



Diversity and distribution of turnip yellows virus infecting *Brassica napus* L. (canola) in the Western Cape, South Africa

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Abstract Turnip yellows virus (TuYV), (genus: *Polerovirus*), poses a significant threat to brassica-ceous crops, particularly *Brassica napus* L. (canola). In Australia and the United Kingdom, TuYV has caused substantial grain yield reductions of up to 50%. This study documents the first extensive survey of TuYV on canola in South Africa, specifically in the Swartland and Overberg regions of the Western Cape. Using RNAseq-derived metaviromes generated from 38 symptomatic plants in 2021, complete TuYV genomes, spanning approximately 5650 nucleotides, were assembled from 30 datasets. Phylogenetic (ORF 5) and phylogenomic analyses revealed two genetically distinct yet geographically undifferentiated populations. For samples collected in the 2022 and 2023 seasons, RT-PCR targeting a 1,284 nucleotide fragment, including ORF 3a, confirmed TuYV presence in a further 245 samples, with phylogenetic analyses

again indicating two distinct phylogroups, based on ORF 3a. These findings suggest a widespread TuYV distribution in the Western Cape, with spread potentially facilitated by various aphid vectors. The existence of distinct, widely distributed variants, implies a longstanding presence in the region, underscoring the need for continued monitoring, management, and resistance testing to mitigate potential severe yield losses.

Keywords *Brassica napus* · Virus diversity · Turnip yellows virus · South Africa

Introduction

Canola (rapeseed, oilseed rape, *Brassica napus* L.) represents cultivars of rapeseed that produce oils that are relatively low in erucic acid. Globally, canola is the third most important oilseed crop after soybean and sunflower (Chataut et al., 2023). In South Africa, canola has been cultivated commercially since the late 1990's (Esterhuizen, 2023), with the vast majority of current production occurring in the Western Cape province where it is grown as a dryland rotation crop with wheat and barley (Tsfamariam et al., 2010). Within the Western Cape, the two major production regions are Swartland and Overberg with consistent year on year increases in yield and area planted, at 128,000 hectares (ha) planted on 2023 (Esterhuizen, 2023) with

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a gross production value of approximately R1 billion (DALRRD, 2021).

In South Africa, canola production is often limited by disease-causing fungi (e.g., *Fusarium*, *Rhizoctonia* and *Sclerotinia* species) and oomycetes from the genus *Pythium* (Lamprecht et al., 2011; Rothmann & McLaren, 2018). Although few viruses impact canola production compared to other grain crops, some viruses are economically important, limiting yields in many canola-growing regions. Among these, turnip yellows virus (TuYV) (family: *Solemoviridae*, genus: *Polerovirus*), is one of the most important canola pathogens causing, 11–46% production losses globally (Ibrahim et al., 2023). TuYV is spread by aphid species in a persistent manner, with *Myzus persicae* being the most common and efficient vector of the virus (Schliephake et al., 2000). In South Africa, TuYV has been reported from limited numbers of canola samples, as well as from cabbage (*Brassica oleracea* L. var. *capitata*) where it was associated with brassica stunting disorder (New et al., 2016).

TuYV infections on canola typically manifest as leaf purpling and stunting, where early season infections are associated with reduced shoot and flower production, which ultimately results in the production of less seed and seed of low quality (Jones et al., 2007). This is because seed from diseased plants typically contain less oil (Congdon et al., 2020) with increased levels of erucic acid and glucosinolate, both of which negatively impact the health of animals and humans (Jay et al., 1999). However, relatively little is known about the genetic variation across different populations of TuYV as different virus variants may be associated with variations in symptom severity, host range and vector transmissibility (Congdon et al., 2023). Such patterns emphasise the need for in depth, regional diversity studies for informing TuYV management strategies.

In South Africa, no virological surveys have been previously performed on canola. In this study, we aimed to identify the causal agent responsible for symptoms resembling those of TuYV in canola plants from the Western Cape. We also explored the distribution and diversity this viral agent, as well as its persistence in the main canola growing regions in South Africa, over a period of three years.

