			Contains protease motifs and carries amino acid sequences that are part of the VPg covalently attached to the 5′ end of the genome	Li et al., 2007
ORF2	P2		Role in replication by carrying the viral RNA-dependent RNA-polymerase (RdRp)	Delfosse et al., 2021
ORF3a	P3a		Long distance movement	Smirnova et al., 2015
ORF3	P3		Major capsid protein, main virion component, role in plant-virus interaction, aphid-virus recognition	Terradot et al., 2001 Lee et al., 2002 Kaplan et al., 2007
ORF4	P4		Systemic spread in plants, phloem-specific movement protein	Tacke et al., 1993 Mayo and Ziegler-Graff, 1996 Ziegler-Graff et al., 1996

ORF	Translated protein	Fusion protein and Read-through protein	Function	Reference
				Lee et al., 2002
ORF5	P5		Symptom induction, accumulation of virus and systemic spread	Brault et al., 1995 Ziegler-Graff et al., 1996 Bruyere et al., 1997
			Transmission efficiency, specificity, persistence of virus in the aphid vector	van den Heuvel et al., 1999
		P3-P5	Acquisition of virus, circulation and inoculation by vector	Brault et al., 2000
			Limits the virus infection to the phloem, virus accumulation and movement	Peter et al., 2009
ORF6	P6		Not determined	Delfosse et al., 2021
ORF7	P7		Role in plant-aphid interaction (regulates phytohormones by inhibition of aphid induction of ethylene, improves aphid fecundity)	Patton et al., 2020

Viruses are mainly detected using enzyme-linked immunosorbent assay (ELISA). Still, it is challenging to use this method to distinguish between BMYV and BChV in case of a co-infection (Viganó and Stevens, 2007). Specific primers in RT-PCR targeting the most variable gene regions can distinguish between viruses. This kind of multiplex method could differentiate between BMYV and BChV simultaneously which tells us whether there is a mixed infection (Hauser et al., 2000b). Currently, there are more advanced detection methods like the multiplex reverse transcription-

polymerase chain reaction (mRT-PCR) combined with Luminex xTAG assay in order to detect multiple viruses causing VY at the same time (Schop et al., 2024). However, recombination between viruses complicates the molecular method of distinction between them. Especially in the case of a recombinant virus, it displays high similarity to more than one virus based on the studied gene region (Kozlowska-Makulska et al., 2015). Once the virus is identified, it helps the farmers to manage better the disease severity occurring due to mixed infections and helps plant breeders to develop resistant cultivars accordingly (Stevens et al., 2005b).

1.3.3 Control measures

Virus yellows disease in sugar beet was kept under control by farmers for over 25 years using neonicotinoid seed treatments. With the ban on this chemical in the EU, this disease made a drastic comeback in 2020, making it difficult for farmers to cultivate sugar beet (Dewar and Qi, 2021). The ban on the insecticide usage was imposed due to the decline in the population of bees. In this era of climate change, especially in temperate countries, aphid migrations are occurring much earlier compared to that observed 30-40 years ago (Aldén et al., 2019). There is increased overwintering survival of aphids, and this poses a great threat to sugar beet cultivation. The current chemical control method is insufficient to cope with mass migrations of aphids in the future. There are several integrated pest management practices and other sustainable methods used to manage VY of sugar beet (https://www.britishsugar.co.uk/perch/resources/virus-yellows-pathwaybrochure-web.pdf).

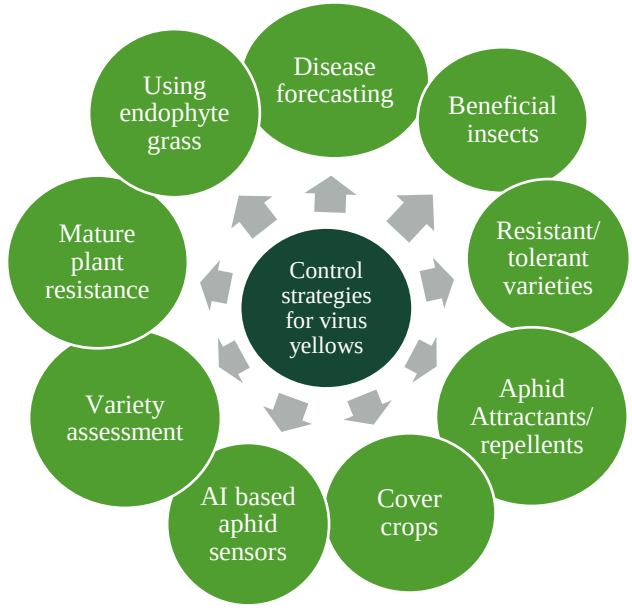


Figure 5 Control methods for VY disease. Adapted from the source: 21149-british-sugar-virus-yellows-pathway-brochure_lr4.pdf.

The major principles for limiting the reductions in yield are preventing virus transmission by vectors, reducing symptoms and creating resistance or tolerance in crop varieties. Aphid monitoring aids in predicting flight activity, assessing risk, and knowing the timing of crop colonization and its intensity of attack on crops so that it helps in implementing proper management practices (Luquet et al., 2023). Aphid attractants that could influence aphid behaviour (pheromones, onion and garlic extracts) can also be used to keep them away from the sugar beet crop (Francis et al., 2022). Cover crops could be used to camouflage sugar beet or to confuse the detection system of aphids, which is also a good strategy for managing the disease (Didenko et al., 2021). Utilizing natural enemies of the aphid vector, like lady bird beetles, parasitic wasps and entomopathogenic fungi, to control the vector would be yet another strategy that could be used to limit this disease (Eilenberg et al., 2009; Ben Fekih et al., 2013). The alternative hosts that serve as infectious sources need to be eliminated before sowing the main crop and careful crop rotation should be done to limit the spread of the disease. The most viable and environmental-friendly method apart from the

other sustainable alternatives for controlling VY in sugar beet, would be to have resistant varieties. Still, no such promising varieties have developed. Mature plant resistance (MPR) is also being deployed to manage VY (Schop et al., 2022). In order to head towards the development of resistant varieties, knowledge of the resistance mechanisms is necessary and available sources should be explored for further studies. Developing resistance by genetic engineering against viruses causing this disease could be exploitable to replace the use of insecticides and hence providing durable strategies for the future (Rollwage et al., 2024).

1.4 Molecular plant breeding methods

Traditional breeding methods involve crosses between elite cultivars with advanced inbred lines that contains novel traits. These processes are tedious as they involve carefully selecting phenotypes to advance generations. While selecting beneficial traits down the generations, there is always a risk of linkage drag, which becomes a hurdle in improving the production of crops (Collard et al., 2005). Molecular breeding methods like marker-assisted selection (MAS) aid in selecting favourable alleles (foreground selection) against undesirable regions of the genome (background selection) in order to improve breeding populations (Rani et al., 2023). Marker-assisted techniques have always been a big boon for plant breeding since they help improving the speed and efficiency of breeding. Predominantly used markers are of two kinds: dominant and co-dominant. Genetic markers that cannot differentiate between homozygous and heterozygous plants and can detect only one allele are known as dominant markers, while those that differentiate between them are called co-dominant markers. Amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and sequence tagged site (STS) (can be used in co-dominant manner as well) are some of the dominant markers used in plant breeding. Currently, co-dominant markers are deployed widely, which include restriction fragment length polymorphism (RFLP), single nucleotide polymorphisms (SNPs), cleaved amplified polymorphic sequences (CAPs), single sequence repeats (SSRs) and transposable elements (TEs). Sequence-based markers like SNPs are most widely used for crop improvement as they cover large variations at the molecular level over the whole genome. SNPs are found at varying

frequencies in coding or non-coding and intergenic regions of the genome (Edwards and Batley, 2010). As the sequencing cost has decreased to a large extent in recent years, next-generation sequencing technology (NGS) is being utilized to produce molecular markers for genotyping a large number of breeding lines (Varshney et al., 2009).

1.4.1 Quantitative genetic approaches

Many traits in plants are genetically complex and are governed by more than one gene. Each of these genes has a small and cumulative effect on the trait of interest. For such traits, the phenotypes in a large population do not belong to discrete classes, but they are rather observed to follow a continuous normal distribution, referred to as quantitative traits. The chromosomal regions or loci contributing to the allelic variation for these quantitative traits are called quantitative trait loci (QTLs). QTL mapping is one of the quantitative genetic approaches that utilizes linkage maps to identify QTLs underlying complex traits like yield or disease resistance in crops (Rani et al., 2023). The primary principle behind QTL mapping is to detect the association between the trait of interest and a genotype (Miles and Wayne, 2008). This technique is facilitated by the help of molecular breeding tools like genetic markers that act as a substitute for phenotypic selection in breeding programs by detecting variation in the DNA (DNA polymorphisms). QTL mapping methods involve several steps (Figure 6). Earlier in the 20th century, the linkage-based mapping approach was predominantly used in plant breeding to disentangle the genetic basis of complex traits. Apart from QTL mapping, genome-wide association studies (GWAS) and genomic selection approaches are commonly used (Ibrahim et al., 2020; Krishnappa et al., 2021). Association mapping studies are usually done in genetically diverse and large population panels. The robustness of this technique is based on the polymorphism existing in the germplasm selected for study and the linkage disequilibrium between the genetic markers.

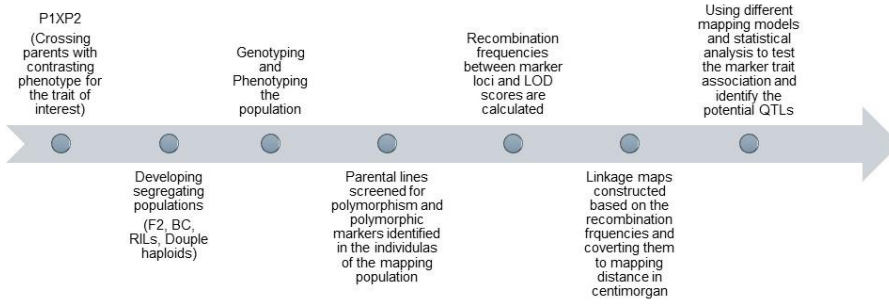


Figure 6 Steps in QTL mapping analysis.

The advantages of GWAS are the better resolution of mapping, less time for research since the need to make the experimental crosses are eliminated, improved allele number and better understanding of all the evolutionary events that occurred in a particular panel of germplasm (Tibbs Cortes et al., 2021; Uffelmann et al., 2021). Genomic selection is another method that identifies the desirable allele controlling a particular trait of interest using molecular markers deployed across the genome (Jannink et al., 2010; Crossa et al., 2017). This technique is highly useful for the traits governed by multiple QTL regions. Phenotypic and genotypic data of a training population is utilized to generate a prediction model. This model is further used to estimate the genetic or breeding values of all the individuals in an experimental breeding population, only based on their genotypes. This enables further prediction of their phenotypes, and the best ones can be selected for future crossings in the breeding program. There are also molecular breeding methods combining QTL mapping and whole genome sequencing called QTLseq (Takagi et al., 2013; Martínez-Guardiola et al., 2024). The prerequisite for performing this technique, similar to QTL mapping, is developing a segregating population for the trait of interest. The population is then phenotyped and the plants that exhibit extreme phenotypes (20-50 plants in each bulk) are selected for further study. Whole genome sequencing is performed on DNA extracted from the bulks. The sequences obtained are aligned to a reference genome, and the SNP indexes of the bulks are calculated. The delta SNP indexes, obtained by subtracting the SNP index of bulk two from the SNP index of bulk one, are ultimately used to interpret the QTLseq results. The genomic regions with ΔSNP values above the confidence threshold (95 or 99% significance level) are considered as candidate QTL regions for governing the trait of interest. A positive or

negative ΔSNP index could mean that the alleles in that genomic region are more frequent in the bulk with the higher or lower phenotypic values, respectively.

1.4.2 Transcriptomic techniques in plant-virus interactions

In recent years, transcriptomic approaches in plant breeding have gained more importance. Genomic studies facilitate an overall understanding of genetic information, whereas transcriptomic studies enable understanding of gene expression patterns at the cellular, tissue or organism level. This method helps in knowing the transcript level changes at different developmental stages, environmental conditions, or even at various time points of exposure to pathogens. In addition, this approach enables detection of all kinds of transcript species like mRNA, small RNAs and non-coding RNAs. The two main types of methodologies in transcriptomics are either based on hybridization or sequencing (Wang et al., 2009). DNA microarray technology is based on hybridization. Technologies like suppression subtractive hybridization (SSH), serial analysis of gene expression (SAGE) and RNA sequencing (RNAseq) are some of the sequence-based approaches in transcriptomics. The RNAseq techniques require cDNA library preparation from high quality RNA in order to allow sequencing via the NGS platform. The cDNA library preparation involves steps like capturing mRNA or depletion of ribosomal RNA (rRNA) followed by converting RNA to cDNA by reverse transcription and adaptor ligation at the ends of the cDNA. In organisms with well-annotated genomes, single-end reads are usually used. In poorly annotated genomes, paired-end reads are used to read the fragments that must be sequenced (Conesa et al., 2016). Sequenced read sizes can range from 35 to 500 base pairs in length. Further, after sequencing, the transcript reads are aligned to the respective reference genome followed by differential gene expression analysis (DESeq) and gene ontology (GO) analysis to unravel the various functional pathways affected. RNAseq is also a good approach for understanding and disentangling mechanisms behind plant-virus interactions in different crops (Zanardo et al., 2019). This method has also been utilized for many years for studying various traits in sugar beet, especially to examine responses to biotic stresses (Fernando Gil et al., 2020; Ghaemi et al., 2020; Holmquist et al., 2021; Rossi, 2023). Combined

transcriptomic and genomic approaches could be used to understand complex interactions between pathogens in host crops.

2. Aim of the study

With the restriction on use of neonicotinoids within the EU to manage aphids and with no cultivars available with resistance to these viruses, it is of utmost importance to develop genetic virus resistance in crops for sustainable disease management and combatting future yield losses. The overall goal of this thesis was to unravel the diversity of viruses associated with virus yellows in Swedish sugar beet and to characterise the resistance against BMYV in a resistance source identified by DLF Beet Seeds AB. The other specific objectives of the manuscripts, which are part of this thesis were:

1. Identify the viruses associated with the VY disease complex in Sweden and determine the relationships between different isolates of viruses by phylogenetic analysis. This knowledge is essential for resistance breeding.

2. Analyse a resistant genotype that has been identified by DLF Beet Seeds AB by:
   - ❖ Determining the responses of the resistant wild beet in comparison to a susceptible cultivar and disentangle the possible mechanisms of defence by transcriptome analysis.
   - ❖ Identifying the genetic determinants of resistance through QTLseq and QTL mapping.

Through this thesis, together with DLF Beet Seeds AB, we aimed to share applied research knowledge and pave the way for resistance breeding to develop better varieties for sugar beet farmers.

3. Results and Discussion

3.1 High incidence of turnip yellows virus in winter oilseed rape

Climate change poses a considerable risk for crops in Sweden being infected by insect-transmitted viruses (Roos et al., 2011). In 2018, the Swedish Board of Agriculture reported an unusually high number of green peach aphids in suction traps in southern Sweden (Aldén et al., 2019). This indicated a high risk for infection of oilseed rape (OSR, *Brassica napus*) with turnip yellows virus (TuYV). TuYV is commonly infecting OSR in many countries, which may result in substantial yield losses (Congdon et al., 2020). Hence, a survey was conducted in the spring of 2019 by collecting random leaf samples from six counties of Sweden covering 46 fields of OSR in order to determine the incidence of TuYV and to study the genetic diversity of TuYV (Figure 2, Paper I). Tests of 20 or 90 samples per field with DAS-ELISA detected TuYV in all the fields except for one field in Hjo, located in Västra Götaland (Table 1, Paper I). The survey unveiled a high incidence of TuYV in OSR, especially in the counties of Östergötland, Kalmar and Skåne with an average TuYV incidence of 75%. The results also indicated that OSR fields in southern and south-eastern Sweden had a much higher virus incidence than fields from central and south-western Sweden. The high incidence of TuYV in OSR in Sweden could be due to climate change, where aphids are more active during milder autumns allowing them to transmit virus to seedlings of winter OSR. Furthermore, this could also have been a consequence of the ban on neonicotinoid insecticides in the EU that were used for managing the aphid vectors. RT-PCR analysis of nine ELISA-

positive samples confirmed TuYV infection in the OSR samples. Comparing the sequences of the CP gene of the Swedish TuYV isolates showed that they were highly identical (99.5 – 100%) (Figure 3, Paper I). Based on nucleotide sequence comparisons and phylogenetic analyses, the Swedish isolates from Hörninge and Lårstad were found to be closely related to TuYV isolate 3b from OSR in the UK (Figures 3 and 4, Paper I), while the other Swedish TuYV isolates were more closely related to pea isolates of TuYV from the UK. High-throughput sequencing of the Swedish isolate from Karpalund using RNA isolated from the leaf sample enabled the assembly of genome sequences for TuYV and two TuYV-associated RNA molecules (TuYVaRNA and TuYVaRNA2). Similar to the CP gene, the complete genome sequence of TuYV-Karpalund was most closely related to pea isolates of TuYV from the UK (Figure 5, Suppl. Figure S2, Paper I). The two TuYVaRNAs were detected for the first time in Sweden and shared high nucleotide sequence identities with German isolates of TuYVaRNA and TuYVaRNA2 from pea (Figure 6, Suppl. Figure S3, Paper I). Recently, TuYV outbreaks have occurred in both legumes and OSR with a high incidence of TuYV in pea in both Germany and the UK (Gaafar and Ziebell, 2019; Gaafar et al., 2020; Fowkes et al., 2021; Filardo et al., 2021). The close relationships of the Swedish isolates of TuYV, TuYVaRNA and TuYVaRNA2 with pea isolates from the UK and Germany indicate that TuYV easily can move between OSR and pea.

