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Research Papers

Spatial distribution and molecular characterization of persistently aphid-transmitted viruses causing yellowing and stunting in faba bean and chickpea crops in Tunisia

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Summary. Field surveys were conducted in the northeastern and northwestern regions of Tunisia between 2013/2014 and 2018/2019 growing seasons to identify viruses that infect faba bean and chickpea crops. Field observations showed that 18.8% of the faba bean and 21.0% of the chickpea fields surveyed had virus-like symptoms. These rates exceeded 20% during the growing seasons from 2013/2014 to 2018/2019, and were most common in the 2014/2015 growing season (28.6% of surveyed faba bean fields and 37.5% of chickpea fields showed virus-like symptoms in more than 20%). Totals of 1,538 faba bean and 1,511 chickpea plant samples showing yellowing and stunting symptoms were collected from 144 faba bean and 124 chickpea fields, . All collected samples were tested by tissue blot immunoassay (TBIA) using six monoclonal antibodies. These results showed that chickpea chlorotic stunt virus (CpCSV; *Polerovirus CPCSV*) was the most prevalent in faba bean and chickpea, with incidences of 20.2% and 37.6%, respectively, followed by beet western yellows virus (BWYV; *Polerovirus BWYV*) (13.5 and 9.7%), bean leafroll virus (BLRV; *Luteovirus phaseoli*) (3.7 and 3.3%), and faba bean necrotic yellows virus (FBNYV; *Nanovirus necroflaviviciae*) detected only in faba bean (6.6% of faba bean samples tested). In addition, TBIA results indicated that single virus infections were more prevalent than mixed infections in both crops. Mixed infections were predominantly co-infections involving viruses in *Polerovirus* (*Solemoviridae*), particularly CpCSV and BWYV (93% in faba bean and 69% in chickpea). Twenty-six samples that reacted positively with different monoclonal antibodies were assessed with reverse transcription-polymerase chain reaction (RT-PCR) using generic and specific primers followed by sequencing of the partial coat protein (*CP*) gene. The sequence analyses confirmed presence of CpCSV, BWYV, BLRV, brassica yellows virus (BrYV; *Polerovirus TUYV*), and turnip yellows virus (TuYV; *Polerovirus TUYV*). Comparative sequence analyses of the Tunisian iso-

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lates indicated that 13 CpCSV sequences had nucleotide sequence similarities of 95 to 99% with the reference isolate (EU541266) belonging to serotype I, and six BLRV isolates had similarities of 96 to 99% with BLRV reference isolate (PP333098). One sample (TuCp265-19) had a mixed infection with CpCSV and BLRV. Six isolates initially detected using BWYV-specific primers were sequenced and analyzed. BLASTn results showed that only three isolates were closely related (98 to 100%) to BWYV (OM419176), while the remaining four isolates were identified as *Polerovirus TUYV* and showed greatest similarity to BrYV (LC428361) and TuYV (OP699039), indicating co-occurrence of two distinct *Polerovirus* species within the analyzed samples. There is no information on the genetic variability of legume viruses in Tunisia, so this study shows that these viruses should be considered when developing disease management strategies to improve faba bean and chickpea production in Tunisia.

Keywords. CpCSV, BWYV, BLRV, *Polerovirus*, mixed infections, sequencing, phylogenetic analyses.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.) are the two major pulse crops grown in West Asia and North Africa (WANA) (Makkouk, 2020). In Tunisia, faba bean and chickpea are among the most widely cultivated legumes, grown in northern Tunisia. In 2024, faba bean covered 55,212 ha, with an average yield of 1.34 ton ha^{-1}, while chickpea covered 6,743 ha with an average yield of 1.43 ton^{-1} (FAOSTAT, 2026). These two crops are key components of the Tunisian cereal-based production system, and their productivity is affected by parasitic weeds, diseases and insect pests. Viruses and their vectors are among the most important obstacles to production of these two key crops, and the disease management practices available to farmers are limited.

Several viruses associated with yellowing and stunting symptoms in cool-season food legumes are among the most internationally important diseases (Makkouk *et al.*, 2003; Makkouk, 2020). These viruses are predominantly transmitted by aphid vectors, either persistently circulative, as reported for the viruses investigated in the present study, or less commonly those that are non-persistently transmitted (Makkouk *et al.*, 2003; Kumar *et al.*, 2008; Kumari *et al.*, 2009; Makkouk *et al.*, 2014). Some of these viruses have been reported in Tunisia, include: faba bean necrotic yellows virus (FBNYV; *Nanovirus necroflaviviciae*, *Nanoviridae*), bean leafroll virus (BLRV; *Luteovirus phaseoli*, *Luteovirus, Tombusviridae*), soybean dwarf virus (SbDV; *Luteovirus glycinis*, *Luteovirus*, *Tombusviridae*), beet western yellows virus (BWYV; *Polerovirus BWYV*, *Polerovirus*, *Solemoviridae*), and chickpea chlorotic stunt virus (CpCSV; *Polerovirus CPCSV*, *Polerovirus*, *Solemoviridae*) (Najar *et al.*, 2000a; 2003; 2011; Kumari *et al.*, 2015). The main aphids transmitting legume viruses are *Aphis craccivora* and *Acyrthosiphon pisum* (Asaad *et al.*, 2009; Abraham and Vetten, 2022).