Materials and methods

Collection of symptomatic material

In 2021, 33 canola plants, four *Raphanus raphanistrum* (wild radish) plants and a *Brassica alba* (white mustard) plant displaying symptoms of virus like disease, were collected from the Swartland and Overberg regions in Western Cape province (South Africa). These two regions are spatially and geographically separated by several of the Cape Fold mountain ranges, as well as climatically with the Swartland region being warmer and drier (Midgeley et al., 2023).

During 2022 and 2023, a further 144 and 107 symptomatic plants were collected, respectively.

Nucleic acid isolation, RNAseq library preparation and sequencing

Leaf material for each sample was pulverised using liquid nitrogen and total RNA was isolated using a modified cetyltrimethylammonium bromide protocol (White et al., 2008). RNA quantity and purity were measured using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNA obtained for samples collected in 2021 were used to prepare RNAseq libraries (Shishkin et al., 2015). Library sequencing was performed using an Illumina NextSeq 2 K (Illumina, San Diego, CA, USA) at the University of Leeds Genomics Facility, Leeds, UK, as paired ends (2 × 100 bp).

Quality control of non-demultiplexed RNAseq data was done using FastQC (Babraham Bioinformatics, Cambridge, UK). Demultiplexing into individual datasets was performed using Je (Girardot et al., 2016). Trimming for quality and adapter content was performed using CLC Genomics Workbench 21 (Qiagen Bioinformatics, Aarhus, Denmark), with the following parameters: Minimum read length: 20 nt and quality threshold: 0.05. Adapter trimming was performed using Illumina universal adapter (5'-AGA TCGGAAGAG-3') and RNAseq adapter (5'-TAC ACGACGCTCTTCCGATCT NNNNNNNNT-3') sequences. Assembly was then performed on each dataset, using SPAdes (Prjibelski et al., 2020) with the *meta* option (Nurk et al., 2017). Plant virus-like contigs were identified using blastx (Altschul et al., 1990) and the “virus” fraction of the NCBI’s

nonredundant database (12–07–2022). Open reading frames (ORFs) were determined using ORF finder (Wheeler et al., 2003).

RT-PCR and sanger sequencing

All samples were subjected to reverse transcription (RT)-PCR to confirm the presence of TuYV, using primers designed in this study: TuYV-F (5' CGG ACA ACT GGA ATT CTG CTC TCA 3') and TuYV-R (5' GGG AAT ACC ATC GAG AAT CGA GGT T 3'). This primer pair targets a 1,284 nucleotide (nt) fragment, binding at 3,031–3,054 nt and 4290–4314 nt (with reference to GenBank accession NC003743) and includes ORF 3a, ORF 3 and ORF 4. A two-step reaction was setup, using M-MuLV reverse transcriptase, murine RNase inhibitor and OneTaq® 2X Master Mix (New England Biolabs, Ipswich, MA, USA), according to instructions. Positive amplicons were purified using ExoSAP-IT™ (Thermo Fischer Scientific Baltics, Vilnius, Lithuania). Sequencing reactions were performed, in both forward and reverse directions, using a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fischer Scientific Baltics, Vilnius, Lithuania), purified using ethanol/EDTA precipitation and submitted to the University of Pretoria, DNA Sanger sequencing facility for capillary electrophoresis, using an ABI 3500xl Genetic Analyzer (Life Technologies Holdings, Singapore). Chromatogram base calling and correction was done using 4peaks (Nucleobytes, Aalsmeer, The Netherlands). Contigs based on forward and reverse sequences were generated using the CAP3 function in Unipro's bioinformatics software UGENE (Okonechnikov et al., 2012).

Phylogenetic analyses and identification of phylogroups

The complete coding and non-coding nucleotide sequences of the genomes were aligned to extant, complete genome sequences of TuYV, using Clustal Omega (Madeira et al., 2019). The best-fit model was determined using MEGA X (Stecher et al., 2020). A maximum-likelihood phylogenomic tree was generated using the general time reversible model (Tavaré et al., 1986) with invariant sites (I) and gamma (G) distribution (n=5) and 1000 bootstrap replicates to estimate branch support.