3.2 Mixed infections of poleroviruses in sugar beet

In 2018, following the ban on chemicals (neonicotinoids) used in Europe for aphid control, there was an increased demand for developing more sustainable practices to manage aphid-transmitted diseases in many crops, including sugar beet. However, to develop cultivars with resistance or tolerance cultivars against viral diseases, it was important to have a better understanding of the diversity of viruses transmitted by aphids. After the ban on neonicotinoids, an outbreak of VY was reported in the UK in 2020 (Dewar and Qi, 2021). Following that, virus monitoring was expanded in many European countries (Hossain et al., 2021). In Sweden, a survey was conducted in October 2019, collecting leaves displaying chlorotic symptoms

in sugar beet from four fields in the county of Skåne in southern Sweden. For six out of twenty-five samples, infection of poleroviruses was identified using TAS-ELISA. These ELISA-positive samples and an additional polerovirus-infected sample from DLF Beet Seed AB were used for molecular and phylogenetic analyses. Testing these samples by RT-PCR targeting the CP gene confirmed infection with poleroviruses, while BYV was not detected in any of the samples. The RT-PCR products were cloned and three clones were sequenced for each of the seven isolates. Sequence analyses revealed infection with BMYV in all samples, whereas BChV was found in five samples (3, 17, 19, 22, 24). This is the first report of BChV in Sweden. For two samples (3 and 24), the analyses also identified triple infection of BMYV, BChV and TuYV. With both pairwise sequence comparisons and phylogenetic analyses, there was a clear demarcation of the clones into BMYV, BChV and TuYV (Figures 3 and 4, Paper I). The Swedish BMYV clones shared highest nucleotide identity at 99.3 to 99.5% to the Broom's Barn isolate of BMYV from the UK (EF107543), and they were all closely related in the phylogenetic tree with a high bootstrap value (Figures 3 and 4, Paper I). The BChV clones shared high nucleotide sequence identity at 99.7 to 100% to European isolates from France (MW367424) and the UK (L39952) (Figure 3, Paper I). These reference isolates were also in the same clade as the Swedish BChV clones in the phylogenetic tree (Figure 4, Paper I). Even though the host range of TuYV is not known to include sugar beet, a few studies have reported TuYV in sugar beet (Pálak, 1979; Newbert, 2016). Our survey found TuYV in two samples along with BMYV and BChV. It is possible that over the years, TuYV has adapted to sugar beet as a host, or that it can infect in the presence of other poleroviruses. Mixed infections are always a risk factor as they may lead to the emergence of new virus variants by recombination and changes in host range (García-Arenal et al., 2003; Yoshida and Tamada, 2019). Poleroviruses are known to be prone to recombinations (Kozlowska-Makulska et al., 2015; Newbert, 2016) and it is therefore important with continuous monitoring for the emergence of new virus variants with potentially increased transmission efficiency, increased virulence, ability to overcome resistance or changed host range.

3.3 Transcriptomic study identifies potential candidate genes for partial resistance to beet mild yellowing virus in wild beet source

Gaining insights into the molecular basis of interactions between BMYV and sugar beet would pave the way towards developing resistance against this virus. In manuscript II, we performed transcriptomic studies, looking at the responses of a resistant genotype (wild beet source) and a susceptible genotype (breeding line of sugar beet) to BMYV. It will also give a general understanding of the biological, cellular and molecular pathways triggered in these genotypes upon infection with BMYV. The ultimate goal was to find candidate genes involved in defence responses to BMYV and understand the underlying resistance mechanism in the wild beet genotype. Treatments in the experiment included plant exposure to viruliferous aphids (Inoculated), healthy aphids without virus (Healthy control) and only insecticide spray (Insecticide control). Inoculation responses to BMYV were observed in inoculated (old) leaves at 0, 1, 4, 14, 21 and 28 days post-inoculation (DPI). Similarly, observations were also made in systemic (young) leaves at 14, 21 and 28 DPI. The wild beet genotype remained green throughout the experiment, whereas symptoms appeared at 14 DPI in the susceptible genotype (Figure 1, Manuscript II). The old, inoculated leaves of the susceptible genotype turned completely yellow and fell off by 28 DPI, while chlorotic symptoms became visible in the second leaf pair.

RT-qPCR analysis showed that the virus titre varied between the genotypes at 14, 21 and 28 DPI in both old inoculated and young systemic leaves (Figure 2, Manuscript II). However, a significant difference was observed only at 21 DPI in the young systemic leaves. The virus titre in the susceptible genotype was always found to be 2-4 fold higher than in the resistant genotype in both old and young leaves at different time points after inoculation. Reduced virus titre and no symptom expression in the resistant genotype imply that it is partially resistant against this virus. There have been previous reports on partial resistance against BMYV using wild sea beet sources and also in other cultivated *Beta* species, which have been introgressed into sugar beet breeding lines (Asher et al., 2001; Grimmer et al., 2008), but no resistance has been reported so far in sugar beet.

The outcome of the gene expression analysis revealed that more genes were significantly expressed (FDR < 0.05 and log2FoldChange > 1) in response to BMYV inoculation in the susceptible genotype (1398 genes) than in the resistant one (235 genes) combining all leaf ages and time points (Table 1). Among the significant DEGs, 137 genes were found to be uniquely expressed in the resistant genotype, and 1157 genes in the susceptible genotype (Figure 3, Manuscript II). This implies that the transcriptome response to BMYV inoculation was much stronger in the susceptible than in the resistant genotype. Fourteen significant DEGs were shortlisted as potential candidate genes for resistance (Table 2, Manuscript II). These genes are known for their functions in immune responses to biotic stresses, including virus infections in plants, in regulating virus accumulation in the host and also for involvement in symptom development in host plants. Seven DEGs in the resistant genotype that were uniquely upregulated are known to be involved in the endoplasmic reticulum protein degradation pathway (ERAD). This could be one of the resistance mechanisms acting in the wild beet genotype by reducing endoplasmic reticulum (ER) stress. Previously, a study on potato leaf roll virus (polerovirus) have reported that the CP – luminal binding protein (BiP) interaction could be the basis for reduced ER cytotoxicity, which is otherwise generated by high levels of viral protein accumulation. It could be hypothesised that a similar mechanism would be operating in BMYV – beet interaction as well because the motif of the CP, which is interacting with BiP, is conserved across many poleroviruses, including BMYV (Figure 8, Manuscript II). The BiP gene was significantly upregulated at 21 DPI in the resistant wild beet genotype.

Table 2 Selected uniquely upregulated genes in the resistant genotype involved in viral protein degradation pathways, plant defence responses to viruses and in symptom development

EL10 gene ID	Annotation	Log2fold change	Time point	Description
EL10Ac5g11039[a]	Thioredoxin-like 1-2, chloroplastic	1.02	21 DPI (old)	Role in preventing oxidative damage of antioxidant enzymes, reducing virus accumulation in the host plant,
		1.17	21 DPI (young)	

				SA-mediated defence responses
EL10Ac3g07016	Putative disease resistance protein RGA3	1.39	28 DPI (young)	Disease resistance protein (CC-NBS-LRR class) family involved in plant defence to pathogens
EL10Ac3g07017	Putative disease resistance protein RGA4	1.65	28 DPI (young)	
EL10Ac2g03638	Cytochrome P450 CYP73A100	1.27	21 DPI (old)	Role in secondary metabolite production and in response to wounding
EL10Ac8g18590	Cytochrome P450 734A1	1.06	28 DPI (young)	
EL10Ac6g15151	Leucine-zipper of ternary complex factor MIP1	1.10	21 DPI (old)	Involved in endoplasmic-reticulum associated protein degradation (ERAD) pathway
EL10Ac4g09930	Luminal-binding protein 4	1.05	21 DPI (old)	
EL10Ac3g07108	EGF domain-specific O-linked N-acetylglucosamine transferase	1.05	21 DPI (old)	
EL10Ac3g06084	Probable E3 ubiquitin ligase SUD1	1.14	14 DPI (young)	
EL10Ac8g20617	Vacuolar protein sorting-associated protein 28 homolog 2	1.38	28 DPI (young)	
EL10Ac3g05286	Calcineurin B-like protein 4	1.14	28 DPI (young)	
EL10Ac4g07519	Probable LRR receptor-like serine/threonine-protein kinase At4g26540	1.10	14 DPI (young)	Role in recognition of PAMPs and triggering immune responses
EL10Ac1g00851	Chalcone synthase	1.24	21 DPI (old)	Role in secondary

				metabolite production
EL10Ac5g10982	Transcription factor TCP15	1.01	28 DPI (young)	Transcription factor family involved in ETI

[a] Upregulated gene in resistant and downregulated in susceptible genotype

The gene ontology (GO) analysis revealed that the top enriched biological processes in the resistant genotype included response to organic cyclic compounds and response to salicylic acid, which are known to play a role in immune responses to plant pathogens including viruses (Hammerbacher et al., 2019; Murphy et al., 2020) (Figure Suppl. 6-A, Manuscript II). On the other hand, DNA damage response, DNA replication, DNA metabolic process and DNA templated DNA replication were the top enriched GO IDs in the susceptible genotype (Figure Suppl. 6-B, Manuscript II). These biological processes are known to be observed as responses to virus infections in host plants due to the pressure on the cellular machinery developed because of oxidative damage caused by reactive oxygen species (Jeong et al., 2023). Three KEGG pathways (Photosynthesis, DNA replication and biosynthesis of secondary metabolites) were enriched in the susceptible genotype, whereas no KEGG pathways were enriched in the resistant genotype (Figure Suppl. 5, Manuscript II). Symptom expression together with photosynthesis pathways enriched in KEGG analysis and downregulation of biological processes related to light harvesting systems (photosystem I and II) and chlorophyll metabolic processes all indicate that there may be drastic changes in chloroplast structure and functions of susceptible plants. Chlorosis is a common symptom of compatible virus infections occurring due to altered chloroplast structure and functions affecting the photosynthesis machinery (Li et al., 2015). It could also be inferred that the defence response to BMYV is weak in the susceptible genotype. As a commonality between both genotypes tested, it was observed that genes connected to production of secondary metabolites display significant changes in expression levels showing that they play a crucial role in the response to BMYV. The potential candidate genes identified by transcriptomics need to be functionally verified to confirm their role in defence responses and to gain further insights into the resistance mechanisms.

3.4 QTLseq reveals genomic regions associated with BMYV symptom expression in an F2 population of wild beet × sugar beet

QTLseq was one among the population genetic analyses used for determining the genomic positions linked to BMYV resistance in the F2 population, which was obtained by crossing a resistant genotype of wild beet to a susceptible genotype of sugar beet (same as used for RNAseq studies). This quantitative genetic method integrates bulk segregant analysis and high throughput whole genome sequencing to identify QTLs associated with a trait of interest at an early stage in a segregating population. Phenotyping for VY can be done in multiple ways. Traditionally, VY disease in sugar beet is phenotyped by looking at the visual symptoms appearing on the leaves with the extent of chlorosis and leaf brittleness, as well as the overall appearance of the plant (Bock et al., 2022). We performed disease scoring (scale from 1-9) on the F2 population by looking at visual symptoms. We found that the population distribution of disease scores was skewed towards the susceptible parent, but displayed a continuous variation (Figure 2, Manuscript III). Plants with extreme phenotypes (30 plants each for resistant and susceptible bulks) were selected for DNA extractions. The DNA for the parental genotypes and pooled DNA for each bulk were genotyped by whole genome sequencing. The QTLseq analysis resulted in identifying 5470 positions linked to the trait using a sliding window analysis (SNP index < 0.4, window size of 2000 kb and step size of 100 kb) with 39 peaks across chromosome 2, 6 and 8 crossing the significant 95% confidence interval (Table 2, Figure 3, Manuscript III). The significant peaks obtained consisted of ΔSNP index values ranging from 0.24 – 0.29 indicating that these genomic positions explained only 24 - 29% of the differences in allele frequencies between the two extreme bulks for phenotypic response to BMYV infection. The continuous distribution of the population and absence of any single genomic region explaining more than 50% variation between the bulks indicate that several genomic regions with minor effects control the trait. The susceptible parent contributed to most of the key genomic regions identified. Among the significant regions identified, Bevul.8G078200 (Eukaryotic translation initiation factor 4E) on chromosome 8 was one of the essential candidate genes identified. It is a well-known gene for susceptibility to poleroviruses (Gallagher, 2013; Rollwage et al., 2024) (Suppl. Table 1, Manuscript III). Further studies are

required to understand the potential role of these genes in determining resistance or susceptible to BMYV in beet.

3.5 Mapping studies identify a significant major QTL associated with leaf virus titre in an S2 population of wild beet × sugar beet

In order to locate the QTLs responsible for resistance to BMYV in the wild beet genotype, an S2 segregating population consisting of 245 inbred lines was evaluated for virus content in leaves and roots using TAS-ELISA. The continuous normal distribution of virus titre in both leaves and roots revealed that a single dominant gene does not govern these traits. Instead, they showed a quantitative nature (Figure 4, Manuscript III). Genotyping was performed using 22000 SNP molecular markers in the S1 parents that produced the S2 individuals. A linkage map was constructed with 2250 polymorphic markers (Figure 5, Manuscript III) and the logarithm of odds (LOD) statistical test was performed to detect significant QTLs using three models: Haley-Knot regression model (H-K), linear mixed model (LMM) and leave one chromosome out model (LOCO). In addition, a multi-QTL analysis was performed using composite interval mapping. With data for the virus titre of leaves, one QTL with significant LOD score was identified in chromosome 1 at 7.6 Mbp using two single QTL models (H-K and LMM) as well as by composite interval mapping. The QTL explained 16.7% of the variation in the trait (Table 3 and 4, Figure 6A, Manuscript III). A LOD score > 3 generally indicates that the molecular marker and the functional gene region are close enough to give a significant marker-trait association. In our study, using leaf virus content data, a QTL (LOD score > 3) explaining more than 10% variation in the trait with a significant additive effect indicates that it is a major QTL for BMYV resistance in the wild beet genotype. In this QTL region, eight genes were identified as potential candidates for future studies because of their known roles in virus resistance in plants: Bevul.1G156900 (Leucine-rich repeat-containing protein), Bevul.1G157000 (Phloem protein 2-like), Bevul.1G157100 (Mitogen-activated protein kinase), Bevul.1G157400 (cAMP-response element binding protein related-bZIP transcription factor family protein), Bevul.1G159500 (Jacalin-like lectin domain), Bevul.1G159800 (Thioredoxin H1), Bevul.1G159900

(Thioredoxin H1) and Bevul.1G160000 (Germin-like protein) (Table 6, Manuscript III). Among these proteins are the Phloem protein 2-like protein (PP2), which previously has been reported to be the phloem partner associated with cucurbit aphid-borne mosaic polerovirus (Bencharki et al., 2010). Furthermore, overexpression of the PP2-encoding gene repressed phloem feeding by aphids in *Arabidopsis thaliana* (Zhang et al., 2011). Functional studies for these candidate genes are required to prove their role in BMYV resistance. With data for the virus content of roots, one significant QTL with substantial LOD scores was detected on chromosome 8 using all three models. However, the variance explained by this QTL was non-significant (Table 3 and 5, Figure 6B, Manuscript III). QTL analysis using data for virus titre of roots could not detect QTLs explaining significant variation in the trait. For future studies on the detection of major QTLs associated with resistance and susceptibility to BMYV, tests of further selfed generations with a larger population size could be performed. Testing the population in the field under more natural conditions would allow us to understand the environmental impact on the identified QTL.

4. Summary of findings and conclusions

The main findings are:

- **Virus diversity study**: TuYV in oilseed rape (OSR) as well as BMYV and BChV in sugar beet are the most common poleroviruses found in these crops in Sweden based on the results for a single year (2019). TuYV was also found in sugar beet. Mixed infections of three viruses and climate change pose a potential threat to sugar beet and OSR crops in the future.

Tests with ELISA, RT-PCR and sequencing showed that TuYV has become very common in OSR plants in Sweden with average infection rates of 75% in the counties Skåne, Kalmar and Östergötland. RT-PCR results revealed that sugar beet plants with VY were infected with both BChV and BMYV (mixed infections), with BChV being reported for the first time in Sweden. Two sugar beet samples had a triple infection of BMYV, BChV and TuYV. Poleroviruses are prone to recombination, and mixed infections in the same host constitute a risk for the emergence of new polerovirus genotypes with changed properties, including broadened host range. The presence of TuYV in sugar beet may be a spillover from OSR. Phylogenetic analysis of the CP gene showed that the Swedish isolates of BMYV, BChV and TuYV were closely related to European isolates. Complete genome sequences of TuYV, TuYVaRNA and TuYVaRNA2 were recovered from a Swedish OSR sample (Karpalund isolate). The Swedish TuYV isolate was found to be closely related to pea isolates from the UK, which indicates that TuYV could be easily transmitted between OSR and pea. TuYVaRNA and TuYVaRNA2 were previously not reported in Sweden.