Serological differentiation of closely related virus species within *Solemoviridae*, particularly *Polerovirus* species, may be limited by cross-reactivity when antibodies are raised against conserved epitopes, highlighting the importance of sequence-based approaches for accurate virus identification (Moukahel *et al.*, 2021; Ademe *et al.*, 2025). Uses of molecular techniques for chickpea showing yellowing/stunting symptoms that showed cross-reactivity between the specific monoclonal antibodies have led to identification of four new viruses in *Polerovirus* that infect chickpea in Sudan (Moukahel *et al.*, 2021).

Accurate identification of viruses affecting legume crops is crucial for breeding for resistance to these diseases they cause and for developing crop disease management methods. Viruses infecting food legume crops can be identified using serology, but molecular approaches have not been widely used to identify additional viruses that may cause economic losses in Tunisia. To address this knowledge gap, field surveys were conducted in major chickpea and faba bean production areas to determine whether new viruses are associated with yellowing/stunting symptoms. Results of these surveys are reported in this paper.

MATERIALS AND METHODS

Field surveys, visual disease assessments, and sample collection

Field surveys were carried out each growing season from 2013/2014 to 2018/2019, in the main faba bean and chickpea growing areas of Tunisia during the flowering and pod setting stages of crop growth. The surveys were carried out in the northeastern regions (Bizerte, and Cap Bon) and northwestern regions (Kef, Jendouba, and Béja) of Tunisia (Figure 1). Totals of 1,538 faba bean and 1,511 chickpea samples, showing symptoms including yellowing, stunting, leaf rolling, and/or reddening, were collected from 144 faba bean and 124 chickpea fields (Table 1). In each field, plants were inspected using a standardized survey format, that recorded crop condition, growth stage, disease symptoms, disease incidence,

Figure 1. A map showing the location of fields surveyed in the northeastern (Bizerte, Cap Bon) and the northwestern (Kef, Jendouba, Béja) regions of Tunisia between 2013-2019.

and aphid populations. Virus incidence was estimated in each field as the percentage of plants exhibiting virus-like symptoms per square meter at different randomly selected locations within the field. Incidence values were then classified into five categories: <1%, 1 to 5%, 6 to 20%, 21 to 50%, and >50%. All samples were placed in labelled plastic bags and transported to the ICARDA Virology Laboratory in Tunis for virus testing. For these, fresh stems from each plant sample were blotted onto nitrocellulose membrane (NCM, 0.45 µm, Bio-Rad, Cat No. 1620115) in ten replicates. Leaves from each sample were freeze-dried for subsequent molecular analysis.

Serological assays

All samples blotted onto NCM were tested by tissue-blot immunoassay (TBIA) using the method of Makkouk and Kumari (1996). A monoclonal antibody (MAb) specific for FBNYV (3-2E9; Franz *et al.*, 1996), and a broad-spectrum legume MAb (5G4; Katul, 1992) that detects legume virus species in *Solemoviridae* and *Tombusviridae*, were used for the assessments. Samples that reacted positively with 5G4 MAb were subsequently tested with specific MAbs for BWYV (A5977; Agdia), BLRV (4B10; Katul, 1992), SbDV (PVAS-650, ATCC, United States of America) and a mixture of three MAbs (5-2B8, 5-3D5 and 5-5B8) produced against a Syrian isolate of CpCSV (Abraham *et al.*, 2006; 2009).

Virus molecular characterizations, genetic diversity and phylogenetic analyses

Based on serological reactions with different MAbs, 26 samples (four from faba bean and 22 from chickpea) (Table 2), representing all the surveyed regions, were selected for further molecular characterizations (seven of these samples only reacted with 5G4 Mab, ten samples reacted positively with 5G4 and CpCSV MAbs, six samples reacted positively with 5G4 and BWYV MAbs, and three samples reacted positively with 5G4 and BLRV MAbs (Table 2). Total RNA was extracted from plant samples, and cDNA syntheses were carried out as described by Moukahel *et al*. (2021).

Virus detections were carried out using reverse transcription-polymerase chain reactions (RT-PCR) that included a pair of generic primers (AS3/Pol3870F) to amplify 370 bp of the partial coat protein (*CP*) gene of several Luteo- and polero-viruses. Samples amplified using a pair of generic primers were reprocessed by multiplex RT-PCR (MP-PCR) (Moukahel *et al*., 2021), using the generic reverse primer AS3 with species-specific primers for CpCSV, BWYV, SbDV, BLRV, phasey bean mild yellows virus (PBMYV; *Polerovirus PBMYV*; *Polerovirus*; *Solemoviridae*), and cucurbit aphid-borne yellows virus (CAbYV; *Polerovirus CABYV, Polerovirus*, *Solemoviridae*). Each MP-PCR amplification mixture was divided in two multiplex master mixes due to the proximity in product sizes for some primers: master mix-I include AS3 with primers BLRV3589F, BWYV3969F, SbDV3731F, and PBMYV3396F (amplify, respectively, 551, 276, 418 and 838 bp of the partial *CP*); and master mix-II consisted of AS3 with primers CpCSV3705F and CABYV3635F (amplify, respectively, 566 and 474 bp). Primers, PCR amplification conditions and electrophoresis analyses were carried out using the protocol described by Moukahel *et al*. (2021).