The amino acid (aa) sequences for ORF 3a (45 aa) (derived from Sanger and genome sequence data) and for ORF 5 (430 aa) (derived from genome sequence data) were aligned using Clustal Omega. Maximum-likelihood phylogenies were generated using the Jones-Taylor-Thornton (JTT) (Jones et al., 1992)+G model. Branch support was estimated using the same model parameters and 1000 pseudoreplicates.

Amino acid similarity matrices were generated using alignments containing relevant sequences of each phylogroup for ORF 5. Matrices were generated using the *generate distance matrix* within Unipro UGENE (Okonechnikov et al., 2012). Cognate sequences of TuYV C20A (MT586597) and TuYV 5509 (MT586587) were selected to represent phylogroups 5 – I and II respectively. Similarity matrices were generated for ORF 3a, using the same approach as for ORF 5, but with the cognate region of TuYV 5513 (MT586592), representing TuYV phylogroup P3a – I, and TuYV 5509 (MT586587) representing TuYV phylogroup P3a—II, according to the demarcation thresholds proposed by Filardo et al., (2021).

Results

Genome sequence of TuYV from South African canola

A total of 194,763,388 pre-trim and 141,104,249 post-trim reads were obtained across all 38 datasets for the 2021 samples, with a post-trim average of 3,713,270 and a range of 417,154 to 9,929,524 reads. Individual datasets are available within a National Center for Biotechnology Information (NCBI) sequence read archive under accession PRJNA998000. Blast results showed that 30 of the 38 datasets were associated with TuYV contigs. No other plant-associated viruses were detected in any of the other datasets. All positive 2021 samples were associated with a complete/near-complete TuYV genome, encoding seven ORFs (ORF 0–2, 3a, and 3–5). The average genome length was 5,676 nt with a range of 5,625–5,703 nt. See Table 1 for sample provenance, sequence lengths, GenBank accession numbers and average coverages.

Table 1 Sample accessions from the 2021 collection period

Sample accession	Host plant	Host cultivar	Region	GenBank accession of TuYV genome	Genome length (nt)	Average coverage (x)
21–0334	Canola	Diamond	Swartland	OR350418	5,639	1,353
21–0335	Canola	Diamond	Swartland	OR350419	5,703	1,229
21–0337	Canola	Diamond	Swartland	-	-	-
21–0338	Canola	Diamond	Swartland	OR350420	5,625	827
21–0339	Canola	Diamond	Swartland	OR350421	5,681	1,290
21–0340	Canola	Diamond	Swartland	OR350422	5,693	1,096
21–0341	Canola	Diamond	Swartland	OR350423	5,666	1,690
21–0342	Canola	Diamond	Swartland	OR350424	5,675	219
21–0343	Canola	Diamond	Swartland	-	-	-
21–0344	Canola	Diamond	Swartland	OR350425	5,665	1,812
21–0345	Wild radish	n/a	Swartland	-	-	-
21–0346	Canola	Diamond	Swartland	OR350426	5,694	742
21–0347	Canola	Diamond	Swartland	OR350427	5,689	138
21–0348	Canola	Diamond	Swartland	OR350428	5,681	644
21–0368	Canola	PN990	Swartland	OR350429	5,685	745
21–0369	Canola	PN990	Swartland	-	-	-
21–0370	Canola	PN990	Swartland	OR350430	5693	784
21–0375	Canola	PN990	Swartland	-	-	-
21–0378	Wild radish	n/a	Swartland	OR350431	5,660	106
21–0379	Canola	PN990	Swartland	OR350432	5,681	1,051
21–0381	Canola	Hyola350TT	Overberg	OR350433	5,685	2,467
21–0385	Canola	Hyola350TT	Overberg	OR350434	5,679	1,455
21–0386	Canola	Hyola350TT	Overberg	OR350435	5,667	1,048
21–0422	Canola	Unknown	Swartland	OR350436	5,666	1,203
21–0423	Canola	Unknown	Swartland	-	-	-
21–0424	Canola	Unknown	Overberg	OR350437	5,664	417
21–0425	Canola	Unknown	Overberg	OR350438	5,680	2,680
21–0426	Canola	Unknown	Overberg	OR350439	5,694	902
21–0428	Canola	Unknown	Overberg	OR350440	5,649	2,310
21–0432	Canola	Unknown	Overberg	OR350441	5,675	2,902
21–0433	Wild radish	n/a	Overberg	-	-	-
21–0434	Canola	Unknown	Overberg	OR350442	5,673	601
21–0435	Canola	Unknown	Overberg	OR350443	5,689	682
21–0436	White mustard	Vitaro	Overberg	OR350444	5,689	2,002
21–0438	Canola	Hyola650TT	Overberg	OR350445	5,676	598
21–0456	Wild radish	n/a	Swartland	OR350446	5,667	329
21–0460	Canola	Unknown	Swartland	OR350447	5,685	128
21–0461	Canola	Unknown	Swartland	-	-	-