- **Virus quantification by RT-qPCR and transcriptome analysis**: With absence of symptoms and reduced virus titre, a genotype of wild beet was found to be partially resistant to BMYV. A susceptible genotype of sugar beet showed a much stronger transcriptomic response than the wild beet. Candidate genes for resistance were identified among upregulated genes in response to BMYV in resistant plants, which also indicated a possible mechanism for resistance.

Plants of the resistant genotype did not display symptoms even at 28 DPI. They had a lower virus titre than the susceptible genotype, indicating partial resistance and not complete resistance or tolerance against BMYV. There were more significant DEGs for the susceptible genotype than the resistant genotype, which indicates that the transcriptome response to BMYV infection was stronger in the susceptible plants than the resistant ones. Seven genes significantly upregulated in the resistant genotype encode proteins involved in protein processing in the ER, which could be one of the mechanisms contributing to the ability of the resistant genotype to manage ER stress induced by BMYV infection.

- **Quantitative genetic studies**: The resistance to BMYV of the wild beet genotype could be explained as a combined effect of multiple loci with minor effects.

Continuous normal distribution displayed by the F2 population together with low ΔSNP index values (0.24 – 0.29) obtained explaining less than 50% variation between the bulks indicate the possibility of several regions in the genome with minor effects being involved in the phenotypic responses to BMYV infection in leaves. By QTL mapping with data for virus content of leaves, one major QTL was detected in chromosome 1 with significant LOD scores using two single QTL analysis models as well as by multi-QTL analysis. The QTL explained 16.7% of the variance in the trait, and this infers that the QTL has a significant association with BMYV resistance in leaves. There were signals for different chromosome regions using data for virus content of roots, but they did not explain any substantial variation in the trait. In order to narrow down the chromosomal regions associated with the trait

and identify the other significant QTLs, mapping studies can be conducted in further selfed generations with larger population sizes.

5. Future perspectives

One of the biggest challenges that the world is facing these days is that of climate change. Climate change impacts multiple sectors, including agriculture, where it has a huge effect on crop production and insect pests. Agricultural pests are adaptable organisms, which respond in several ways to climate change. They are seen to enhance their overwintering survival and also broaden their geographic and host ranges in response to the increased temperatures in temperate regions. Due to these adaptations, plant diseases transmitted by insects also increase in various crops. In order to meet the demands of the growing population in food production and simultaneously combat climate change, we should keep in mind the need to achieve sustainability through agricultural practices. To achieve this goal, integrated pest management approaches are carried out where the chemical usage is limited and enhanced sustainable practices are carried out for managing pests and diseases. Multiple strategies exist to manage VY in sugar beet (Figure 5).

Disease forecasting is the first and foremost step in any disease management strategy. The virus diversity study done in OSR and sugar beet clearly showed that mixed infections of poleroviruses, which are transmitted by aphids along with the worsening climate change can be a potential threat in the future. Insect surveillance needs to be improved in temperate countries like Sweden, where the aphid activity could indicate future viral disease transmission. Currently, aphid monitoring and counting based on artificial intelligence for better predicting their activity are being used and could aid in controlling early infections of crops (Gao et al., 2024). Based on when the

aphids arrive in the sugar beet crop, infection risks could be forecasted before the crop season and necessary measures to be taken could be conveyed to the farmers before the crop season, which would help them managing the disease better. Virus testing also needs to be conducted more frequently in crops like OSR, sugar beet and legumes as well as in weeds that are hosts for poleroviruses to look for the appearance of new virus genotypes, which could be dangerous in the future.

Cover crops can be grown ahead of sugar beet that could trap aphids and improve soil structure and nutrition, which would benefit the sugar beet yield. However, cover crops could also act as virus sources, and hence, it is important that the cover crop is ploughed down. Fields should be monitored for any possible sources of aphid-infested or overwintering crop plants, which could be potential hosts for these viruses, and proper hygiene of the field should be maintained before planting sugar beet. Predators of aphids, like lady bird beetles or lace wings, could be utilised to reduce the number of viral vectors. Volatiles like pheromones or other natural substances like onion or garlic extracts attract aphids and keep them away from the sugar beet crop. New approaches are also being tested where endophytic grasses carrying natural toxins against aphids are grown before sugar beet, which enables transferring this natural resistance to beets (https://bbro.co.uk/media/51018/23-jan-vy-integrated-approach.pdf).

Mature plant resistance (MPR) is yet another mechanism that could be utilised in managing aphids transmitting VY (Schop et al., 2022). Sugar beet plants at the 10-12 leaf stage significantly affect the fecundity and survival rate of aphids conferring MPR compared to younger plants. Genotypes differ in their levels of MPR to aphids and appropriate selection of the genotype for planting can help manage VY. Exploiting the sugar beet germplasm in order to develop resistant or tolerant cultivars is a crucial VY management strategy for the future. Variety assessment needs to be done in parallel with developing new cultivars to monitor their resistance/tolerance level regularly and accordingly, better choices can be recommended to farmers for planting. The knowledge obtained through this research would lay a strong foundation for future studies to understand BMYV-sugar beet interactions better. Genomic regions have been identified that are linked to BMYV resistance and susceptibility in wild beet × sugar beet breeding populations. Further

mapping in selfed generations with larger population sizes will help detecting other QTLs for resistance. Furthermore, functional studies on the shortlisted candidate genes from the transcriptomic studies would enable an understanding of their role in the immune responses to BMYV in sugar beet. These results would provide a strong groundwork for resistance breeding against VY. This project aimed to contribute towards developing durable resistance to BMYV and help farmers prevent severe crop losses due to virus infections.

References

Agranovsky, A. A., & Lesemann, D.-E. (2011). Closterovirus. In: Tidona, C. & Darai, G. (Eds) The Springer Index of Viruses. New York, NY: Springer, pp. 327–333.

Alazem, M., & Lin, N. S. (2015). Roles of plant hormones in the regulation of host–virus interactions. *Molecular Plant Pathology*, 16(5), 529-540.

Alcaide-Loridan, C., & Jupin, I. (2012). Ubiquitin and plant viruses, let's play together!. *Plant physiology*, 160(1), 72-82.

Aldén, A., Berg, G., Christerson, T., Gerdtsson, A., Söderlind, C., & Östlund, R. (2019). Växtskyddsåret 2019 – Hallands, Skånes och Blekinge län. JO19:8, Jordbruksverket, Jönköping, Sweden.

Asher, M. J., Luterbacher, M. C., & Frese, L. (2001). Wild *Beta* species as a source of resistance to sugar-beet pests and diseases. *International Sugar Journal*, 103, 447-451.

Atkinson, N. J., & Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany*, 63(10), 3523-3543.

Baitha, A., Srivastava, S., & Misra, V. (2022). Insect-pests of sugar beet and their integrated management. In: Misra, V., Srivastava, S., & Mall, A. K. (Eds) Sugar Beet Cultivation, Management and Processing. Singapore: Springer, pp. 643-657.

Ben Fekih, I., Boukhris-Bouhachem, S., Eilenberg, J., Allagui, M. B., & Jensen, A. B. (2013). The occurrence of two species of Entomophthorales (Entomophthoromycota), pathogens of *Sitobion avenae* and *Myzus persicae* (Hemiptera: Aphididae), in Tunisia. *BioMed Research International*, 2013(1), 838145.

Ben Rejeb, I., Pastor, V., & Mauch-Mani, B. (2014). Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants*, 3(4), 458-475.

Bencharki, B., Boissinot, S., Revollon, S., Ziegler-Graff, V., Erdinger, M., Wiss, L., Dinant, S., Renard, D., Beuve, M., Lemaitre-Guillier, C. & Brault, V. (2010). Phloem protein partners of Cucurbit aphid borne yellows virus: possible involvement of phloem proteins in virus transmission by aphids. *Molecular Plant-Microbe interactions*, 23(6), 799-810.

Biancardi, E. (2005). Genetics and breeding of sugar beet. Boca Raton: CRC Press.

Biancardi, E., McGrath, J. M., Panella, L. W., Lewellen, R. T., & Stevanato, P. (2010). Sugar beet. In: Bradshaw, J. E. (Ed.) Root and Tuber Crops. New York: Springer, pp. 173-219.

Biancardi, E., Panella, L. W., & Lewellen, R. T. (2012). *Beta maritima*: The Origin of Beets. New York: Springer.

Björling, K., & Möllerström, G. (1974). Incidence and importance of beet yellowing viruses in Sweden 1946 to 1973. *Socker Handlingar*, 26, 1–14.

Bock, C. H., Chiang, K.-S., & Del Ponte, E. M. (2022). Plant disease severity estimated visually: a century of research, best practices, and opportunities for improving methods and practices to maximize accuracy. *Tropical Plant Pathology*, 47, 25–42.

Boissinot, S., Pichon, E., Sorin, C., Piccini, C., Scheidecker, D., Ziegler-Graff, V., & Brault, V. (2017). Systemic propagation of a fluorescent infectious clone of a polerovirus following inoculation by agrobacteria and aphids. *Viruses*, 9(7), 166.

Boller, T., & Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology*, 60(1), 379-406.

Borgolte, S., Varrelmann, M., & Hossain, R. (2024). Time point of virus yellows infection is crucial for yield losses in sugar beet, and co-infection with beet mosaic virus is negligible under field conditions. *Plant Pathology*, 73(8), 2056-2070

Bosemark, N. O. (1979). Genetic poverty of the sugar beet in Europe. In: Zeven, A. C., & van Harten, A. M. (Eds) Proceedings of the Conference Broadening the Genetic Base of Crops, Wageningen, Netherlands, pp. 29-35.

Brault, V., Mutterer, J., Scheidecker, D., Simonis, M. T., Herrbach, E., Richards, K., & Ziegler-Graff, V. (2000). Effects of point mutations in the readthrough domain of the beet western yellows virus minor capsid protein on virus accumulation in planta and on transmission by aphids. *Journal of Virology*, 74(3), 1140-1148.

Brault, V., van den Heuvel, J. F., Verbeek, M., Ziegler-Graff, V., Reutenauer, A., Herrbach, E., Garaud, J., Guilley, H., Richards, K., & Jonard, G. (1995). Aphid transmission of beet western yellows luteovirus requires the minor capsid read-through protein P74. *The EMBO Journal*, 14(4), 650-659.

Bruyere, A., Brault, V., Ziegler-Graff, V., Simonis, M. T., Van den Heuvel, J. F. J. M., Richards, K., Guilley, H., Jonard, G., & Herrbach, E. (1997). Effects of mutations in the beet western yellows virus readthrough protein on its expression and packaging and on virus accumulation, symptoms, and aphid transmission. *Virology*, 230(2), 323-334.

Cai, D., Kleine, M., Kifle, S., Harloff, H. J., Sandal, N. N., Marcker, K. A., Klein-Lankhorst, R. M., Salentijn, E. M., Lange, W., Stiekema, W. J., Wyss, U., Grundler, F. M., Florian M. W., & Jung, C. (1997). Positional cloning of a gene for nematode resistance in sugar beet. *Science*, 275(5301), 832-834.

Calil, I. P., & Fontes, E. P. (2017). Plant immunity against viruses: antiviral immune receptors in focus. *Annals of Botany*, 119(5), 711-723.

Cascardo, R. S., Arantes, I. L., Silva, T. F., Sachetto-Martins, G., Vaslin, M. F., & Corrêa, R. L. (2015). Function and diversity of P0 proteins among cotton leafroll dwarf virus isolates. *Virology Journal*, 12, 1-10.

Cavaco, A. R., Matos, A. R., & Figueiredo, A. (2021). Speaking the language of lipids: the cross-talk between plants and pathogens in defence and disease. *Cellular and Molecular Life Sciences*, 78(9), 4399-4415.

Chowdhury, I. A., Yan, G., & Khan, M. (2022). Diseases caused by nematodes on the sugar beet. In: Misra, V., Srivastava, S., & Mall, A. K. (Eds). Singapore: Springer, pp. 737-749.

Clover, G. R. G., Azam-Ali, S. N., Jaggard, K. W., & Smith, H. G. (1999). The effects of beet yellows virus on the growth and physiology of sugar beet (*Beta vulgaris*). *Plant Pathology*, 48, 129–138.

Collard, B. C., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, 142, 169-196.

Conesa, A., Madrigal, P., Tarazona, S., Gomez-Cabrero, D., Cervera, A., McPherson, A., Szcześniak, M. W., Gaffney, D.J., Elo, L. L., Zhang, X., & Mortazavi, A. (2016). A survey of best practices for RNA-seq data analysis. *Genome Biology*, 17, 1-19.

Congdon, B. S., Baulch, J. R., & Coutts, B. A. (2020). Impact of turnip yellows virus infection on seed yield of an open-pollinated and hybrid canola cultivar when inoculated at different growth stages. *Virus Research*, 277, 197847.

Cooke, D. A., & Scott, J. E. (2012). The sugar beet crop. London: Chapman & Hall.

Coons, G. H. (1975). Interspecific hybrids between *Beta vulgaris* L. and the wild species of *Beta*. *Journal of the American Society of Sugar Beet Technologists*, 18, 281-306.

Coons, G. H., Owen, F. V., & Stewart, D. (1955). Improvement of the sugar beet in the United States. *Advances in Agronomy*, 7, 89-139.

Creamer, R. (2020) Beet curly top virus transmission, epidemiology, and management. In: Awasthi, L. P. (Ed.) Applied Plant Virology. Academic Press, pp. 521-527.

Crossa, J., Pérez-Rodríguez, P., Cuevas, J., Montesinos-López, O., Jarquín, D., De Los Campos, G. G., Burgueño, J., González-Camacho, J. M., Pérez-Elizalde, S., Beyene, Y., Dreisigacker, S., Singh, R., Zhang, X., Gowda, M., Roorkiwal, M., Rutkoski, J., & Varshney, R. K. (2017). Genomic selection in plant breeding: methods, models, and perspectives. *Trends in Plant Science*, 22(11), 961-975.

de Koeijer, K. J., & van der Werf, W. (1999). Effects of beet yellows virus and beet mild yellowing virus on leaf area dynamics of sugar beet (*Beta vulgaris* L.). *Field Crops Research*, 61(2), 163-177.

Delfosse, V. C., Agrofoglio, Y. C., Casse, M. F., Kresic, I. B., Hopp, H. E., Ziegler-Graff, V., & Distéfano, A. J. (2014). The P0 protein encoded by cotton leafroll dwarf virus (CLRDV) inhibits local but not systemic RNA silencing. *Virus Research*, 180, 70-75.

Delfosse, V. C., Barrios Barón, M. P., & Distéfano, A. J. (2021). What we know about poleroviruses: Advances in understanding the functions of polerovirus proteins. *Plant Pathology*, 70(5), 1047-1061.

Dewar, A. M., & Qi, A. (2021). The virus yellows epidemic in sugar beet in the UK in 2020 and the adverse effect of the EU ban on neonicotinoids on sugar beet production. *Outlooks on Pest Management*, 32(2), 53-59.

Didenko, N., Konovalova, V., Razzaghi, S., Bandaogo, A., Bardhan, S., & Sundermeier, A. (2021). Cover crops for pests and soil-borne disease control and insect diversity. In: Islam, R., & Sherman, B. (Eds) Cover Crops and Sustainable Agriculture. Boca Raton: CRC Press, pp. 84-98

Ding, S. W. (2010). RNA-based antiviral immunity. *Nature Reviews Immunology*, 10(9), 632-644.

Dodds, P. N., & Rathjen, J. P. (2010). Plant immunity: towards an integrated view of plant–pathogen interactions. *Nature Reviews Genetics*, 11(8), 539-548.

Dohm, J. C., Lange, C., Holtgräwe, D., Sörensen, T. R., Borchardt, D., Schulz, B., Lehrach, H., Weisshaar, B., & Himmelbauer, H. (2012). Palaeohexaploid ancestry for Caryophyllales inferred from extensive gene-based physical and genetic mapping of the sugar beet genome (Beta vulgaris). *The Plant Journal*, 70(3), 528-540.

Dohm, J. C., Minoche, A. E., Holtgräwe, D., Capella-Gutiérrez, S., Zakrzewski, F., Tafer, H., Rupp, O., Sörensen, T .R., Stracke, R., Reinhardt, R. and Goesmann, A., Kraft, T., Schulz, B., Stadler, P. F., Schmidt, T., Gabaldón, T., Lehrach, H., Weisshaar, B., & Himmelbauer, H. (2014). The genome of the recently domesticated crop plant sugar beet (Beta vulgaris). *Nature*, 505(7484), 546-549.

Dolja, V. V., & Koonin, E. V. (2013). The closterovirus-derived gene expression and RNA interference vectors as tools for research and plant biotechnology. *Frontiers in Microbiology*, 4, 83.