High-quality PCR products were directly sequenced in both directions using the Sanger method by Macrogen, South Korea. The sequences were compared with available database sequences (Table 3), using the basic local alignment search tool BLAST program (Altschul *et al*., 1997; 2005). Sequences were analyzed to estimate nucleotide polymorphism parameters with DnaSP software version 6.0 (Rozas *et al*., 2017). Virus genetic diversity was quantified using haplotype diversity (Hd), numbers of haplotype (h) (Tajima, 1989), numbers of segregating sites (S), and nucleotide diversity (Pi) (Jukes and Cantor, 1969). Pairwise sequence identities were calculated using the Sequence Demarcation Tool (SDT) v1.2 (Muhire *et al*., 2014). Phylogenetic relationships were inferred using the Maximum Likelihood method based on Jukes-Cantor model with 1,000 bootstrap replicates. Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

Table 1. Results of serological tests (tissue blot immunoassay, TBIA) for faba bean and chickpea symptomatic samples collected from different regions of Tunisia during 2013-2019.

Crop / Season	Region	Number of fields surveyed	Number of samples tested	Number of samples reacted positively with MAbs[a]						% of virus infection based on the TBIA results of the 1st test
				1st test		2nd test for samples, which reacted with 5G4				
				FBNYV	5G4	CpCSV	BWYV	BLRV	Unidentified	
Faba bean										
2013/2014	Bizerte	10	141	0	34	17	11	4	0	24.1
	Cap Bon	6	75	0	24	14	8	0	6	32.0
	Kef	5	79	0	15	15	0	0	0	19.0
	Béja	2	32	0	6	6	0	0	0	18.8
	Jendouba	5	81	0	21	15	5	0	2	25.9
2014/2015	Bizerte	17	268	0	68	32	19	0	18	25.4
	Cap Bon	16	172	0	64	5	30	0	30	37.2
	Kef	2	19	0	6	0	2	0	4	31.6
	Béja	14	140	0	48	12	18	0	12	34.3
2016/2017	Bizerte	4	36	23	18	5	0	11	2	100.0
	Cap Bon	3	25	8	11	2	0	5	4	76.0
	Kef	6	49	29	10	0	4	7	0	79.6
	Béja	7	50	10	17	1	0	6	10	54.0
	Jendouba	2	12	1	0	0	0	0	0	8.3
2017/2018	Bizerte	7	86	10	63	33	47	3	0	84.9
	Cap Bon	8	114	0	105	98	43	1	2	92.1
	Kef	3	17	0	13	12	1	0	4	76.5
	Béja	4	38	5	28	27	16	0	0	86.8
	Jendouba	2	12	0	12	12	1	0	0	100.0
2018/2019	Bizerte	8	32	11	14	3	0	9	1	78.1
	Cap Bon	5	22	3	0	0	0	0	0	13.6
	Kef	2	12	2	3	1	0	1	0	41.7
	Béja	3	18	0	6	0	0	4	2	33.3
	Jendouba	3	8	0	8	0	2	6	0	100.0
Subtotal faba bean		144	1538	102	594	310	207	57	97	45.3
Chickpea										
2014/2015	Bizerte	29	504	0	342	262	26	0	54	67.9
	Cap Bon	4	73	0	6	3	4	0	1	8.2
	Béja	7	148	0	61	59	15	0	0	41.2
	Jendouba	8	158	0	71	55	16	0	4	44.9
2015/2016	Bizerte	4	32	0	22	13	6	0	3	68.8
	Cap Bon	9	78	0	34	30	9	0	0	43.6
	Kef	3	21	0	16	4	11	0	1	76.2
	Béja	3	28	0	7	2	1	0	4	25.0
	Jendouba	6	78	0	69	42	28	0	0	88.5
2016/2017	Bizerte	6	56	0	22	10	6	19	0	39.2
	Cap Bon	7	63	0	42	36	12	13	0	66.7
	Kef	3	18	0	13	12	0	0	1	72.2
	Béja	5	41	0	21	9	4	0	8	51.2
	Jendouba	4	30	0	6	2	0	0	4	20.0
2018/2019	Bizerte	3	12	0	4	1	1	0	2	33.3
	Cap Bon	12	91	0	38	6	4	16	12	41.8
	Kef	4	21	0	19	15	0	0	4	90.5
	Béja	6	47	0	14	6	3	1	4	29.8
	Jendouba	1	12	0	1	1	0	0	10	8.3
Subtotal chickpea		124	1511	0	808	568	146	49	112	53.5
Total		268	3049	102	1402	878	353	106	209	49.3

[a] FBNYV: faba bean necrotic yellows virus (3-2E9 MAb); 5G4: broad-spectrum legume luteovirid monoclonal antibody; CpCSV: chickpea chlorotic stunt virus (mixture of 5-2B8, 5-3D5 and 5-5B8 MAbs); BWYV: beet western yellows virus (A5977 MAb from Agdia); BLRV: bean leafroll virus (4B10 MAb).