These samples are associated with high-throughput sequencing data and in cases where turnip yellows virus (TuYV) was present, details of a complete/near complete viral genome is provided. *nt* nucleotides, *x* = average coverage, — = no TuYV detected

RT-PCR detection of TuYV in canola

To screen for the presence of TuYV in canola samples, we designed a RT-PCR procedure based on primers targeting a region including ORF 3a, 3 and 4. Application of this method on nucleic acids obtained from the 2021 samples yielded results identical to those recorded using whole genome sequencing (i.e., all of the samples lacking TuYV, according to NGS, tested negative in the RT-PCR, while those containing TuYV sequences tested positive). We accordingly applied the RT-PCR method for detecting TuYV in the canola samples collected during 2022 and 2023.

Overall, of the 251 samples collected during 2022 and 2023, 245 tested positive for TuYV with RT-PCR and of these 189 were associated with Sanger sequencing data (<https://doi.org/10.5281/zenodo.17647952>) (Supplementary Table 1). Of the 8 RNAseq datasets that were not associated with TuYV contigs, none were associated with “typical” TuYV symptoms. However, of the four samples that tested negative with RT-PCR, all were associated

with TuYV symptoms. Conversely, from those that tested positive with RT-PCR, two were considered to be asymptomatic. However, TuYV was shown to be most reliably detected from the apical parts of infected canola plants, with the oldest (and often most symptomatic) leaves providing the lowest levels of diagnostic reliability (Congdon et al., 2020). Therefore, we cannot exclude the possibility that the four negative RT-PCRs were due to our sampling of lower, symptomatic leaves with low viral titres. Nevertheless, all but two positive canola samples showed foliar symptoms, ranging in intensity and the commonalities of symptom intensity decreasing from the lower to upper leaves was consistently observed. Leaf discoloration ranged from deep maroon to brick red and was consistently interveinal. The positive samples of wild radish displayed symptoms of general chlorosis, while the white mustard positive plant was associated with non-descript reddening, probably not associated with TuYV (Fig. 1).

The information for the samples collected during the 2021 season are listed in Table 1, while