Draycott, A. P. (Ed.). (2008). Sugar beet. Oxford: Blackwell Publishing Ltd.

Duffus, J. E. (1973). The yellowing virus diseases of beet. *Advances in Virus Research*, 18, 347-386.

Duffus, J. E., & Russell, G. E. (1970). Serological and host range evidence for the occurrence of beet western yellows virus in Europe. *Phytopathology*, 60(1), 199-1202.

Dunning, A. & Byford, W. (1982) Pests, Diseases and Disorders of the Sugar Beet. Deleplanque: Brooms Barn Experimental Station.

Edwards, D., & Batley, J. (2010). Plant genome sequencing: applications for crop improvement. *Plant Biotechnology Journal*, 8(1), 2-9.

Eilenberg, J., Meyling, N. V., & Jensen, A. B. (2009). Insect pathogenic fungi in biological control: status and future challenges. IOBC WPRS Bulletin, 7-10.

Farhaoui, A., Tahiri, A., Khadiri, M., El Alami, N., & Lahlali, R. (2023). Fungal root rots of sugar beets: A review of common causal agents and management strategies. *Gesunde Pflanzen*, 75(5), 1411-1440.

Fernando Gil, J., Wibberg, D., Eini, O., Savenkov, E. I., Varrelmann, M., & Liebe, S. (2020). Comparative transcriptome analysis provides molecular insights into the interaction of *Beet necrotic yellow vein virus* and *Beet soil-borne mosaic virus* with their host sugar beet. *Viruses*, 12(1), 76.

Filardo, F., Nancarrow, N., Kehoe, M., McTaggart, A. R., Congdon, B., Kumari, S., Aftab, M., Trębicki, P., Rodoni, B., Thomas, J., & Sharman, M. (2021). Genetic diversity and recombination between turnip yellows virus strains in Australia. *Archives of Virology*, 166(3), 813-829.

Fowkes, A. R., McGreig, S., Pufal, H., Duffy, S., Howard, B., Adams, I. P., Macarthur, R., Weekes, R., & Fox, A. (2021). Integrating high throughput sequencing into survey design reveals turnip yellows virus and soybean dwarf virus in pea (*Pisum sativum*) in the United Kingdom. *Viruses*, 13, 2530.

Francis, F., Then, C., Francis, A., Gbangbo, Y. A. C., Iannello, L., & Ben Fekih, I. (2022). Complementary strategies for biological control of aphids and related virus transmission in sugar beet to replace neonicotinoids. *Agriculture*, 12(10), 1663.

Freeman, B., & Beattie, G. (2008). An overview of plant defences against pathogens and herbivores. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2008-0226-01.

Gaafar, Y. Z., & Ziebell, H. (2019). Two divergent isolates of turnip yellows virus from pea and rapeseed and first report of turnip yellows virus-associated RNA in Germany. *Microbiology Resource Announcements*, 8, e00214-19.

Gaafar, Y. Z., Herz, K., Hartrick, J., Fletcher, J., Blouin, A. G., MacDiarmid, R., & Ziebell, H. (2020). Investigating the pea virome in Germany—Old friends and new players in the field (s). *Frontiers in Microbiology*, 11, 583242.

Galewski, P. J., & Eujayl, I. (2022). A roadmap to durable BCTV resistance using long-read genome assembly of genetic stock KDH13. *Plant Molecular Biology Reporter*, 40(1), 176-187.

Galewski, P., & McGrath, J. M. (2020). Genetic diversity among cultivated beets (Beta vulgaris) assessed via population-based whole genome sequences. *BMC Genomics*, 21, 1-14.

Gallagher, E. W. (2013). A molecular genetic analysis of resistance to poleroviruses in sugar beet and oilseed rape. Doctoral dissertation, University of East Anglia.

Gallet, R., Michalakis, Y., & Blanc, S. (2018). Vector-transmission of plant viruses and constraints imposed by virus–vector interactions. *Current Opinion in Virology*, 33, 144-150

Gao, X., Xue, W., Lennox, C., Stevens, M., & Gao, J. (2024). Developing a hybrid convolutional neural network for automatic aphid counting in sugar beet fields. *Computers and Electronics in Agriculture*, 220, 108910.

García-Arenal, F., Fraile, A., & Malpica, J. M. (2003). Variation and evolution of plant virus populations. *International Microbiology*, 6, 225-232.

Ghaemi, R., Pourjam, E., Safaie, N., Verstraeten, B., Mahmoudi, S. B., Mehrabi, R., De Meyer, T., & Kyndt, T. (2020). Molecular insights into the compatible and incompatible interactions between sugar beet and the beet cyst nematode. *BMC Plant Biology*, 20, 1-16.

Gill, U. S., Lee, S., & Mysore, K. S. (2015). Host versus nonhost resistance: distinct wars with similar arsenals. *Phytopathology*, 105(5), 580-587.

Gray, S., & Gildow, F. E. (2003). Luteovirus-aphid interactions. *Annual Review of Phytopathology*, 41(1), 539-566.

Grimmer, M. K, Bean, K. M., Luterbacher, M. C., Stevens, M., & Asher, M.J. (2008). Beet mild yellowing virus resistance derived from wild and cultivated *Beta* germplasm. *Plant Breeding*, 127(3), 315-318.

Hammerbacher A, Coutinho TA, Gershenzon J. (2019). Roles of plant volatiles in defence against microbial pathogens and microbial exploitation of volatiles. *Plant, Cell & Environment*. 42(10):2827-43.

Hauser, S., Stevens, M., Mougel, C., Smith, H. G., Fritsch, C., Herrbach, E., & Lemaire, O. (2000b). Biological, serological, and molecular variability suggest three distinct polerovirus species infecting beet or rape. *Phytopathology*, 90(5), 460-466.

Hauser, S., Weber, C., Vetter, G., Stevens, M., Beuve, M., & Lemaire, O. (2000a). Improved detection and differentiation of poleroviruses infecting beet or rape by multiplex RT-PCR. *Journal of Virological Methods*, 89(1-2), 11-21.

Hipper, C., Brault, V., Ziegler-Graff, V., & Revers, F. (2013). Viral and cellular factors involved in phloem transport of plant viruses. *Frontiers in Plant science*, 4, 154.

Holmquist, L., Dölfors, F., Fogelqvist, J., Cohn, J., Kraft, T., & Dixelius, C. (2021). Major latex protein-like encoding genes contribute to *Rhizoctonia solani* defence responses in sugar beet. *Molecular Genetics and Genomics*, 296, 155-164.

Hossain, M. S., ElSayed, A. I., Moore, M., & Dietz, K. J. (2017). Redox and reactive oxygen species network in acclimation for salinity tolerance in sugar beet. *Journal of Experimental Botany*, 68(5), 1283-1298.

Hossain, R., Menzel, W., Lachmann, C., & Varrelmann, M. (2021). New insights into virus yellows distribution in Europe and effects of beet yellows virus,

beet mild yellowing virus, and beet chlorosis virus on sugar beet yield following field inoculation. *Plant Pathology*, 70(3), 584-593.

Hunger, S., Gaspero, G. D., Möhring, S., Bellin, D., Schäfer-Pregl, R., Borchardt, D. C., Durel, C.E., Werber, M., Weisshaar, B., Salamini, F., & Schneider, K. (2003). Isolation and linkage analysis of expressed disease-resistance gene analogues of sugar beet (*Beta vulgaris* L.). *Genome*, 46(1), 70-82.

Ibrahim, A. K., Zhang, L., Niyitanga, S., Afzal, M. Z., Xu, Y., Zhang, L., Zhang, L., & Qi, J. (2020). Principles and approaches of association mapping in plant breeding. *Tropical Plant Biology*, 13, 212-224.

Jaggard, K. W., Dewar, A. M., & Pidgeon, J. D. (1998). The relative effects of drought stress and virus yellows on the yield of sugar beet in the UK, 1980–95. *The Journal of Agricultural Science*, 130(3), 337-343.

Jannink, J. L., Lorenz, A. J., & Iwata, H. (2010). Genomic selection in plant breeding: from theory to practice. *Briefings in Functional Genomics*, 9(2), 166-177.

Jeger, M. J. (2023). Tolerance of plant virus disease: Its genetic, physiological, and epidemiological significance. *Food and Energy Security*, 12(6), e440.

Jeong, H. W., Ryu, T. H., Lee, H. J., Kim, K. H., & Jeong, R. D. (2023). DNA Damage Triggers the Activation of Immune Response to Viral Pathogens via Salicylic Acid in Plants. *The Plant Pathology Journal*, 39(5), 449.

Kaplan, I. B., Lee, L., Ripoll, D. R., Palukaitis, P., Gildow, F., & Gray, S. M. (2007). Point mutations in the potato leafroll virus major capsid protein alter virion stability and aphid transmission. *Journal of General Virology*, 88(6), 1821-1830.

Karakotov, S. D., Apasov, I. V., Nalbandyan, A. A., Vasilchenko, E. N., & Fedulova, T. P. (2021). Modern issues of sugar beet (Beta vulgaris L.) hybrid breeding. *Vavilov Journal of Genetics and Breeding*, 25(4), 394.

Kaya, R., & Yılmaz, N. D. K. (2016). Distribution of some aphid-borne viruses infecting sugar beet in Turkey. *Sugar Industry-Zuckerindustrie*, 141(12), 747-752.

Khechmar, S., Chesnais, Q., Villeroy, C., Brault, V., & Drucker, M. (2024). Interplay between a polerovirus and a closterovirus decreases aphid transmission of the polerovirus. *Microbiology Spectrum*, 12(11), e01115-24.

Kørner, C. J., Klauser, D., Niehl, A., Domínguez-Ferreras, A., Chinchilla, D., Boller, T., Heinlein, M., & Hann, D. R. (2013). The immunity regulator BAK1 contributes to resistance against diverse RNA viruses. *Molecular Plant-Microbe Interactions*, 26(11), 1271-1280.

Kozlowska-Makulska, A., Beuve, M., Syller, J., Szyndel, M. S., Lemaire, O., Bouzoubaa, S., & Herrbach, E: (2009). Aphid transmissibility of different European beet polerovirus isolates. *European Journal of Plant Pathology*, 125, 337-341.

Kozlowska-Makulska, A., Guilley, H., Szyndel, M. S., Beuve, M., Lemaire, O., Herrbach, E., & Bouzoubaa, S. (2010). P0 proteins of European beet-infecting poleroviruses display variable RNA silencing suppression activity. *Journal of General Virology*, 91(4), 1082-1091.

Kozlowska-Makulska, A., Hasiow-Jaroszewska, B., Szyndel, M. S., Herrbach, E., Bouzoubaa, S., Lemaire, O., & Beuve, M. (2015). Phylogenetic relationships and the occurrence of interspecific recombination between beet chlorosis virus (BChV) and Beet mild yellowing virus (BMYV). *Archives of Virology*, 160, 429-433.

Krishnappa, G., Savadi, S., Tyagi, B. S., Singh, S. K., Mamrutha, H. M., Kumar, S., Mishra, C.N., Khan, H., Gangadhara, K., Uday, G., & Singh, G. P. (2021). Integrated genomic selection for rapid improvement of crops. *Genomics*, 113(3), 1070-1086.

Kumar, S., Korra, T., Thakur, R., Arutselvan, R., Kashyap, A. S., Nehela, Y., Chaplygin, V., Minkina, T & Keswani, C. (2023). Role of plant secondary metabolites in defence and transcriptional regulation in response to biotic stress. *Plant Stress*, 8, 100154.

Langer, R. H. M., & Hill, G. D. (1991). Agricultural plants. Cambridge University Press.

LaTourrette, K., Holste, N. M., & Garcia-Ruiz, H. (2021). Polerovirus genomic variation. *Virus Evolution*, 7(2), veab102.

Lee, L., Palukaitis, P., & Gray, S. M. (2002). Host-dependent requirement for the Potato leafroll virus 17-kda protein in virus movement. *Molecular Plant-Microbe Interactions*, 15(10), 1086-1094.

Lewellen, R. T., Wisler, G. C., Liu, H. Y., Kaffka, S. R., Sears, J. L., & Duffus, J. E. (1999). Reaction of sugar beet breeding lines and hybrids to beet chlorosis luteovirus. *Journal of Sugar Beet Research*, 36, 76.

Li, X., Halpin, C., & Ryan, M. D. (2007). A novel cleavage site within the potato leafroll virus P1 polyprotein. *Journal of General Virology*, 88(5), 1620-1623.

Li, Y., Cui, H., Cui, X., & Wang, A. (2016). The altered photosynthetic machinery during compatible virus infection. *Current Opinion in Virology*, 17, 19-24.

Limburg, D. D., Mauk, P. A., & Godfrey, L. D. (1997). Characteristics of beet yellows closterovirus transmission to sugar beets by *Aphis fabae*. *Phytopathology*, 87(7), 766-771.

Luquet, M., Poggi, S., Buchard, C., Plantegenest, M., & Tricault, Y. (2023). Predicting the seasonal flight activity of *Myzus persicae*, the main aphid vector of Virus Yellows in sugar beet. *Pest Management Science*, 79(11), 4508-4520.

Majumdar, A., Sharma, A., & Belludi, R. (2023). Natural and engineered resistance mechanisms in plants against phytoviruses. *Pathogens*, 12(4), 619.

Majumdar, R., Galewski, P. J., Eujayl, I., Minocha, R., Vincill, E., & Strausbaugh, C. A. (2022). Regulatory roles of small non-coding RNAs in sugar beet resistance against beet curly top virus. *Frontiers in Plant Science*, 12, 780877.

Mandadi, K. K., & Scholthof, K. B. G. (2013). Plant immune responses against viruses: how does a virus cause disease? *The Plant Cell*, 25(5), 1489-1505.

Martínez-Guardiola, C., Parreño, R., & Candela, H. (2024). MAPtools: command-line tools for mapping-by-sequencing and QTL-Seq analysis and visualization. *Plant Methods*, 20(1), 107.

Mayo, M. A., & Ziegler-Graff, V. (1996). Molecular biology of luteoviruses. *Advances in Virus Research*, 46(1), 413-460.

McGrath, J. M., Funk, A., Galewski, P., Ou, S., Townsend, B., Davenport, K., Daligault, H., Johnson, S., Lee, J., Hastie, A. and Darracq, A., Willems, G., Barnes, S., Liachko, I., Sullivan, S., Koren, S., Phillippy, A., Wang, J., Liu, T., Pulman, J., Childs, K., Yocum, A., Fermin, D., Mutasa-Göttgens, E., Stevanato, P., Taguchi, K & Dorn, K. M. (2023). A contiguous de novo genome assembly of sugar beet EL10 *(Beta vulgaris* L.). *DNA Research*, 30(1), dsac033.

McGrath, J. M., & Panella, L. (2018). Sugar beet breeding. *Plant Breeding Reviews*, 42, 167-218.

Miles, C., & Wayne, M. (2008). Quantitative trait locus (QTL) analysis. *Nature Education*, 1(1), 208.

Monteiro, F., Romeiras, M. M., Batista, D., & Duarte, M. C. (2013). Biodiversity assessment of sugar beet species and its wild relatives: linking ecological data with new genetic approaches. *American Journal of Plant Sciences*, 4, 35902.

Murphy, A. M., Zhou, T., & Carr, J. P. (2020). An update on salicylic acid biosynthesis, its induction and potential exploitation by plant viruses. *Current Opinion in Virology*, 42, 8-17.

Muthamilarasan, M., & Prasad, M. (2013). Plant innate immunity: an updated insight into defence mechanism. *Journal of Biosciences*, 38, 433-449.

Newbert, M. J. (2016). The genetic diversity of Turnip yellows virus in oilseed rape (*Brassica napus*) in Europe, pathogenic determinants, new sources of resistance and host range. Doctoral dissertation, University of Warwick.

Newman, M. A., Sundelin, T., Nielsen, J. T., & Erbs, G. (2013). MAMP (microbe-associated molecular pattern) triggered immunity in plants. *Frontiers in Plant Science*, 4, 139.

Ngou, B. P. M., Ahn, H. K., Ding, P., & Jones, J. D. (2021). Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature*, 592(7852), 110-115.

Okole, N., Ispizua Yamati, F. R., Hossain, R., Varrelmann, M., Mahlein, A. K., & Heim, R. H. (2024). Aerial low-altitude remote sensing and deep learning

for in-field disease incidence scoring of virus yellows in sugar beet. *Plant Pathology*. https://doi.org/10.1111/ppa.13973.

Osman, T. A., Coutts, R. H., & Buck, K. W. (2006). In vitro synthesis of minus-strand RNA by an isolated cereal yellow dwarf virus RNA-dependent RNA polymerase requires VPg and a stem-loop structure at the 3′ end of the virus RNA. *Journal of Virology*, 80(21), 10743-10751.

Pagán, I., & Holmes, E. C. (2010). Long-term evolution of the *Luteoviridae*: time scale and mode of virus speciation. *Journal of Virology*, 84(12), 6177-6187.

Pálak, J. (1979). Occurrence of beet western yellows virus in sugar beet in Czechoslovakia. *Biologia Plantarum*, 21(4), 275-279.