All samples were negative for Soybean dwarf virus (PVAS-650 MAb from ATCC, USA).

RESULTS

Assessments of virus-like symptoms in the field

During field visits, colonies of the potential virus-transmitting aphids *Aphis fabae, Aphis craccivora, Acyrthosiphon pisum* and *Myzus persicae* were observed congregating on the stems, leaves and shoots of young faba bean plants. Faba bean plants exhibiting symptoms commonly associated with virus diseases were collected, including yellowing, chlorosis, stunting and leaf rolling. Virus-like symptom incidence was estimated based on these observable field symptoms. Symptom incidence varied among seasons, crop types, and surveyed regions. Approximately 18.8% of faba bean and 21.8% of chickpea fields had more 20% of plants with virus-like symptoms during the 2013/2014 to 2018/2019 growing seasons, with the 2014/2015 growing season having the most severe diseases (28.6% of faba bean and 37.5% chickpea fields had more than 20% of plants with virus-like symptoms) (Figure 2). Not all symptomatic plants were confirmed to be virus-infected; as similar symptoms may also result from other factors, such as abiotic stresses.

Virus identifications based on serological tests

Serological results (TBIA) from 1,538 faba bean and 1,511 chickpea symptomatic samples showed that overall incidence of the detected viruses in chickpea samples was 53.5%, whereas in faba bean this was 45.3% (Table 1). Greatest incidence was recorded for luteoviruses, where 808 chickpea (53.5%) and 594 faba bean samples (38.6%) reacted positively with the broad-spectrum legume virus MAb (5G4). In contrast, FBNYV was detected in 102 (6.6%) of the faba bean samples (Table 1). Based on serological tests with the MAbs used in this study, 54.7% of the faba bean samples and 46.5% of the chickpea samples were virus-free (Table 1).

When samples that had reacted positively to the 5G4 MAb were retested with the specific MAbs, CpCSV, BWYV, and BLRV were detected, respectively, in 310, 207 and 57 faba bean samples, and in 568, 146 and 46 chickpea samples. SbDV was not detected in any of the assessed samples. In addition, 209 samples (97 from faba bean and 112 from chickpea) reacted only with 5G4 MAb, but not with the specific MAbs used in the study (Table 1).

Mixed virus infections

Mixed virus infections were less frequent than single infections in both crop types. In faba bean, 123 out of 594 (20.7%) 5G4-positive samples showed mixed infections, whereas in chickpea, 71 out of 808 (8.8%) 5G4-positive samples had mixed infections (Table 4). These infections were mostly CpCSV + BWYV co-infections, which accounted 114 of 123 mixed infections (92.7%) in faba bean and 50 of the 71 mixed infections (69%) in chickpea. Other combinations were less frequent, including CpCSV + BLRV (detected in one faba bean and 13 chickpea samples), while triple infections were rare, occurring in two faba bean and three chickpea samples (CpCSV + BWYV + BLRV) and in one faba bean sample (CpCSV + BWYV + FBNYV) (Table 4).

Regionally, CpCSV and BWYV co-infections were most prevalent in Bizerte, representing 59% of mixed infections in faba bean and 52% in chickpea, followed by Cap Bon where they accounted for 37% of mixed infections in faba bean and 26% of those in chickpea (Figure 3). Overall, CpCSV was the most frequently detected virus in mixed infections affecting the chickpea and faba bean crops.

Molecular characterizations of viruses

The generic primer pair AS3/Pol3870F amplified a fragment of approx. 370 bp, corresponding to a partial *CP* gene of luteo- and polero-viruses. MP-PCR using specific primers detected CpCSV, BWYV and BLRV in, respectively, four, six and two samples, with the expected amplicon size of each virus (566 bp for CpCSV, 276 bp for BWYV, and 551 bp for BLRV). In addition, two mixed infections were detected: isolate TuCp265-19 (CpCSV + BLRV) and isolate TuCp275-19 (CpCSV + BWYV) (Table 2). Eleven samples were amplified by the generic primer pair AS3/Pol3870F, but not by the specific primers used. Therefore, these together, in addition to those amplified with specific primers, were subjected to Sanger sequencing (Table 2). No sample was amplified with the primers for SbDV, PBMYV, or CAbYV.

Analysis of 26 partial *CP* genes (one amplicon from 24 samples, and two amplicons from one sample TuCp265-19) confirmed presence of CpCSV (in 13 samples), BLRV (in six samples), BWYV (in three samples), brassica yellows virus (BrYV; *Polerovirus TUYV; Polerovirus; Solemoviridae*) (two samples) and turnip yellows virus (TuYV; *Polerovirus TUYV*) (two samples) (Table 2). BLASTn analyses showed that Tunisian CpCSV isolates shared 95%-99% nucleotide similarity with the Moroccan faba bean CpCSV-serotype I isolate (EU541266) (Table 2, Figure 4).