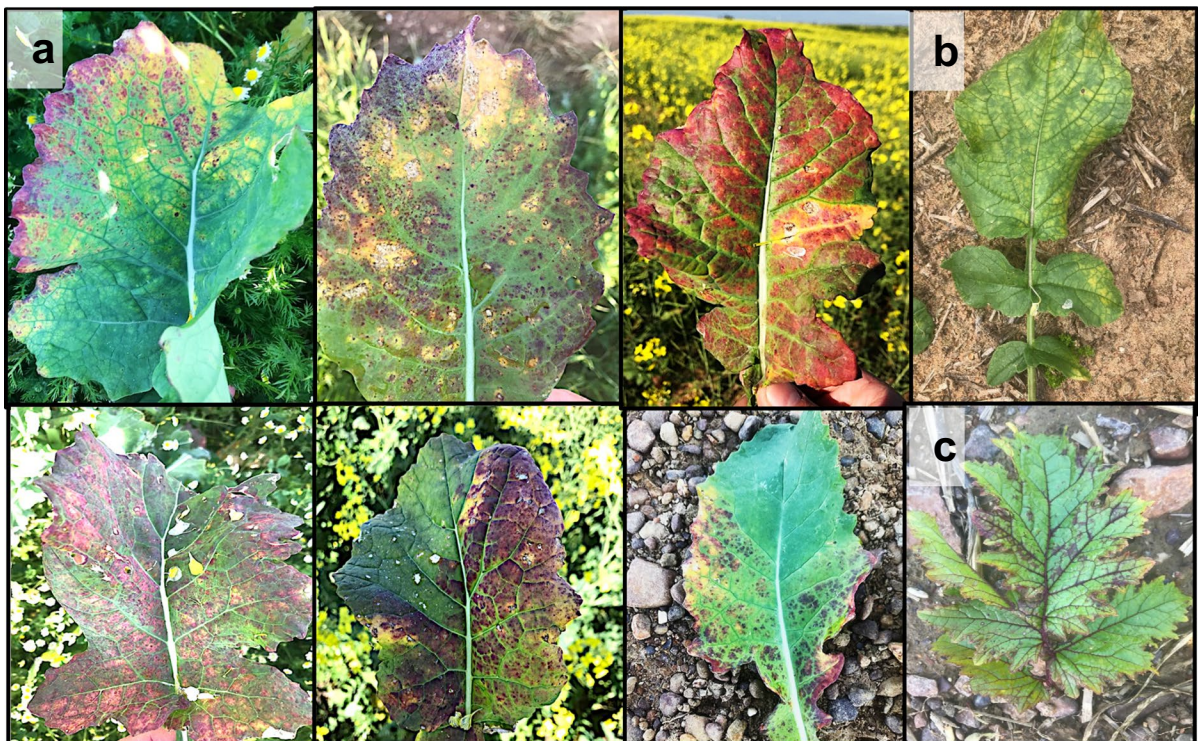


Fig. 1 Representative symptoms associated with (a) canola, (b) *Raphanus raphanistrum* (wild radish) plants and (c) *Brassica alba* (white mustard)

those for subsequent samples are provided in Supplementary Table 1. Symptom images for all samples can be viewed using the following link: <https://doi.org/10.5281/zenodo.14188108>. File names correspond to the respective sample accession number, appended either with P (positive) or N (negative), based on either RNAseq or RT-PCR results. A map, showing the approximate sampling locations can be found at bit.ly/4jdbk89.

South African canola TuYV is closely related to those from Australian canola

The phylogenomic tree showed that TuYV variants from this study clustered into two distinct clades (abridged tree, Fig. 2; full tree, Supplementary Fig. 1), both of which contain the sequences of TuYV from Australian canola (Filardo et al., 2021). However, grouping into these clades did not correspond to the geographic location of the sample collection site. The close relationship of the South African canola TuYV variants with those from Australian canola was