Pathak, A. D., Srivastava, S., Misra, V., Mall, A. K., & Srivastava, S. (2022). Evolution and history of sugar beet in the world: An overview. In: Misra, V., Srivastava, S., & Mall, A. K. (Eds) Sugar Beet Cultivation, Management and Processing, pp. 3-10.

Patton, M. F., Bak, A., Sayre, J. M., Heck, M. L., & Casteel, C. L. (2020). A polerovirus, Potato leafroll virus, alters plant–vector interactions using three viral proteins. *Plant, Cell & Environment*, 43(2), 387-399.

Paudel, D. B., & Sanfaçon, H. (2018). Exploring the diversity of mechanisms associated with plant tolerance to virus infection. *Frontiers in Plant Science*, 9, 1575.

Pazhouhandeh, M., Dieterle, M., Marrocco, K., Lechner, E., Berry, B., Brault, V., Hemmer, O., Kretsch, T., Richards, K. E., Genschik, P., & Ziegler-Graff, V. (2006). F-box-like domain in the polerovirus protein P0 is required for silencing suppressor function. *Proceedings of the National Academy of Sciences*, 103(6), 1994-1999.

Peter, K. A., Liang, D., Palukaitis, P., & Gray, S. M. (2008). Small deletions in the potato leafroll virus readthrough protein affect particle morphology, aphid transmission, virus movement and accumulation. *Journal of General Virology*, 89(8), 2037-2045.

Rana, A. K., Gupta, V. K., Newbold, J., Roberts, D., Rees, R. M., Krishnamurthy, S., & Thakur, V. K. (2022). Sugar beet pulp: Resurgence and trailblazing journey towards a circular bioeconomy. *Fuel*, 312, 122953.

Rani, K., Kumar, M., Razzaq, A., Ajay, B. C., Kona, P., Bera, S. K., & Wani, S. H. (2023). Recent advances in molecular marker technology for QTL mapping in plants. In: Wani, S. H., Wang, D., & Singh, G. P. (Eds) QTL Mapping in Crop Improvement. Cambridge (MA): Academic Press, pp. 1-15.

Revers, F., & Nicaise, V. (2014). Plant resistance to infection by viruses. eLS.

Richardson, K. (2010). Traditional breeding in sugar beet. *Sugar Tech*, 12, 181-186.

Rodriguez-Medina, C., Boissinot, S., Chapuis, S., Gereige, D., Rastegar, M., Erdinger, M., Revers, F., Ziegler-Graff, V., & Brault, V. (2015). A protein kinase binds the C-terminal domain of the readthrough protein of *Turnip yellows virus* and regulates virus accumulation. *Virology*, 486, pp.44-53.

Rollwage, L., Van Houtte, H., Hossain, R., Wynant, N., Willems, G., & Varrelmann, M. (2024). Recessive resistance against beet chlorosis virus is conferred by the eukaryotic translation initiation factor (iso) 4E in *Beta vulgaris*. *Plant Biotechnology Journal*, 22(8), 2129-2141.

Roos, J., Hopkins, R., Kvarnheden, A., & Dixelius, C. (2011). The impact of global warming on plant diseases and insect vectors in Sweden. *European Journal of Plant Pathology*, 129, 9-19.

Rossi, V. (2023). Exploring resistance to *Aphanomyces cochlioides* in sugar beet. Doctoral dissertation, Swedish University of Agricultural Sciences, No. 2023: 45.

Sadowy, E., Maasen, A., Juszczuk, M., David, C., Zagórski-Ostoja, W., Gronenborn, B., & Hulanicka, M. D. (2001). The ORF0 product of *Potato leafroll virus* is indispensable for virus accumulation. *Journal of General Virology*, 82(6), 1529-1532.

Savitsky, V. F. (1950). Monogerm sugar beets in the United States. *Proceedings American Society of Sugar Beet Technologists*, 6, 156-159.

Schliephake, E., Graichen, K., & Rabenstein, F. (2000). Investigations on the vector transmission of the Beet mild yellowing virus (BMYV) and the Turnip yellows virus (TuYV). *Journal of Plant Diseases and Protection*, 107, 81-87.

Schneider, K., Kulosa, D., Soerensen, T. R., Möhring, S., Heine, M., Durstewitz, G., Polley, A., Weber, E., Jamsari, Lein, J., Hohmann, U., Tahiro, E., Bernd Weisshaar, B., Schulz, B., Koch, G., Jung, C., & Ganal, M. (2007). Analysis of DNA polymorphisms in sugar beet (*Beta vulgaris* L.) and development of an SNP-based map of expressed genes. *Theoretical and Applied Genetics*, 115(5), 601.

Schop, S., Kloth, K. J., Raaijmakers, E., & van Der Vlugt, R. A. A. (2022). The effect of mature plant resistance in sugar beet (*Beta vulgaris* spp. *vulgaris*) on survival, fecundity and behaviour of green peach aphids (*Myzus persicae*). *Bulletin of Entomological Research*, 112(5), 707-714.

Schop, S., van den Ham, F., van Oorschot, E., Grapendaal, S. R., Raaijmakers, E., & van der Vlugt, R. A. (2024). Development of a one-step multiplex reverse transcription-polymerase chain reaction and Luminex xTAG assay for the simultaneous detection of yellowing viruses infecting sugar beet. *Plant Pathology*, 73, 1533-1541.

Schwichtenberg, K., Wenke, T., Zakrzewski, F., Seibt, K. M., Minoche, A., Dohm, J. C., Weisshaar, B., Himmelbauer, H., & Schmidt, T. (2016). Diversification, evolution and methylation of short interspersed nuclear element families in sugar beet and related Amaranthaceae species. *The Plant Journal*, 85(2), 229-244.

Smirnova, E., Firth, A. E., Miller, W. A., Scheidecker, D., Brault, V., Reinbold, C., Rakotondrafara, A. M., Chung, B. Y.-W., & Ziegler-Graff, V. (2015).

Discovery of a small non-AUG-initiated ORF in poleroviruses and luteoviruses that is required for long-distance movement. *PLoS Pathogens*, 11(5), e1004868.

Smith, H. G., & Hallsworth, P. B. (1990). The effects of yellowing viruses on yield of sugar beet in field trials, 1985 and 1987. *Annals of Applied Biology*, 116, 503–511.

Sõmera, M., Fargette, D., Hébrard, E., Sarmiento, C., & ICTV Report Consortium. (2021). ICTV virus taxonomy profile: *Solemoviridae* 2021. *Journal of General Virology*, 102(12), 001707.

Stevanato, P., Chiodi, C., Broccanello, C., Concheri, G., Biancardi, E., Pavli, O., & Skaracis, G. (2019). Sustainability of the sugar beet crop. *Sugar Tech*, 21(5), 1-14.

Stevens, M., & Bowen, S. (2021). Virus feature: learning through adversity. Aphids and virus control in 2021. *Sugar Beet Review*, 89(1), 10-15.

Stevens, M., Freeman, B., Liu, H. Y., Herrbach, E., & Lemaire, O. (2005a). Beet poleroviruses: close friends or distant relatives? *Molecular Plant Pathology*, 6(1), 1-9.

Stevens, M., Hallsworth, P. B., & Smith, H. G. (2004). The effects of Beet mild yellowing virus and Beet chlorosis virus on the yield of UK field-grown sugar beet in 1997, 1999 and 2000. *Annals of Applied Biology*, 144(1), 113-119.

Stevens, M., Patron, N. J., Dolby, C. A., Weekes, R., Hallsworth, P. B., Lemaire, O., & Smith, H. G. (2005b). Distribution and properties of geographically distinct isolates of sugar beet yellowing viruses. *Plant Pathology*, 54(2), 100-107.

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist*, 203(1), 32-43.

Tacke, E., Schmitz, J., Prüfer, D., & Rohde, W. (1993). Mutational analysis of the nucleic acid-binding 17 kDa phosphoprotein of potato leafroll luteovirus identifies an amphipathic α-helix as the domain for protein/protein interactions. *Virology*, 197(1), 274-282.

Takagi, H., Abe, A., Yoshida, K., Kosugi, S., Natsume, S., Mitsuoka, C., Uemura, A., Utsushi, H., Tamiru, M., Takuno, S., Innan, H., Cano, L.M., Kamoun, S & Terauchi, R. (2013). QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *The Plant Journal*, 74(1), 174-183.

Tamada, T. (2016). General features of beet necrotic yellow vein virus. In: Biancardi, E., Tamada, T. (eds) Rhizomania. Springer, Cham, 55-83.

Tan, W., Li, K., Liu, D., & Xing, W. (2023). Cercospora leaf spot disease of sugar beet. *Plant Signaling & Behavior*, 18(1), 2214765.

Terradot, L., Souchet, M., Tran, V., & Ducray-Bourdin, D. G. (2001). Analysis of a three-dimensional structure of Potato leafroll virus coat protein obtained by homology modeling. *Virology*, 286(1), 72-82.

Tian, Y., Fan, L., Thurau, T., Jung, C., & Cai, D. (2004). The absence of TIR-type resistance gene analogues in the sugar beet (*Beta vulgaris* L.) genome. *Journal of Molecular Evolution*, 58(1), 40-53.

Tibbs Cortes, L., Zhang, Z., & Yu, J. (2021). Status and prospects of genome-wide association studies in plants. *The Plant Genome*, 14(1), e20077.

Uffelmann, E., Huang, Q. Q., Munung, N. S., De Vries, J., Okada, Y., Martin, A. R., Martin, H.C., Lappalainen, T., & Posthuma, D. (2021). Genome-wide association studies. *Nature Reviews Methods Primers*, 1(1), 59.

Üstün, S., Hafrén, A., & Hofius, D. (2017). Autophagy as a mediator of life and death in plants. *Current Opinion in Plant Biology*, 40, 122-130.

van Den Heuvel, J. F. J. M. (1999). Fate of a luteovirus in the haemolymph of an aphid. In Luteoviridae (pp. 112-119). CABI.

Varshney, R. K., Nayak, S. N., May, G. D., & Jackson, S. A. (2009). Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends in Biotechnology*, 27(9), 522-530.

Verchot, J. (2016). Plant virus infection and the ubiquitin proteasome machinery: arms race along the endoplasmic reticulum. *Viruses*, 8(11), 314.

Viganó, F. & Stevens, M. (2007). Development of a multiplex immunocapture-RT-PCR for simultaneous detection of BMYV and BChV in plants and single aphids. *Journal of Virological Methods*, 146(1), 196–201.

Walker, P. J., Siddell, S. G., Lefkowitz, E. J., Mushegian, A. R., Adriaenssens, E. M., Alfenas-Zerbini, P., Davison, A.J., Dempsey, D.M., Dutilh, B.E., García, M. L., Harrach, B., Harrison, R. L., Hendrickson, R .C., Junglen, S., Knowles, N. J., Krupovic, M., Kuhn, J. H., Lambert, A. J., Łobocka, M., Nibert, M. L, Oksanen, H. M., Orton, R. J., Robertson, D. L., Rubino, L., Sabanadzovic, S., Simmonds, P., Smith, D. B., Suzuki, N., Van Dooerslaer, K., Vandamme, A. M., Varsani, A., & Zerbini, F. M. (2021). Changes to virus taxonomy and to the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2021). *Archives of Virology*, 166(9), 2633-2648.

Wan, J., He, M., Hou, Q., Zou, L., Yang, Y., Wei, Y., & Chen, X. (2021). Cell wall associated immunity in plants. *Stress Biology*, 1(1), 3.

Wang, X., Jiang, N., Liu, J., Liu, W., & Wang, G. L. (2014). The role of effectors and host immunity in plant–necrotrophic fungal interactions. *Virulence*, 5(7), 722-732.

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, 10(1), 57-63.

Wintermantel, W. M. (2005). Co-infection of *Beet mosaic virus* with beet yellowing viruses leads to increased symptom expression on sugar beet. *Plant Disease*, 89(3), 325–331.

Yoshida, N., & Tamada, T. (2019). Host range and molecular analysis of *Beet leaf yellowing virus*, *Beet western yellows virus*-JP and *Brassica yellows virus* in Japan. *Plant Pathology*, 68(6), 1045-1058.

Yu, B., Chen, M., Grin, I., & Ma, C. (2020). Mechanisms of sugar beet response to biotic and abiotic stresses. Mechanisms of genome protection and repair, 167-194.

Zanardo, L. G., de Souza, G. B., & Alves, M. S. (2019). Transcriptomics of plant–virus interactions: A review. *Theoretical and Experimental Plant Physiology*, 31, 103-125.

Zhang, C., Shi, H., Chen, L., Wang, X., Lü, B., Zhang, S., Liang, Y., Liu, R., Qian, J., Sun, W., You, Z., & Dong, H. (2011). Harpin-induced expression and transgenic overexpression of the phloem protein gene *AtPP2-A1* in Arabidopsis repress phloem feeding of the green peach aphid *Myzus persicae*. *BMC Plant Biology*, 11, 1-19.

Zicari, S., Zhang, R., & Kaffka, S. (2019). Sugar beet. In Integrated processing technologies for food and agricultural by-products (pp. 331-351). Academic Press. ELSEVIER.

Ziegler-Graff, V., Brault, V., Mutterer, J. D., Simonis, M. T., Herrbach, E., Guilley, H., Richards, K. E., & Jonard, G. (1996). The coat protein of beet western yellows luteovirus is essential for systemic infection but the viral gene products P29 and P19 are dispensable for systemic infection and aphid transmission. *Molecular Plant-Microbe Interactions*, 9(6), 501-510.

Zipfel, C. (2008). Pattern-recognition receptors in plant innate immunity. *Current Opinion in Immunology*, 20(1), 10-16.

Popular science summary

Sugar is an inevitable part of day-to-day life be it for making home-made as well as processed goods, alcoholic beverages, soft drinks and dairy products or it could be part of bioplastics, cosmetics, biofuels and medicines. Commercially, it plays an important role in food-processing industries. Globally, sugar is produced mainly from sugar cane and sugar beet. Sugar beet is the main source of sugar especially in countries with a temperate climate. Around 20% of the sugar produced in the world comes from sugar beet, where the sucrose content in different cultivars ranges from 13-22%. However, the sugar production and productivity are often affected by a plethora of environmental stress factors as well as by pests and diseases. Among the diseases affecting the crop, there are several diseases caused by viral pathogens, which are transmitted by different vectors (e.g., protozoa, leafhoppers or aphids). Virus yellows (VY) disease of sugar beet is transmitted mainly by the green peach aphid and the black bean aphid, and the disease may result in a yield loss of up to 30%. This disease is associated with multiple viruses and hence very complex. In Europe, these viruses are beet mild yellowing virus (BMYV), beet chlorosis virus (BChV), beet yellows virus (BYV) and beet mosaic virus (BtMV). Recently, turnip yellows virus (TuYV) is also seen to infect sugar beet even though it before was not considered as a host. Neonicotinoid chemicals were previously used for managing the aphid vectors transmitting these viruses. Currently, the use of these chemicals is restricted in EU and alternate ways to manage VY need to be explored. One of the best ways to do that is to develop cultivars with durable virus resistance. In this project, our focus was on BMYV as it is the most common among the viruses in the VY-complex in Europe as well as in Sweden. To develop resistant or tolerant cultivars, insights into the virus-sugar beet interactions need to be gained through studies. We performed

experiments using a resistant genotype of wild beet and a susceptible genotype of sugar beet, looking at the responses to BMYV infection using different molecular techniques. Virus quantifications were done at different time points after inoculation with BMYV. The results showed that the virus content in the resistant plants was always lower than in the susceptible plants. The lack of symptom expression and lower virus titre revealed that the wild beet genotype was partially resistant against BMYV. Fourteen potential candidate genes were identified for resistance against BMYV. These genes are known from other studies to have a role in defence responses to viruses in plants and may be involved in the response that protects the plant from the stress caused by the virus infection. Therefore, they are of high interest for future studies. We also identified genome regions linked to susceptibility and resistance to BMYV. Collectively our studies enabled identification of the most common poleroviruses (BMYV, BChV and TuYV) causing VY in Sweden as well as candidate genes for defence responses and genome regions for BMYV resistance in the wild beet genotype. This would lay a foundation for breeding programs to develop resistant cultivars of sugar beet that could be used by farmers in sugar beet cultivation and limit the usage of chemical insecticides.