Among the 13 CpCSV isolates, five isolates (GenBank accession numbers MT739413, MT739414, MT739415, MT739416, and MT739418) were 100% iden-

Table 2. Geographic origins, comparison and identity with reference GenBank accessions of chickpea and faba bean isolates collected from Tunisia in 2016, 2018, and 2019.

Isolate code[a]	Crop	Region	Genbank accession number	TBIA reaction with MAbs	RT-PCR reaction with primers[b]	Virus sequences Blastn reference GenBank accessions	Blastn similarity %
TuCp08-19	Chickpea	Cap Bon	PQ526601	5G4, CpCSV	Generic only	CpCSV; EU541266	98
TuCp15-19	Chickpea	Cap Bon	PQ526602	5G4, CpCSV	Generic only	CpCSV; EU541266	98
TuCp37-19	Chickpea	Cap Bon	PQ526603	5G4, CpCSV	Generic only	CpCSV; EU541266	98
TuCp98-19	Chickpea	Bizerte	PQ526604	5G4, CpCSV	Generic, CpCSV	CpCSV; EU541266	98
TuCp100-19	Chickpea	Bizerte	PQ526605	5G4, CpCSV	Generic only	CpCSV; EU541266	98
TuCp108-19	Chickpea	Bizerte	PQ526606	5G4, BLRV	Generic only	CpCSV; EU541266	98
TuCp160-19	Chickpea	Jendouba	PQ526607	5G4, CpCSV	Generic, CpCSV	CpCSV; EU541266	95
TuCp195-19	Chickpea	Beja	PQ526608	5G4	Generic, CpCSV	CpCSV; EU541266	99
TuCp207-19	Chickpea	Beja	PQ526609	5G4, CpCSV	Generic only	CpCSV; EU541266	98
TuCp261-19	Chickpea	Beja	PQ526610	5G4	Generic only	CpCSV; EU541266	98
TuCp265-19	Chickpea	Beja	PQ526611	5G4, CpCSV	Generic, CpCSV, BLRV	CpCSV; EU541266	98
TuCp290-19	Chickpea	Cap Bon	PQ526612	5G4, BLRV	Generic only	CpCSV; EU541266	99
TuFa109-18	Faba bean	Cap Bon	MT739415	5G4, CpCSV	Generic, CpCSV	CpCSV; EU541266	98
TuFa62-16	Faba bean	Bizerte	PQ526614	5G4, BWYV	Generic, BWYV	BWYV; OM419176	100
TuFa72-16	Faba bean	Bizerte	PQ526615	5G4, BWYV	Generic, BWYV	BWYV; OM419176	98
TuCp58-19	Chickpea	Cap Bon	PQ526617	5G4, BWYV	Generic, BWYV	BWYV; OM419176	99
TuCp104-19	Chickpea	Bizerte	PQ526618	5G4	Generic, BWYV	BrYV; LC428361	98
TuCp275-19	Chickpea	Cap Bon	PQ526620	5G4	Generic, CpCSV, BWYV	BrYV; LC428361	91
TuCp16-19	Chickpea	Cap Bon	PQ526616	5G4, BWYV	Generic, BWYV	TuYv; OP699039	96
TuCp229-19	Chickpea	Beja	PQ526619	5G4, BWYV	Generic, BWYV	TuYV; OP699039	94
TuFa101-18	Faba bean	Cap Bon	PQ526622	5G4	Generic, BLRV	BLRV; PP333098	98
TuCp36-19	Chickpea	Cap Bon	PQ526623	5G4, BLRV	Generic, BLRV	BLRV; PP333098	99
TuCp38-19	Chickpea	Cap Bon	PQ526624	5G4	Generic only	BLRV; PP333098	96
TuCp172-19	Chickpea	Jendouba	PQ526625	5G4	Generic only	BLRV; PP333098	96
TuCp258-19	Chickpea	Beja	PQ526626	5G4, BWYV	Generic only	BLRV; PP333098	98
TuCp265-19	Chickpea	Beja	PQ526627	5G4, CpCSV	Generic, CpCSV, BLRV	BLRV; PP333098	98

[a] The last two numbers refer to year of collection.