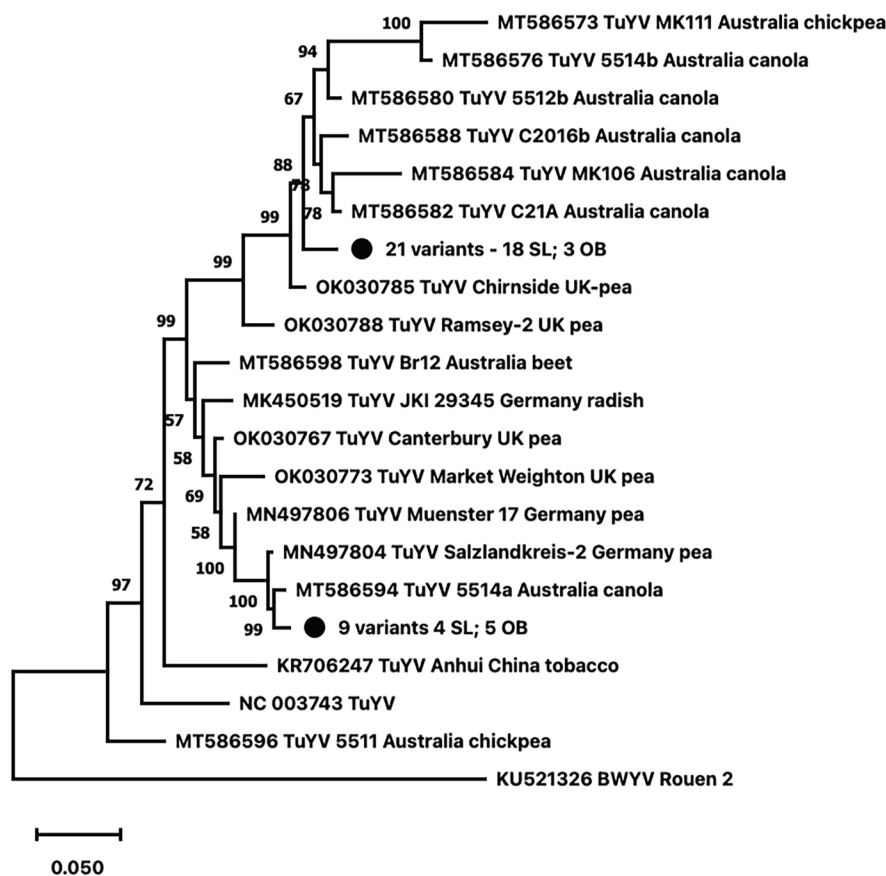


Fig. 2 Maximum likelihood phylogeny illustrating the phylogenetic relationships between the complete/near complete genomes of turnip yellows virus from this and selected references from GenBank. The phylogeny represents the tree with the highest log likelihood and was generated in MEGA X using the General Time Reversible model (Tavaré et al., 1986) with a proportion of invariant sites and gamma distribution to account for among-site rate variation. Bootstrapping was applied (1000 replicates) and the percentage of trees in which

the associated taxa clustered together is shown next to the branches. Bootstrap percentages lower than 50 are not shown. Phylogroups from this study are indicated with filled black circles, with numbers representing the how many variants are associated with each collapsed branch. SL – Swartland, OB—Overberg regions in the Western Cape. A representative genome of beet western yellows virus (BWYV) was used as an outgroup. The scale bar shows substitutions per site

supported by phylogenetic analyses of the ORF 5 and ORF 3a datasets (Supplementary Figs. 2 and 4).

The ORF 5 dataset contained sequences obtained from the genome data of the 30 TuYV associated with the 2021 canola samples, as well as global reference sequences of TuYV, brassica yellows virus (BrYV) and beet western yellows virus (BWYV). South African variants clustered into two of the three phylogroups previously identified by Filardo et al (2021) in Australia. Here, nine of our variants clustered with group P5 – I (four were from Swartland and five from Overberg), while 21 clustered with group P5 – II (19 were from Swartland and two from Overberg). None of the variants from the current study clustered within group P5—III. Based on amino acid similarity (Supplementary Fig. 3), our P5 – I and II variants shared 89–100% and 90–100% amino acid identity (AAI), respectively, with those from Australia (Supplementary Figs. 2 and 3).

The ORF 3a dataset contained sequences extracted from the genomes of associated with the 30 TuYV-positive samples collected in 2021, as well as 108 and 81 sequences of the RT-PCR amplicons generated from the samples collected in 2022 and 2023, respectively. The alignment also included global reference sequences of TuYV, brassica yellows virus (BrYV) and beet western yellows virus (BWYV). The ORF 3a phylogeny also clustered our sequences into 2 of the phylogroups (P3a – I and II) identified in Australian canola by Filardo et al. (2021). A total of 58 variants formed part of phylogroup P3a – I (36 from Overberg and 22 from Swartland) and corresponded to P5 – I. Based on amino acid similarity (Supplementary Fig. 5) to a P3a – I representative (MT586592) from Australian canola, the South African variants in this phylogroup shared 96–100% AAI. Among the South African P3a – I variants, pair-wise AAI values also ranged from 96%–100%. A total of South African 161 variants clustered in P3a – II (60 from Overberg and 101 from Swartland), which corresponded to P5 – II. Our P3a – II shared 98–100% AAI with Australian representative (MT586587) from this phylogroup. The South African members of this phylogroup shared 98–100% AAI.