Populärvetenskaplig sammanfattning

Socker är en oundviklig del av vårt dagliga liv och används både hemma och industriellt för att producera varor, alkoholdrycker, läsk och mejeriprodukter eller ingår i bioplaster, kosmetika, biobränsle och läkemedel. Det har kommersiellt en viktig roll i livsmedelsindustrin. Socker produceras globalt huvudsakligen från sockerrör och sockerbetor. Speciellt i länder med tempererat klimat är sockerbetor den största sockerkällan. Omkring 20% av det socker som produceras i världen kommer från sockerbetor där sackaroshalten i olika sorter varierar från 13 till 22%. Produktionen av socker och produktiviteten påverkas dock ofta av en mängd stressfaktorer i miljön och av sjukdomar och skadegörare. Bland de sjukdomar som påverkar grödan finns flera orsakade av virus överförda med olika vektorer (t.ex. protozoer, stritar eller bladlöss). Sjukdomen virusgulsot hos sockerbeta överförs huvudsakligen av persikbladlus och betbladlus, och sjukdomen kan resultera i en skördeförlust på upp till 30%. Sjukdomen är kopplad till flera virus och är därmed komplex. I Europa är dessa virus beet mild yellowing virus (BMYV, milt betvirusgulsot), beet chlorosis virus (BChV), beet yellows virus (BYV, allmän betvirusgulsot) och beet mosaic virus (BtMV, betmosaik). Turnip yellows virus (TuYV, rapsrödsot) har nyligen också setts infektera sockerbeta även om det tidigare inte ansågs vara en virusvärd. Kemikalier av typen neonikotinoider användes tidigare för kontroll av de bladlusvektorer som överför dessa virus. Användandet av dessa kemikalier är numera begränsat inom EU och alternativa sätt för att hantera virusgulsot behöver utforskas. Ett av de bästa sätten att göra detta är utvecklandet av sorter med hållbar virusresistens. Vårt fokus var i det här projektet på BMYV eftersom det i Europa såväl som i Sverige är vanligast bland de virus som ingår i virusgulsotkomplexet. För att utveckla sorter med

resistens eller tolerans måste kunskap om interaktioner mellan virus och sockerbeta erhållas genom studier. Vi utförde experiment med en resistent genotyp av vildväxande beta och en mottaglig genotyp av sockerbeta, och undersökte responsen på BMYV-infektion med olika molekylära tekniker. Virusmängden kvantifierades vid olika tidpunkter efter inokulering med BMYV. Resultaten visade att virusmängden alltid var lägre i resistenta än i mottagliga plantor. Avsaknaden av symtom och lägre virusmängd visade att den vildväxande betgenotypen var partiellt resistent mot BMYV. Fjorton potentiella kandidatgener identifierades för resistens mot BMYV. Man vet från tidigare studier att dessa gener deltar i växters försvarsrespons mot virus och kan vara involverade i den respons som försvarar växten mot stress orsakad av virusinfektion. De är därför av stort intresse för framtida studier. Vi identifierade också genomregioner kopplade till mottaglighet och resistens mot BMYV. Sammantaget möjliggjorde studierna identifiering av de vanligaste polerovirus (BMYV, BChV och TuYV) som orsakar virusgulsot i Sverige samt kandidatgener för försvarsrespons och genomregioner för resistens mot BMYV i en vildväxande betgenotyp. Detta bildar en grund för förädlingsprogram där resistenta sockerbetssorter tas fram som kan användas av lantbrukare i sockerbetsodling och begränsa användningen av kemiska insektsmedel.

Acknowledgements

I believe that the teachers are the divine representatives who help in sustaining knowledge, demolishing the weeds of ignorance and showing the right path to the students to accomplish their dreams. With that, I would like to pay my utmost respect and gratitude to all my Gurus (teachers). I would like to express my deepest respect and gratitude to my main supervisor Anders Kvarnheden and the best teacher I ever had in my life. I fall short of words when I have to express my emotions towards you as my supervisor. Thank you for being so humble, never losing patience, helping me handle all tough situations so smoothly and above all, for always having time for me. You are such a gem of a person and thank you for giving me freedom to express my thoughts no matter what, without any hesitation.

My industrial co-supervisor from DLF Beet seeds AB, Britt louise Lennefors. What can I say! This project would have been impossible without you. You inspire me in several ways as a woman in the role of an eminent researcher, a wonderful manager, a dedicated mother and a beautiful soul inside out. Thank you for always being there for me from day one and caring me as a mother. I never missed my mother during the days I spent with you. My visits to Skåne for performing my experiments had been always so fruitful under your supervision. Huge thanks for always helping me out and consistently putting effort to bring the best out of my PhD work. Without your supervision, these five years would have been difficult for me. Your colleagues from DLF Beet seeds AB have also put a lot of effort for the success of my experiments. Big thanks to all of them.

To my co-supervisors, Per Snell, Erik Alexandersson and Eugene Savenkov. Thanks for all the help extended and sharing valuable feedbacks throughout this journey.

My doctoral research involves a wide range of topics and it has been an enriching experience to learn new skills. Highly indebted to Abu Bakar Siddique for teaching me the basic bioinformatic skills and for always being there for me to guide me while performing the bioinformatic analysis. Thanks for all the fruitful discussions we had which made me understand the complexity of the bioinformatic analysis correctly. I would like to also thank the SLUBI members Amrei Binzer-Panchal and Nicholas Delhomme for all the support and help extended.

To Sajeevan Radha Sivarajan, it was a nice experience to work with you. A sincere thanks for all the fruitful time spent during the experiments and sharing your valuable knowledge with me through your guidance.

I am highly grateful to Anna Westerbergh, who is such a warm hearted person. Thank you for always enquiring if I am doing good whenever we meet around in the department and having deep conversations on different topics. Special thanks for stepping in to help in the last moment with my manuscript.

My friends and colleagues, Silvana Moreno, Yousef Rahimi and Elham Yazdkhasti, thank you for all the help and support. You guys mean a lot to me. Laxmi Mishra! You came very late into my life during this PhD journey but the friendship that we built with each other during this short time is so beautiful. Thank you for all the care, for making sure that I am always doing good and for being my comfort zone to share whatever I feel or go through. My other colleagues at department, Girma Bedada Chala and Martha Rendón Anaya, big thanks for your valuable feedbacks on my manuscript. Highly appreciate the help provided by Qing Heijkenskjöld and Lotta Ann-Charlotte Olsson for various support provided during this journey of my PhD program. Special thanks to my former and current colleagues Aayushi, Katalin, Shridhar, Martina, Pruthvi, Nirbhay, Shailja, Mansi, Swetha, Punya, Nilam and Sonam for being around as a wonderful social circle at SLU.

Lastly, my family for being the biggest pillar of strength during this entire journey. I would like to thank my parents for all their love and support. My husband, Rajesh Debnath, who left his job back in India and relocated to Sweden to be with me and to support me during this entire journey of PhD. Without you, I would have been lost. Thank you for being with me during all the difficult times of life. Aaravi, my sweet little daughter, you came to this world amidst this PhD journey and brought lot of hope and happiness with you. You made me realise all the strengths within me and gave me hope to live this life to experience the beautiful days yet to come, thank you.

I

Phytopathology® • 2023 • 113:1788-1798 • https://doi.org/10.1094/PHYTO-11-22-0446-V

Virology

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

Vinitha Puthanveed,[1] Khushwant Singh,[1,2] Efstratia Poimenopoulou,[1] Josefin Pettersson,[1] Abu Bakar Siddique,[1] and Anders Kvarnheden[1,†]

[1] Department of Plant Biology, Linnean Center for Plant Biology, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden
[2] Division of Crop Protection and Plant Health, Crop Research Institute, Prague 161 06, Czech Republic
Accepted for publication 18 February 2023.

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

†Corresponding author: A. Kvarnheden; anders.kvarnheden@slu.se

Current address for K. Singh: Botany and Plant Science, University of California Riverside, Riverside, CA 92521, U.S.A.

Funding: Support was provided by SLU Grogrund and the Swedish Board of Agriculture.

e-Xtra: Supplementary material is available online.

The author(s) declare no conflict of interest.

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

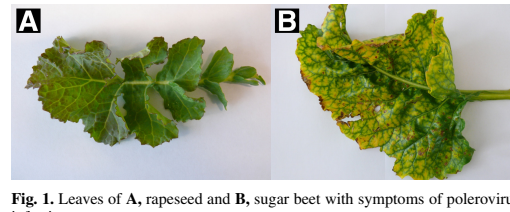


Fig. 1. Leaves of **A,** rapeseed and **B,** sugar beet with symptoms of polerovirus infection.

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

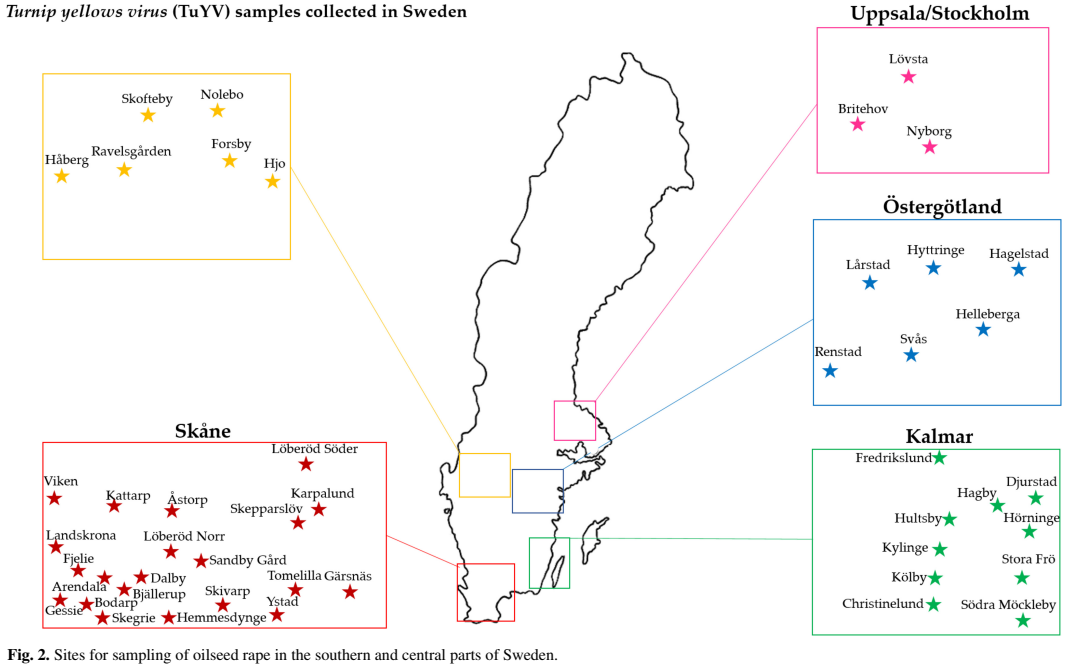


Fig. 2. Sites for sampling of oilseed rape in the southern and central parts of Sweden.

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

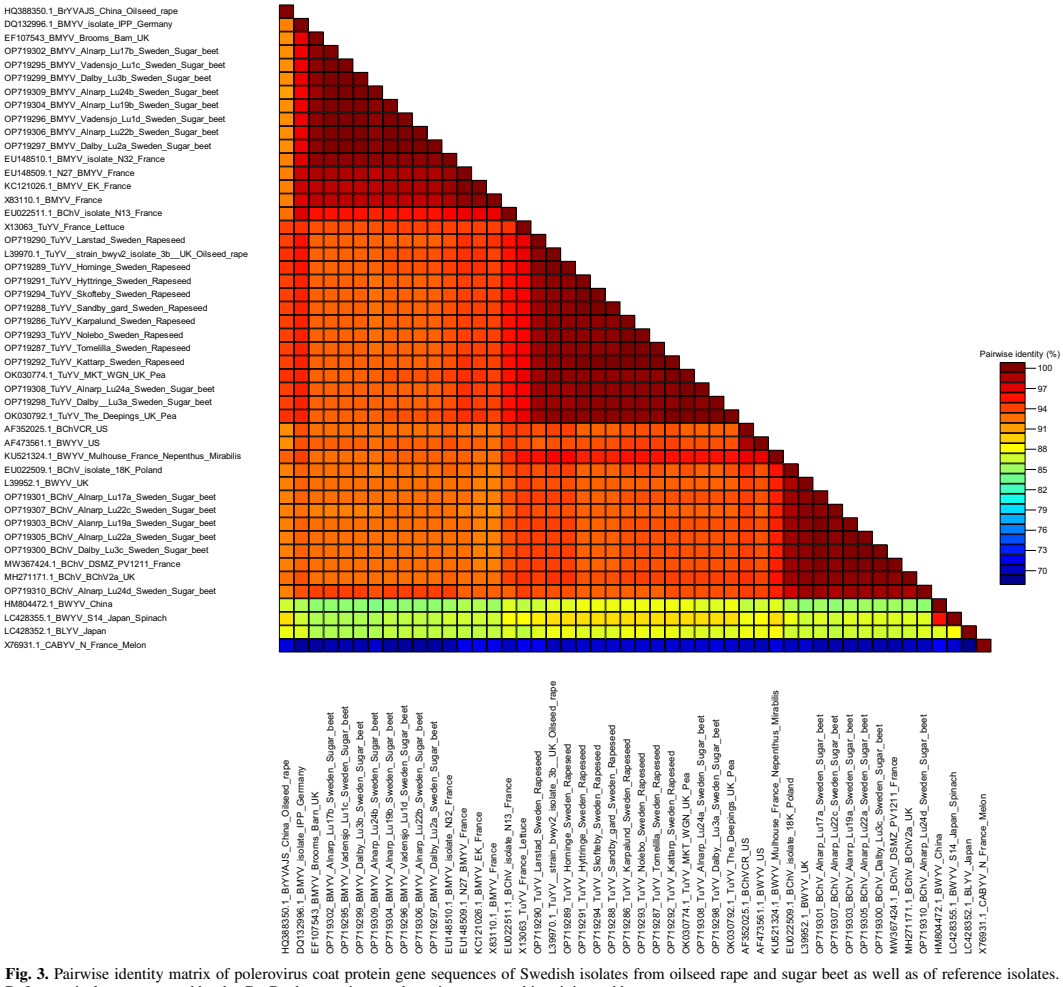


Fig. 3. Pairwise identity matrix of polerovirus coat protein gene sequences of Swedish isolates from oilseed rape and sugar beet as well as of reference isolates. Reference isolates are named by the GenBank accession number, virus, geographic origin, and host.

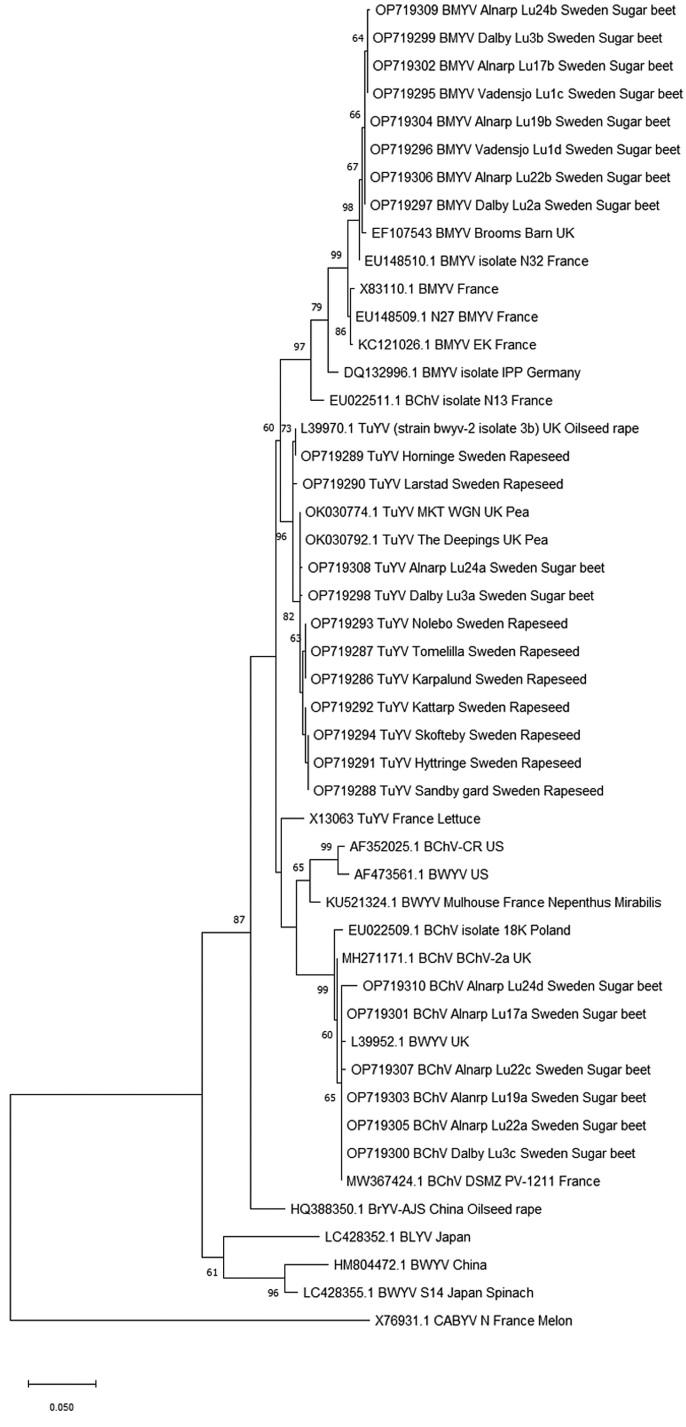


Fig. 4. Maximum likelihood tree of the coat protein gene from Swedish isolates of turnip yellows virus (TuYV), beet mild yellowing virus (BMYV), and beet chlorosis virus (BChV). Reference isolates of TuYV, BMYV, BChV, beet western yellows virus (BWYV), cucurbit aphid-borne yellows virus (CABYV), and 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