[b] Generic primers (AS3/Pol3870F); CpCSV: chickpea chlorotic stunt virus (CpCSV3705F primers); BWYV: beet western yellows virus (BWYV3969F primers); BLRV: bean leafroll virus (BLRV3589F primers).

tical to each other, and therefore only isolate MT739415 was used as the representative sequence for the whole set. Sequence analyses of seven isolates amplified with BWYV-specific primers revealed that three isolates shared 98 to 100% similarity with BWYV from coriander in Cyprus (OM419176) (Table 2, Figure 5 A), whereas four isolates showed 91 to 99% similarity with TuYV. Among these, two isolates were closely related (94 to 99%) to a TuYV isolate from the Czech Republic (OP699039), while two clustered with a Japanese rapeseed isolate (91 to 98%) previously described as BrYV (LC428361), currently classified within the *Polerovirus TUYV* species (Table 2, Figure 5 B). The *CP* of the five Tunisian BLRV isolates shared 96 to 99% nucleotide identity with a BLRV isolate originated from alfalfa in Spain (PP333098) (Table 2, Figure 6). All 26 *CP* sequences generated in this study were deposited in GenBank under the accession number listed in Table 2.

Genetic diversity

The *CP* sequences of 26 Tunisian virus isolates from this study (Table 2) together with 47 references sequences retrieved from GenBank (Table 3) were analysed using DnaSP version 6.0 (Rozas *et al*., 2017). For CpCSV, analysis of the complete dataset (24 sequences) revealed 16 polymorphic sites (S), ten haplotypes (hd), and high haplotype diversity (Hd = 0.841), with nucleotide diversity Pi (JC = 0.0266). However, Tunisian CpCSV showed no variable sites, indicating very low local genetic variability. For TuYV, analysis of the four Tunisian isolates revealed three haplotypes (h = 3), with high haplotype diver-

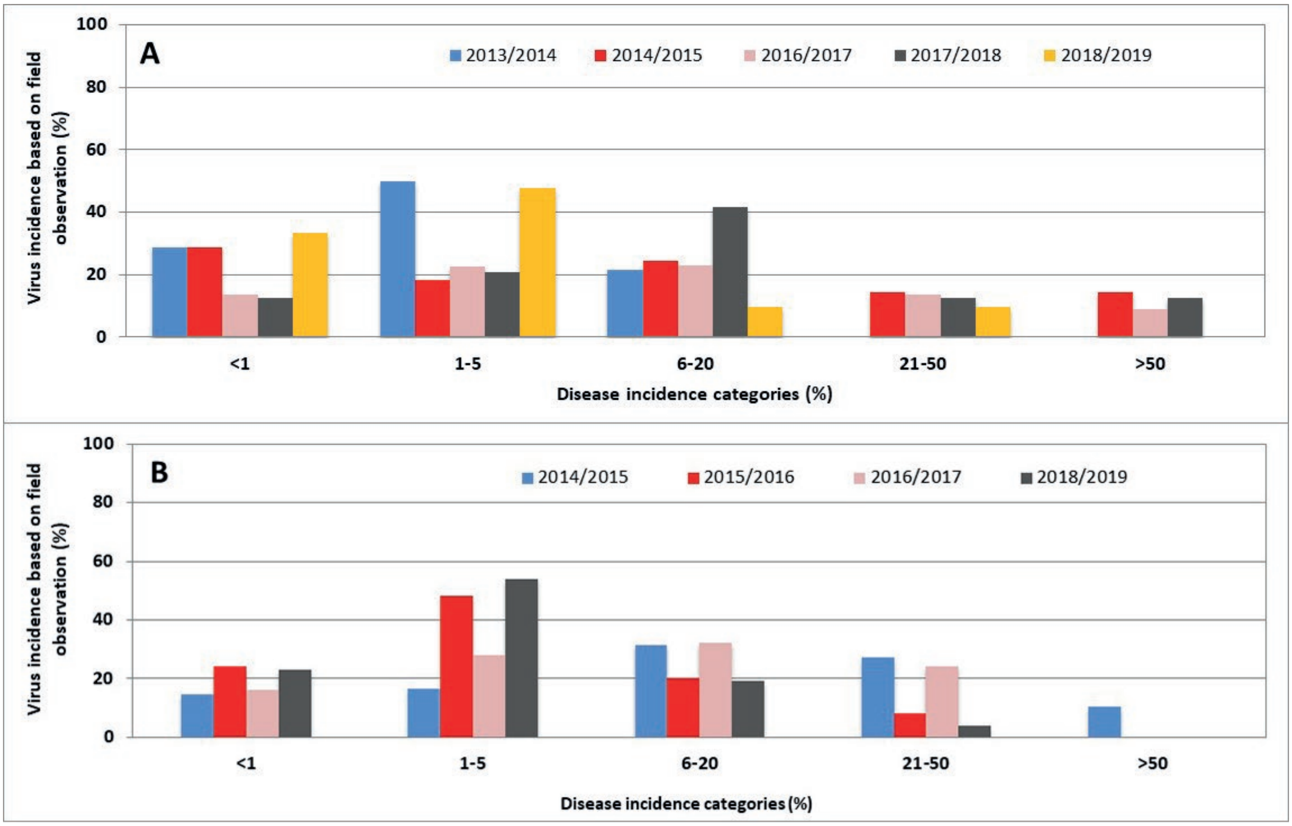

Figure 2. Fields of faba bean (A) and chickpea (B) showing virus-like symptoms during surveys conducted in Tunisia between 2013–2019.