When considering collection dates, a total of 9 P3a – I variants were obtained from the 2021 samples, sharing 96–100% AAI. From the 2022 and 2023 samples, 32 and 17 P3a – I variants were obtained, with those from 2022 sharing 91–96% AAI and those from

Table 2 Distribution of P3a–I and P3a–II variants detected in canola samples collected from two regions, namely Swartland (SL) and Overberg (OB), across three growing seasons (2021–2023)

Year	Region	P3a–I	P3a–II	Total
2021	SL	1	18	19
2021	OB	8	3	11
2022	SL	6	25	31
2022	OB	26	51	77
2023	SL	15	58	73
2023	OB	2	6	8
		58	161	219

Values represent the number of variants identified per region and year. Totals are shown per row and across variant groups

2023 sharing 98–100% AAI. From the 2021 samples contained 21 P3a – II variants that shared 100% AAI. Seventy-six and 64 P3a – II variants were obtained from the 2022 and 2023 samples, with AAI-values of 100% and 98–100%, respectively. These numbers are summarised in Table 2.

Discussion

The findings presented in this study suggest that TuYV may represent an underreported issue in South African canola production, mirroring challenges observed in Australia, where symptoms of TuYV infection are often misattributed to nutrient or environmental stress (Jones et al., 2021). Although nitrogen and phosphorus deficiencies can cause purpling and bronzing of leaves (DAFWA, 2015; Parker, 2009), TuYV symptoms typically differ by presenting more intensely on older leaves, often with as a more red colour than purple, and exhibiting interveinal discolouration and upper leaf chlorosis as symptoms progress. In South Africa, while fungal pathogens have historically been regarded as primary yield-limiting factors in canola (Lamprecht et al., 2011; Rothmann & McLaren, 2018), viruses like TuYV have received less attention. Although TuYV has been documented locally, it has primarily been associated with brassica stunting disorder in cabbage (New et al., 2016). Nevertheless, producers in the Western Cape appear to make the same assumptions as their Australian counterparts and often attribute virus-like symptoms in canola to nutrient deficiencies.

In this study, we confirm the presence of TuYV on canola, and show that the virus is widespread and persistent within the industry. The variants from this study appear to group phylogenetically, with variants from Australian canola. The phylogenomic tree from our study shows that variants of BrYV group with variants of TuYV, with similar observations for ORF 3a and 5 phylogenies. This supports the notion that BrYV and TuYV are conspecific (Filardo et al., 2021), but ICTV current species demarcation thresholds for poleroviruses supports TuYV and BrYV as two separate species (Umar et al., 2022).

In this study, a portion of the genome encoding for ORFs 3a, 3 and 4, was selected as the target gene for RT-PCR amplification of the samples collected in 2022/23. ORF 3a has been shown to be relatively diverse (Filardo et al., 2021), making it a good candidate for TuYV population studies. However, any future work on variant identifications should be done based on ORF 5, coding for the most variable TuYV gene (Filardo et al., 2021), providing the most information on host range, transmission and virulence (Congdon et al., 2023). Although whole-genome characterisation remains the most robust method for TuYV variant classification (Congdon et al., 2023; Filardo et al., 2021), it remains unfeasible for surveys involving hundreds of samples. Pragmatically, ORF 5 has been shown to have the greatest power for resolving between variants of TuYV and BWYV (Filardo et al., 2021).

According to ORF 5 phylogenies, TuYV variants cluster into three groups, with P5-II identified as a recombinant of P5-I and P5-III (Filardo et al., 2021). These groups differ in their host range, transmissibility, and virulence, with P5-I representatives showing the highest capacity for aphid transmission (82%), viral titre, and symptom severity, followed by a decrease in P5-II and an even further decrease in P5-III (Congdon et al., 2023). In our study, the ORF 5 phylogeny showed that most of the variants associated with complete genomes group within P5-II (21 variants), with the remainder grouping with P5-I (9 variants). This is corroborated by the phylogenomic tree.

The phylogenetic analysis of ORF 3a reaffirmed the close relationship between South African Turnip yellows virus (TuYV) variants and Australian canola-infecting strains, with clustering patterns reflecting those previously described by Filardo et al., (2021).

Variants grouped into the P3a-I and P3a-II clades, consistent with their corresponding P5 groupings. As with P5-II, variants from the P3a-II group comprised of the most variants. Interestingly, P5-I variants, which appear to have the greatest propensity for transmission, were detected at levels roughly 50% lower than P5-II, suggesting that factors other than transmissibility alone is responsible for shaping South African canola TuYV populations. This also shows that although the most virulent variant is present in the South Africa, it does not appear to be dominant. However, the factors shaping TuYV population structures, as well the determining the levels of resistance present within cultivars grown in South Africa will require further research.