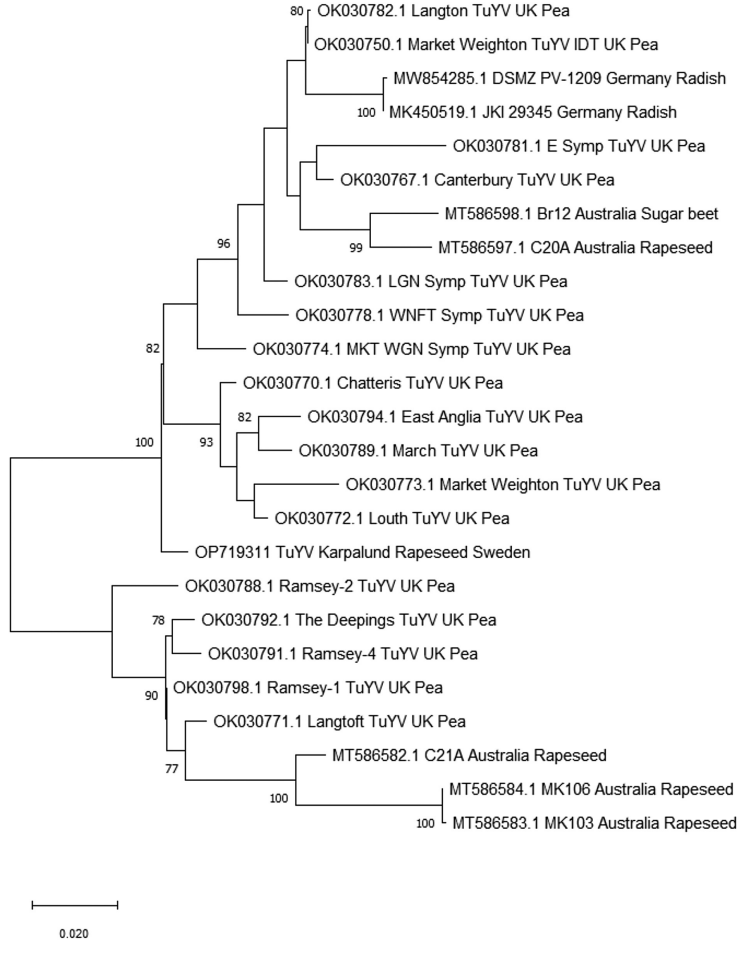


Fig. 5. Maximum likelihood tree with the whole genome of the Swedish isolate of turnip yellows virus (TuYV) from oilseed rape in Karpalund. Reference isolates of TuYV from different regions of the world were included in the analysis, and they are named by GenBank accession number, virus, geographic origin,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

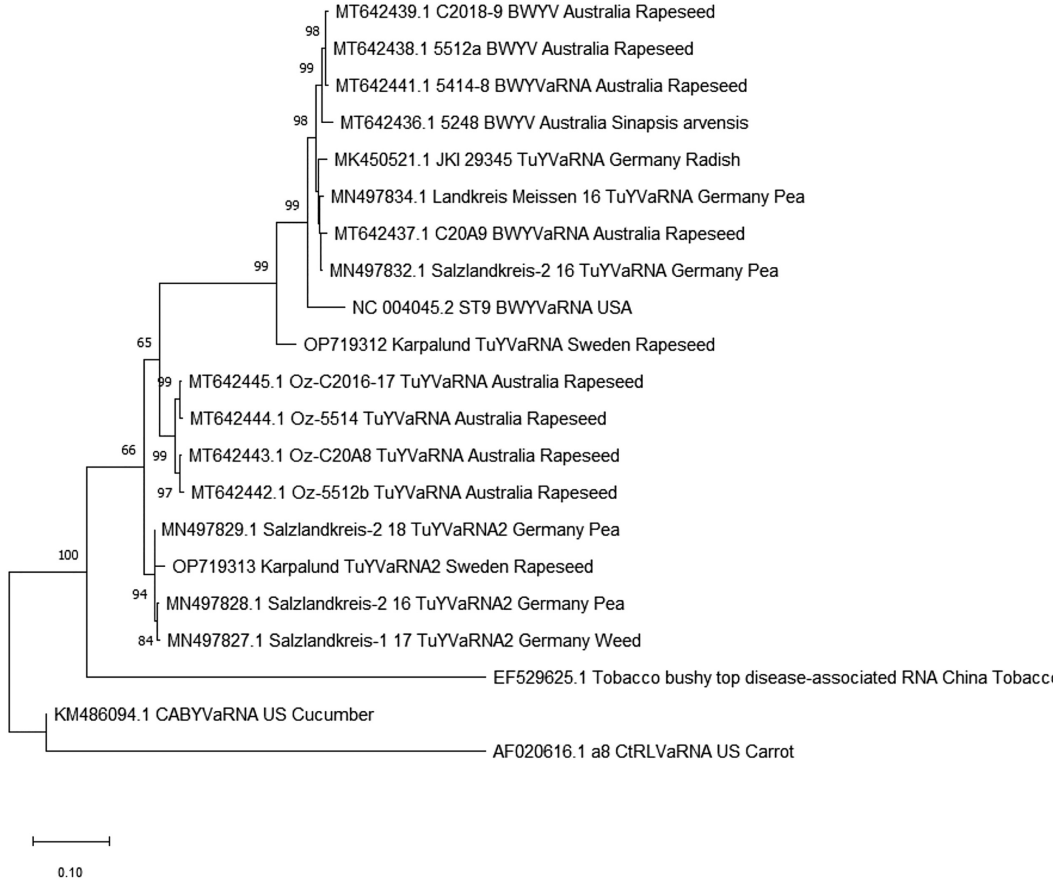


Fig. 6. Maximum likelihood tree with the sequences of the Swedish isolates of turnip yellows virus-associated RNA and turnip yellows virus-associated RNA2 from oilseed rape in Karpalund. Reference isolates of polerovirus-associated RNAs were included in the analysis, and they are named by GenBank accession 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.

Acknowledgments

We thank the Swedish Board of Agriculture, Nordic Beet Research, and DLF Beet Seed for assistance with sample collection. HTS was performed by the SNP&SEQ Technology Platform in Uppsala. The facility is part of the National Genomics Infrastructure Sweden and Science for Life Laboratory. The SNP&SEQ Platform is also supported by the Swedish Research Council and the Knut and Alice Wallenberg Foundation. The data handling was enabled by resources provided by the Swedish National Infrastructure for Computing (SNIC) at UPPMAX (Uppsala Multidisciplinary Center for Advanced Computational Science) partially funded by the Swedish Research Council through a grant, and we acknowledge their assistance concerning technical and implementational aspects. Support by SLUBI (SLU bioinformatics infrastructure) is gratefully acknowledged.

Literature Cited

Abraham, A. D., Menzel, W., Lesemann, D.-E., Varrelmann, M., and Vetten, H. J. 2006. Chickpea chlorotic stunt virus: A new polerovirus infecting cool-season food legumes in Ethiopia. Phytopathology 96:437-446.

Abraham, A. D., Varrelmann, M., and Vetten, H. J. 2008. Molecular evidence for the occurrence of two new luteoviruses in cool season food legumes in Northeast Africa. Afr. J. Biotechnol. 7:414-420.

Adams, I. P., Skelton, A., Macarthur, R., Hodges, T., Hinds, H., Flint, L., Nath, P. D., Boonham, N., and Fox, A. 2014. Carrot yellow leaf virus is associated with carrot internal necrosis. PLoS One 9:e109125.

Aldén, A., Berg, G., Christerson, T., Gerdtsson, A., Söderlind, C., and Östlund, R. 2019. Växtskyddsåret 2019 – Hallands, Skånes och Blekinge län. JO19:8, Jordbruksverket, Jönköping, Sweden.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Asare-Bediako, E., Jones, J. E., Hambidge, A. J., Stevens, M., Mead, A., Jenner, C. E., and Walsh, J. A. 2020. The incidence of turnip yellows virus in oilseed H crops (*Brassica napus* L.) in three different regions of England over three consecutive growing seasons and the relationship with the abundance of flying *Myzus persicae*. Ann. Appl. Biol. 176:130-137.

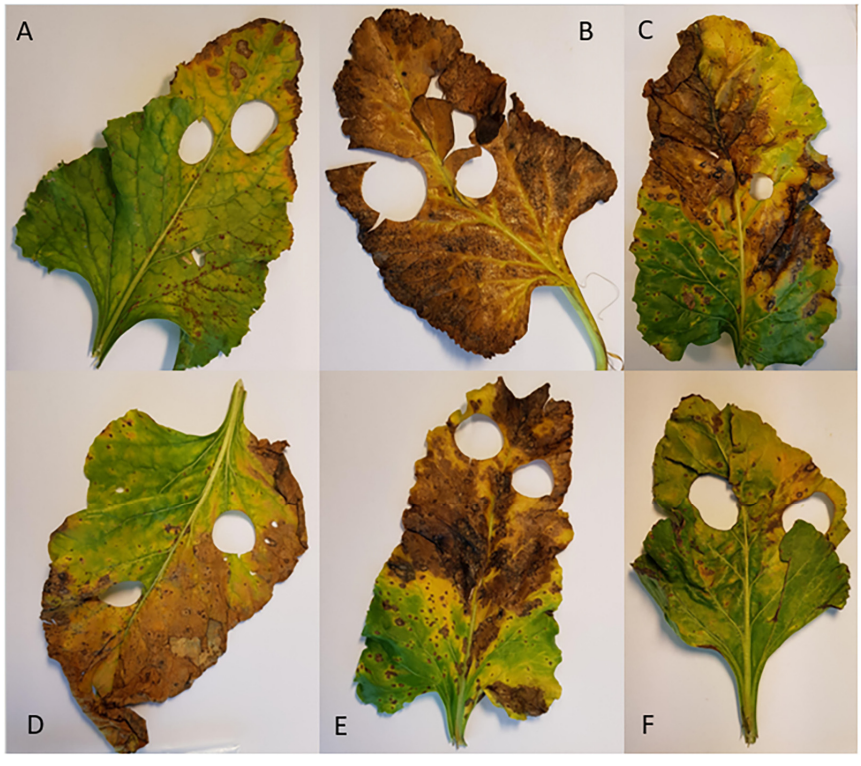
Beuve, M., Stevens, M., Wintermantel, W. M., Liu, H. Y., Hauser, S., and Lemaire, O. 2008. Biological and molecular characterization of an American sugar beet-infecting Beet western yellows virus isolate. Plant Dis. 92:51-60.

Bortolamiol, D., Pazhouhandeh, M., Marrocco, K., Genschik, P., and Ziegler-Graff, V. 2007. The polerovirus F box protein P0 targets ARGONAUTE1 to suppress RNA silencing. Curr. Biol. 17:1615-1621.

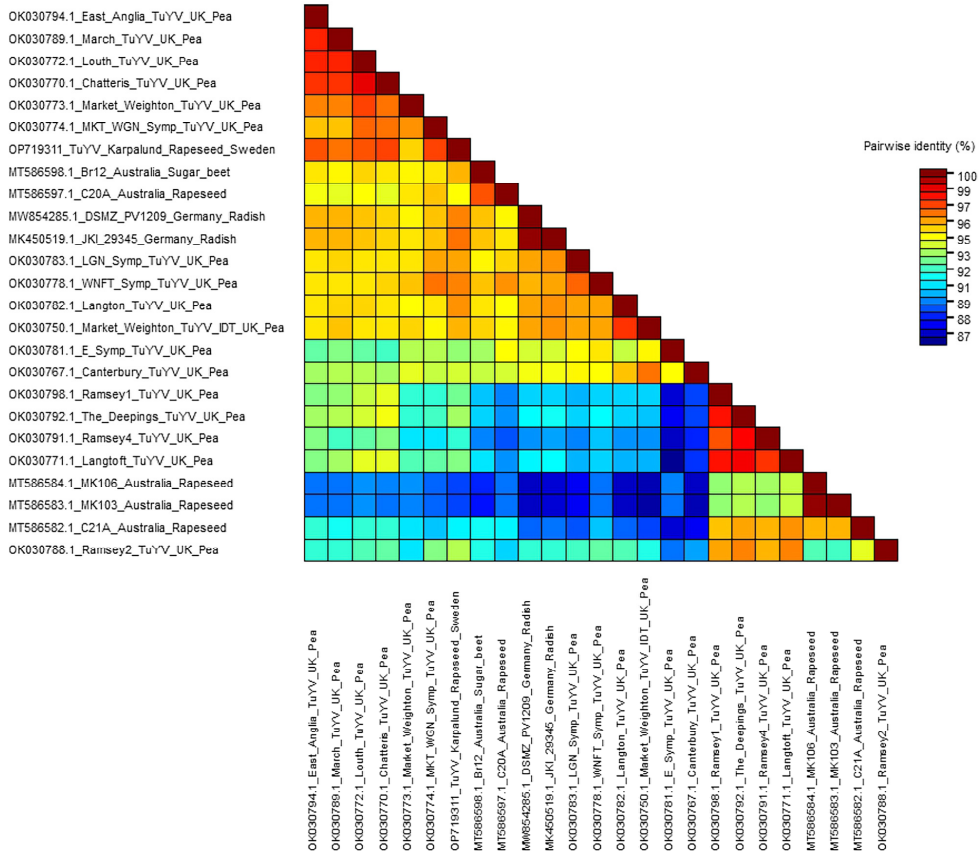
Brault, V., Bergdoll, M., Mutterer, J., Prasad, V., Pfeffer, S., Erdinger, M., Richards, K. E., and Ziegler-Graff, V. 2003. Effects of point mutations in