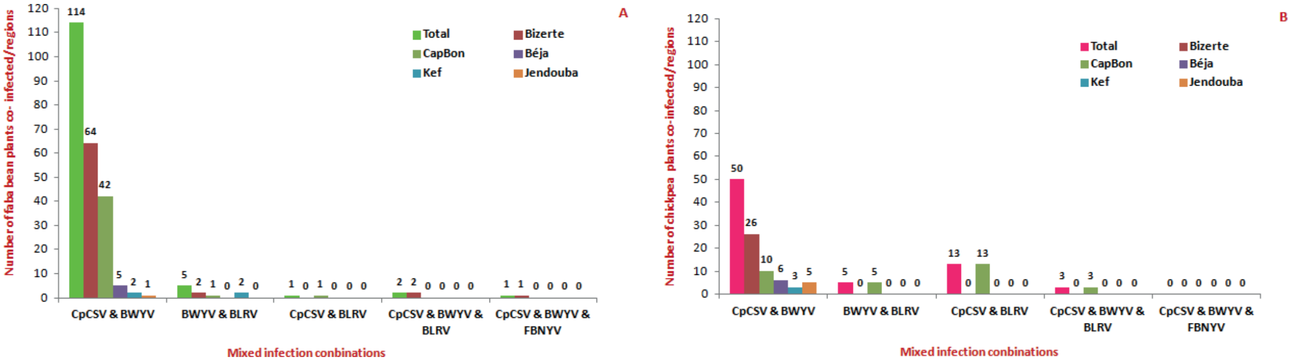

Figure 3. Distribution of mixed infection combinations involving chickpea chlorotic stunt virus (CpCSV), beet western yellows virus (BWYV), bean leafroll virus (BLRV), and baba bean necrotic yellows virus (FBNYV) across five regions of Tunisia, based on serological Tissue-Blot Immunoassay (TBIA) of surveys conducted in faba bean (A) and chickpea (B) crops (2013-2019). Bars indicate to the number of plants co-infected by each virus combination per region, relative to the total mixed-infected plants.

sity (Hd = 0.833), 26 polymorphic sites, and nucleotide diversity Pi (JC) = 0.058. When the complete dataset of 14 sequences was analyzed, nine haplotypes were detected (Hd= 0.879) with the same 26 polymorphic sites and Pi (JC) = 0.058. For BWYV, analysis of nine sequences revealed 35 polymorphic sites, resulting in six haplotypes (h = 6) and high haplotype diversity (Hd = 0.889). Nucleotide diversity was high (Pi (JC) = 0.0586), with an average 11.99 nucleotide differences, indicating substantial genetic differentiation among the BWYV isolates.

Analysis restricted to the three Tunisian BWYV variants identified five polymorphic sites, forming three dis-

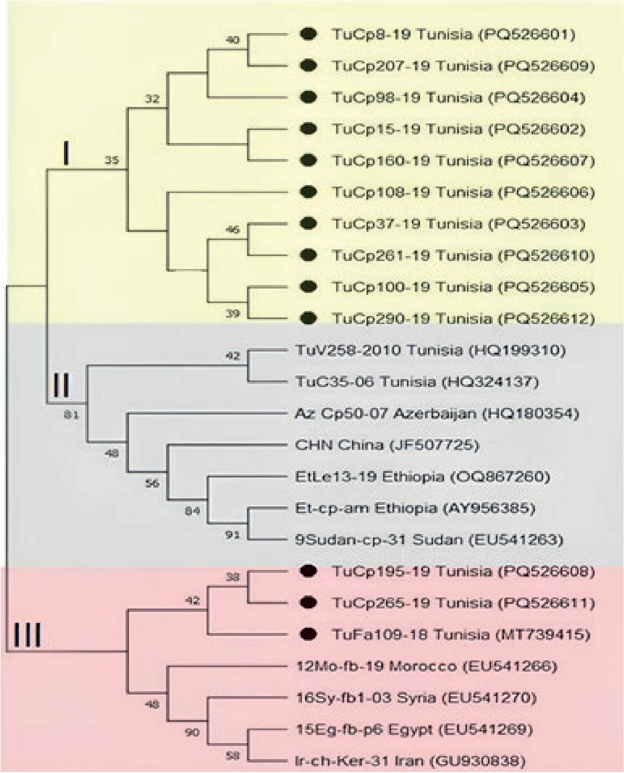

Figure 4. Maximum likelihood phylogenetic analysis of the partial CP gene of 24 chickpea chlorotic stunt virus (CpCSV, species *Polerovirus CPCSV*) isolates from chickpea and faba bean. Isolates from this study are indicated by black circles.