This study has demonstrated the widespread and persistent presence of TuYV for several seasons, in canola from the Western Cape. While it likely presents an ongoing threat to production, further research is needed in several key areas to better understand the extent. Although TuYV is the most damaging virus affecting canola, the damage appears to depend on the growth stage at which infection occurs, with the greatest yield losses associated with infections at pre-flowering stages (Congdon et al., 2020). Infection and time of infection is inextricably linked to aphid population patterns. Pre-flowering infection correlates with increased aphid populations and activity during the first half (autumn/early winter) of the growing season (Slavenko et al., 2024). Generally, early season planting has been associated with improved yield (Midgeley et al., 2023; Shafighi et al., 2021). However, these warmer conditions likely correlate with increased numbers aphid flights and the movement of aphids from “green bridges” (refuge hosts for TuYV) to canola (Slavenko et al., 2024). In our study, all samples were collected at growth stage 6 (flowering) or later and therefore no data for pre-flowering infection was collected.

Wild radish could be acting as a bridge for TuYV to canola and in the current study we present the first report of TuYV infection in wild radish in South Africa. TuYV has a wide host range, shown to infect species from at least twelve different plant families (Slavikova et al., 2022). Wild radish is one of many species of weeds that has the capacity to act as a reservoir host (Vizi et al., 2023). Wild radish is of particular interest, since it is a pervasive invasive in the crop production systems of the Western Cape, where

it is known to be a reservoir host of several non-viral pathogens (Morris & Knox-Davies, 1980). Wild radish is considered a winter annual, it has been shown to survive beyond the winter months in temperate climates, and to act as an effective reservoir for cucurbit chlorotic yellows virus (Kavalappara et al., 2022). While the capacity of wild radish to act as a bridging reservoir for TuYV in the Western Cape has not been considered, the potential for it to fulfil this function should be investigated, particularly around source of perennial water.

Further research should also include determining the stages of the onset of infection across several seasons in the Western Cape and linked to an aphid modelling study such as that of Slavenko et al., (2024). This information will provide insights into the spread of TuYV on canola in South Africa. Mild autumn and winter temperatures are associated with increased aphid activity and a concomitant increase in TuYV incidence (Juergens et al., 2010). Increases in both temperature and drought conditions are expected to exacerbate the effects of TuYV infections on canola (Lago et al., 2024), with near future increases in temperature and drought incidence both being predicted in the Western Cape under climate change scenarios (Naik & Abiodun, 2024). However, practical management of TuYV will likely remain difficult, since insecticidal treatments of aphid vectors have been shown to be ineffective (Bass et al., 2014) and together with the incompatibility of increased pesticide use with the current drive for ecological sustainability. Should vector control prove to be unfeasible, the planting of resistant canola varieties could form part of a practical TuYV control strategy. It has recently been shown that varieties of canola display varying levels of resistance to TuYV (Ibrahim et al., 2023) and is another avenue of research that should be considered for germplasm used in the South African context.

This study confirms that TuYV is not only present but also widespread and persistent in canola across the Swartland and Overberg regions. Phylogenetic and phylogenomic analyses revealed that South African TuYV variants group closely with those from Australia. Although the seemingly less transmissible group II variants are present at higher incidences, other ecological or agronomic factors may be influencing TuYV population structure in the region. The continued misdiagnosis of TuYV symptoms as nutrient deficiencies likely contributes to underreporting

and underestimation of its impact on yield. Sampling at later growth stages, as was done in this study, may limit the ability to assess the full effect of early-season infections, which are known to be more damaging. Therefore, future work should aim to monitor TuYV infection timing across seasons and link this to aphid vector activity and climatic conditions to better understand disease dynamics and inform local management strategies.

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Data availability The data that support the findings of this study are openly available in NCBI public databases.

Code availability Not applicable.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. This manuscript constitutes original research and has not been previously published nor submitted for consideration at any other journal. All authors have agreed to the submission of the manuscript, which complies with the ethical standards of the journal.

Conflict of interest All authors declare that they have no conflicts of interest.

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