the major capsid protein of beet western yellows virus on capsid formation, virus accumulation, and aphid transmission. J. Virol. 77:3247-3256.
Brault, V., Périgon, S., Reinbold, C., Erdinger, M., Scheidecker, D., Herrbach, E., Richards, K. E., and Ziegler-Graff, V. 2005. The polerovirus minor capsid protein determines vector specificity and intestinal tropism in the aphid. J. Virol. 79:9685-9693.
Brault, V., van den Heuvel, J. F. J. M., Verbeek, M., Ziegler-Graff, V., Reutenauer, A., Herrbach, E., Garaud, J. C., Guilley, H., Richards, K. E., and Jonard, G. 1995. Aphid transmission of Beet western yellows luteovirus requires the minor capsid read-through protein P74. EMBO J. 14:650-659.
Chen, S., Zhou, Y., Chen, Y., and Gu, J. 2018. fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884-i890.
Chin, L. S., Foster, J. L., and Falk, B. W. 1993. The beet western yellows virus ST9-associated RNA shares structural and nucleotide sequence homology with carmo-like viruses. Virology 192:473-482.
Clavel, M., Lechner, E., Incarbone, M., Vincent, T., Cognat, V., Smirnova, E., Lecorbeiller, M., Brault, V., Ziegler-Graff, V., and Genschik, P. 2021. Atypical molecular features of RNA silencing against the phloem-restricted polerovirus TuYV. Nucleic Acids Res. 49:11274-11293.
Coleman, A. 2013. Control of Turnip yellows virus: Assessing impact on oilseed rape quality traits and dissecting circulative transmission by aphids. Ph.D. Thesis, The University of East Anglia, Norwich, U.K.
Congdon, B. S., Baulch, J. R., and Coutts, B. A. 2020. Impact of *Turnip yellows virus* infection on seed yield of an open-pollinated and hybrid cultivar when inoculated at different growth stages. Virus Res. 277:197847.
Coutts, B. A., Hawkes, J. R., and Jones, R. A. C. 2006. Occurrence of Beet western yellows virus and its aphid vectors in over-summering broad-leafed weeds and volunteer crop plants in the grainbelt region of south-western Australia. Aust. J. Agric. Res. 57:975-982.
Dewar, A. M. 2017. The adverse impact of the neonicotinoid seed treatment ban on crop protection in oilseed rape in the United Kingdom. Pest Manag. Sci. 73:1305-1309.
Duffus, J. E., and Russell, G. E. 1970. Serological and host range evidence for the occurrence of beet western yellows virus in Europe. Phytopathology 60:1199-1202.
Elena, S. F., Bedhomme, S., Carrasco, P., Cuevas, J. M., de la Iglesia, F., Lafforgue, G., Lalic, J., Pròsper, A., Tromas, N., and Zwart, M. P. 2011. The evolutionary genetics of emerging plant RNA viruses. Mol. Plant-Microbe Interact. 24:287-293.
Falk, B. W., Chin, L. S., and Duffus, J. E. 1989. Complementary DNA cloning and hybridization analysis of beet western yellows luteovirus RNAs. J. Gen. Virol. 70:1301-1309.
Falk, B. W., and Duffus, J. E. 1984. Identification of small single- and double-stranded RNAs associated with severe symptoms in beet western yellows virus-infected *Capsella bursa-pastoris*. Phytopathology 74:1224-1229.
Filardo, F., Nancarrow, N., Kehoe, M., McTaggart, A. R., Congdon, B., Kumari, S., Aftab, M., Trebicki, P., Rodoni, B., Thomas, J., and Sharman, M. 2021. Genetic diversity and recombination between turnip yellows virus strains in Australia. Arch. Virol. 166:813-829.
Fowkes, A. R., McGreig, S., Pufal, H., Duffy, S., Howard, B., Adams, I. P., Macarthur, R., Weekes, R., and Fox, A. 2021. Integrating high throughput sequencing into survey design reveals turnip yellows virus and soybean dwarf virus in pea (*Pisum sativum*) in the United Kingdom. Viruses 13:2530.
Freeman, A. J., and Aftab, M. 2011. Effective management of viruses in pulse crops in south eastern Australia should include management of weeds. Australas. Plant Pathol. 40:430-441.
Gaafar, Y. Z., Herz, K., Hartrick, J., Fletcher, J., Blouin, A. G., MacDiarmid, R., and Ziebell, H. 2020. Investigating the pea virome in Germany–Old friends and new players in the field(s). Front. Microbiol. 11:583242.
Gaafar, Y. Z., and Ziebell, H. 2019. Two divergent isolates of turnip yellows virus from pea and rapeseed and first report of turnip yellows virus-associated RNA in Germany. Microbiol. Resour. Announc. 8:e00214-19.
García-Arenal, F., Fraile, A., and Malpica, J. M. 2003. Variation and evolution of plant virus populations. Int. Microbiol. 6:225-232.
Gibbs, A., Fargette, D., García-Arenal, F., and Gibbs, M. 2010. Time–the emerging dimension of plant virus studies. J. Gen. Virol. 91:13-22.
Graichen, K., and Schliephake, E. 1999. Infestation of winter oilseed rape by turnip yellows Luteovirus and its effect on yield in Germany. Pages 131-136 in: Proceedings of 10th International Rapeseed Congress—New horizons for an old crop. N. Wratten and P. A. Salisbury, eds. International Consultative Group for Rapeseed Research, Canberra, Australia.
Grubaugh, N. D., Gangavarapu, K., Quick, J., Matteson, N. L., De Jesus, J. G., Main, B. J., Tan, A. L., Paul, L. M., Brackney, D. E., Grewal, S., Gurfield, N., Van Rompay, K. K. A., Isern, S., Michael, S. F., Coffey, L. L., Loman, N. J., and Andersen, K. G. 2019. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. Genome Biol. 20:1-19.
Hackenberg, D., Asare-Bediako, E., Baker, A., Walley, P., Jenner, C., Greer, S., Bramham, L., Batley, J., Edwards, D., Delourme, R., Barker, G., Teakle, G., and Walsh, J. 2020. Identification and QTL mapping of resistance to Turnip yellows virus (TuYV) in oilseed rape, *Brassica napus*. Theor. Appl. Genet. 133:383-393.
Hardwick, N. V., Davies, J. M. L., and Wright, D. M. 1994. The incidence of three virus diseases of winter oilseed rape in England and Wales in the 1991/92 and 1992/93 growing seasons. Plant Pathol. 43:1045-1049.
Hipper, C., Monsion, B., Bortolamiol-Bécet, D., Ziegler-Graff, V., and Brault, V. 2014. Formation of virions is strictly required for turnip yellows virus long-distance movement in plants. J. Gen. Virol. 95:496-505.
Jay, C. N., Rossall, S., and Smith, H. G. 1999. Effects of beet western yellows virus on growth and yield of oilseed rape (*Brassica napus*). J. Agric. Sci. 133:131-139.
Jones, R. A. C. 2009. Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. Virus Res. 141:113-130.
Jones, R. A. C., Coutts, B. A., and Hawkes, J. 2007. Yield-limiting potential of *Beet western yellows virus* in *Brassica napus*. Aust. J. Agric. Res. 58: 788-801.
Kozlowska-Makulska, A., Hasiow-Jaroszewska, B., Szyndel, M. S., Herrbach, E., Bouzoubaa, S., Lemaire, O., and Beuve, M. 2015. Phylogenetic relationships and the occurrence of interspecific recombination between beet chlorosis virus (BChV) and Beet mild yellowing virus (BMYV). Arch. Virol. 160:429-433.
Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35:1547-1549.
Langmead, B., and Salzberg, S. L. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9:357-359.
Li, D., Liu, C. M., Luo, R., Sadakane, K., and Lam, T. W. 2015. MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31:1674-1676.
Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997.
Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., and Marth, G., Abecasis, G., Durbin, R., and 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map (SAM) format and SAMtools. Bioinformatics 25:2078-2079.
Lundin, O. 2021. Consequences of the neonicotinoid seed treatment ban on oilseed rape production–what can be learnt from the Swedish experience? Pest Manag. Sci. 77:3815-3819.
Maurier, F., Beury, D., Fléchon, L., Varré, J. S., Touzet, H., Goffard, A., Hot, D., and Caboche, S. 2019. A complete protocol for whole-genome sequencing of virus from clinical samples: Application to coronavirus OC43. Virology 531:141-148.
Mayo, M. A. 2002. ICTV at the Paris ICV: Results of the plenary session and the binomial ballot. Arch. Virol. 147:2254-2260.
Mikheenko, A., Prjibelski, A., Saveliev, V., Antipov, D., and Gurevich, A. 2018. Versatile genome assembly evaluation with QUAST-LG. Bioinformatics 34:i142-i150.
Monci, F., Sánchez-Campos, S., Navas-Castillo, J., and Moriones, E. 2002. A natural recombinant between the geminiviruses Tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. Virology 303:317-326.
Muhire, B. M., Varsani, A., and Martin, D. P. 2014. SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9:e108277.
New, S. A., Van Heerden, S. W., Pietersen, G., and Esterhuizen, L. L. 2016. First report of a *Turnip yellows virus* in association with the brassica stunting disorder in South Africa. Plant Dis. 100:2341.
Newbert, M. J. 2016. The genetic diversity of Turnip yellows virus in oilseed rape (*Brassica napus*) in Europe, pathogenic determinants, new sources of resistance and host range. Ph.D. Thesis, University of Warwick, Coventry, U.K.
Nilsson, C. 2000. Förekomst av Turnip yellows virus i några höstrapsfält 1999/2000. Pages 11B1-B2 in: "Rapport från växtodlings- och växtskyddsdagar i Växjö den 6 och 7 december 2000". Meddelande från södra jordbruksförsöksdistriktet Nr 51.
Ondov, B. D., Bergman, N. H., and Phillippy, A. M. 2011. Interactive metagenomic visualization in a Web browser. BMC Bioinform. 12:1-10.
Pálak, J. 1979. Occurrence of beet western yellows virus in sugar beet in Czechoslovakia. Biol. Plant. 21:275-279.
Passmore, B. K., Sanger, M., Chin, L. S., Falk, B. W., and Bruening, G. 1993. Beet western yellows virus-associated RNA: An independently replicating RNA that stimulates virus accumulation. Proc. Natl Acad. Sci. U.S.A. 90:10168-10172.
Peter, K. A., Gildow, F., Palukaitis, P., and Gray, S. M. 2009. The C terminus of the polerovirus P5 readthrough domain limits virus infection to the phloem. J. Virol. 83:5419-5429.

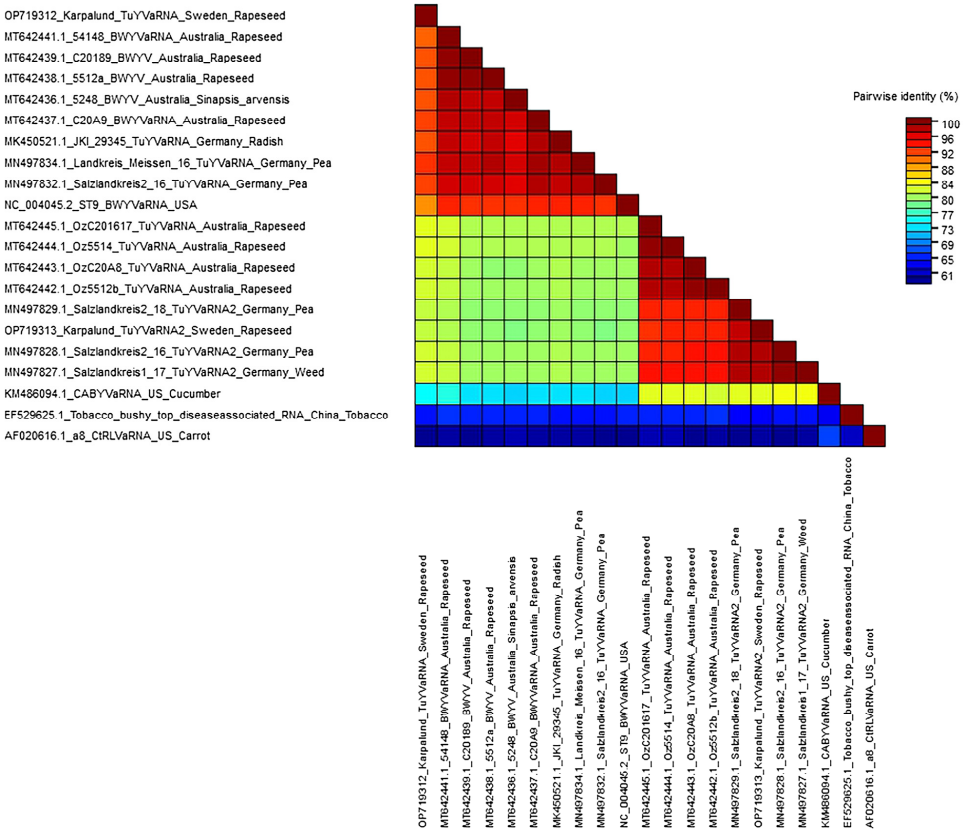
Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A., and Korobeynikov, A. 2020. Using SPAdes de novo assembler. Curr. Protoc. Bioinform. 70:e102.
Rodriguez-Medina, C., Boissinot, S., Chapuis, S., Gereige, D., Rastegar, M., Erdinger, M., Revers, F., Ziegler-Graff, V., and Brault, V. 2015. A 231 protein kinase binds the C-terminal domain of the readthrough protein of Turnip yellows virus and regulates virus accumulation. Virology 486:44-53.
Roos, J., Hopkins, R., Kvarnheden, A., and Dixelius, C. 2011. The impact of global warming on plant diseases and insect vectors in Sweden. Eur. J. Plant Pathol. 129:9-19.
Sanger, M., Passmore, B., Falk, B. W., Bruening, G., Ding, B., and Lucas, W. J. 1994. Symptom severity of beet western yellows virus strain ST9 is conferred by the ST9-associated RNA and is not associated with virus release from the phloem. Virology 200:48-55.
Schliephake, E., Graichen, K., and Rabenstein, F. 2000. Investigations on the vector transmission of the Beet mild yellowing virus (BMYV) and the Turnip yellows virus (TuYV). J. Plant Dis. Protect. 107:81-87.
Schravesande, W. E. W., van Wijk, J. P., and Verhage, A. 2021. Complete genome sequence of novel polerovirus-associated RNA infecting pepper (*Capsicum* spp.) in South Africa. Microbiol. Resour. Announc. 10:e01215-20.
Scott, C., and Bilsborrow, P. E. 2019. The impact of the EU neonicotinoid seed-dressing ban on oilseed rape production in England. Pest Manag. Sci. 75:125-133.
Shahraeen, N., Farzadfar, S. H., and Lesemann, D. E. 2003. Incidence of viruses infecting winter oilseed rape (*Brassica napus* ssp. *oleifera*) in Iran. J. Phytopathol. 151:614-616.
Sigvald, R. 2005. Virus i höstoljeväxter. Faktablad om växtskydd. Jordbruk 126 J. Swedish University of Agricultural Sciences, Uppsala, Sweden.
Smirnova, E., Firth, A. E., Miller, W. A., Scheidecker, D., Brault, V., Reinbold, C., Rakotondrafara, A. M., Chung, B. Y.-W., and Ziegler-Graff, V. 2015. Discovery of a small non-AUG-initiated ORF in poleroviruses and luteoviruses that is required for long-distance movement. PLoS Pathog. 11:e1004868.
Smith, H. G., and Hinckes, J. A. 1985. Studies on beet western yellows virus in oilseed rape (*Brassica napus* ssp. *oleifera*) and sugar beet (*Beta vulgaris*). Ann. Appl. Biol. 107:473-484.
Sõmera, M., Fargette, D., Hébrard, E., and Sarmiento, C. 2021. ICTV Report Consortium 2021. ICTV Virus Taxonomy Profile: *Solemoviridae*. J. Gen. Virol. 102:001707.
Stevens, M., Freeman, B., Liu, H.-Y., Herrbach, E., and Lemaire, O. 2005. Beet poleroviruses: Close friends or distant relatives? Mol. Plant Pathol. 6:1-9.
Stevens, M., McGrann, G., and Clark, B. 2008. Turnip yellows virus (syn Beet western yellows virus): An emerging threat to European oilseed rape production? HGCA Res. Rev. 69:1-37.
Stevens, M., Smith, H. G., and Hallsworth, P. B. 1995. Detection of the luteoviruses, beet mild yellowing virus and beet western yellows virus, in aphids caught in sugar-beet and oilseed rape crops. Ann. Appl. Biol. 127:309-320.
Tan, S. T., Liu, F., Lv, J., Liu, Q. L., Luo, H. M., Xu, Y., Ma, Y., Chen, X. J., Lan, P. X., Chen, H. R., Cao, M. J., and Li, F. 2021. Identification of two novel poleroviruses and the occurrence of Tobacco bushy top disease causal agents in natural plants. Sci. Rep. 11:1-14.
Tang, J., Quinn, B. D., and Clover, G. R. G. 2009. First report of *Carrot red leaf virus*-associated RNA co-infecting carrot with *Carrot red leaf virus* and *Carrot mottle mimic virus* to cause carrot motley dwarf disease in New Zealand. Australas. Plant Dis. Notes 4:15-16.
Veidt, I., Lot, H., Leiser, M., Scheidecker, D., Guilley, H., Richards, K., and Jonard, G. 1988. Nucleotide sequence of beet western yellows virus RNA. Nucleic Acids Res. 16:9917-9932.
Walsh, J. A., Perrin, R. M., Miller, A., and Laycock, D. S. 1989. Studies on beet western yellows virus in winter oilseed rape (*Brassica napus ssp. oleifera*) and the effect of insecticidal treatment on its spread. Crop Prot. 8:137-143.
Wang, F., Wu, Q. F., Zhou, B. G., Gao, Z. L., and Xu, D. F. 2015. First report of turnip yellows virus in tobacco in China. Plant Dis. 99:1870.
Wood, D. E., Lu, J., and Langmead, B. 2019. Improved metagenomic analysis with Kraken 2. Genome Biol. 20:1-13.
Yoshida, N., and Tamada, T. 2019. Host range and molecular analysis of *Beet leaf yellowing virus*, *Beet western yellows virus*-JP and *Brassica yellows virus* in Japan. Plant Pathol. 68:1045-1058.
Zheng, X., Koopmann, B., Ulber, B., and von Tiedemann, A. 2020. A global survey on diseases and pests in oilseed rape—Current challenges and innovative strategies of control. Front. Agron. 2:590908.
Ziegler-Graff, V., Brault, V., Mutterer, J. D., Simonis, M. T., Herrbach, E., Guilley, H., Richards, K. E., and Jonard, G. 1996. The coat protein of Beet western yellow luteovirus is essential for systemic infection but the viral gene products P29 and P19 are dispensable for systemic infection and aphid transmission. Mol. Plant-Microbe Interact. 9:501-510.



Supplementary Figure S1. Sugar beet samples that tested positive in ELISA for infection with polerovirus. A, B, C, D, E and F correspond to sample 2, 3, 17, 19, 22 and 24, respectively (Table 1). Sequence analyses of the coat protein gene showed triple infection in samples 3 and 24 with beet mild yellowing virus, beet chlorosis virus and turnip yellows virus.



Supplementary Figure S2. Pairwise identity matrix of turnip yellows virus (TuYV) genome sequences for the Swedish isolate from oilseed rape in Karpalund and reference isolates. Reference isolates are named by GenBank accession number, virus, geographic origin and host.



Supplementary Figure S3. Pairwise identity matrix of turnip yellows virus-associated RNA (TuYVaRNA) and turnip yellows virus-associated RNA2 (TuYVaRNA2) sequences for the Swedish isolate from oilseed rape in Karpalund and reference isolates. Reference isolates are named by GenBank accession number, virus, geographic origin and host.

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2025:2

Virus yellows (VY) disease is transmitted by aphid vectors and the disease may result in significant yield losses in sugar beet. This doctoral thesis explored the diversity of viruses causing VY disease in Swedish sugar beet. Interactions between beet mild yellowing virus (BMYV) and its host were studied in depth at the transcriptional level and genomic regions underlying resistance and susceptibility to BMYV was mapped in a resistant genotype of wild beet and a susceptible genotype of sugar beet. These findings lay a foundation for future resistance breeding.

Vinitha Puthanveed received her graduate education at the Department of Plant Biology, SLU, Uppsala, Sweden. She did her MSc in Plant Sciences at Wageningen University and Research, Wageningen, the Netherlands and received her BSc in Agriculture at Tamil Nadu Agricultural University, Trichy, India.
Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-437-6
ISBN (electronic version) 978-91-8046-487-1