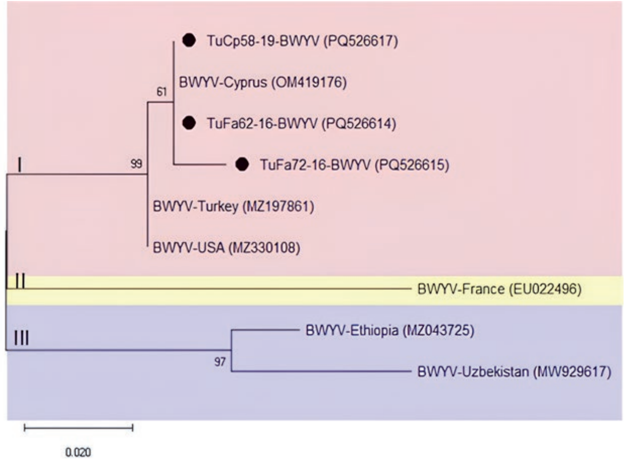

Figure 5. Maximum likelihood phylogenetic analysis of the partial CP gene of 9 beet western yellows virus (BWYV, species *Polerovirus BWYV*) isolates from chickpea and faba bean. Isolates from this study are indicated by black circles.

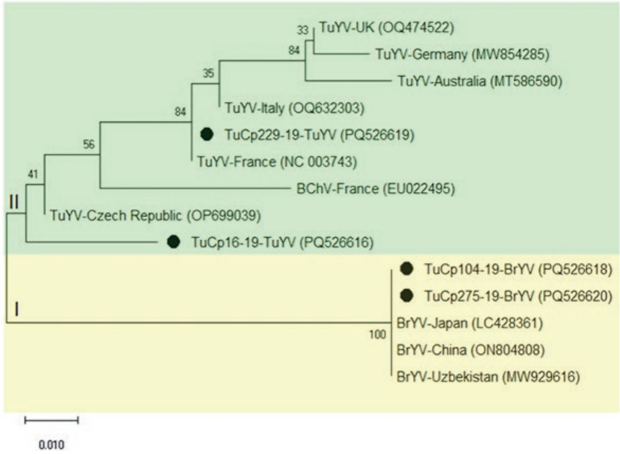

Figure 6. Maximum likelihood phylogenetic analysis of the partial CP gene of 14 TuYV/BrYV (species *Polerovirus TUYV*) isolates from chickpea and faba bean. Isolates from this study are indicated by black circles.

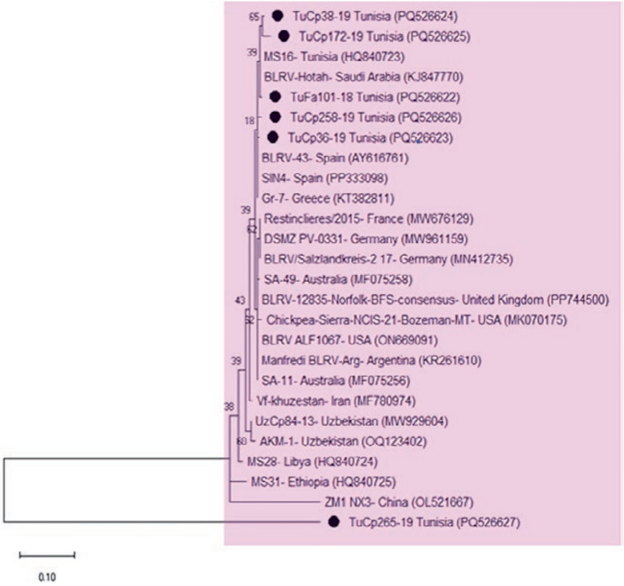

Figure 7. Maximum likelihood phylogenetic analysis of the partial CP gene of 26 bean leafroll virus (BLRV, species *Luteovirus phaseoli*) isolates from chickpea and faba bean. Isolates from this study are indicated by black circles.

tinct haplotypes (Hd = 1.0), suggesting clear genetic differentiation among these isolates. For BLRV, analysis of the complete dataset (26 isolates) revealed 18 haplotypes with high haplotype diversity (Hd = 0.957) and nucleotide diversity of approx. Pi (JC) = 0.1, based on 39 variable sites detected among the CP sequences.

Phylogenetic analyses for specific viruses

CpCSV - The Maximum Likelihood (ML) phylogenetically grouped CpCSV isolates (Figure 4), into three well-defined groups (I, II, and III). Group I comprised most of the new Tunisian isolates, which clustered closely together, indicating high genetic relatedness and suggesting local diversification of CpCSV within Tunisia. All isolates within this subgroup originated only from chickpea, and were collected during the survey conducted in 2019, which indicates existence of a temporally and host-associated lineage. Group II included earlier reported Tunisian isolates together with isolates from Azerbaijan, China, Ethiopia, or Sudan, reflecting a geographically mixed lineage and possible historical introductions. The older Tunisian variants were recovered from two different host plants (chickpea and faba bean), indicating broad host adaptation. In contrast, Group III clustered several Tunisian isolates with isolates from Morocco, Syria, Egypt, and Iran, forming a distinct regional lineage independent of sampling year or host, and indicating ongoing circulation of CpCSV across North Africa and the Middle East. Overall, the phylogenetic structure this virus highlights substantial genetic diversity within CpCSV, and indicates that Tunisian isolates are distributed across multiple evolutionary lineages.

BWYV - The ML phylogenetic analysis resolved BWYV isolates into three clades (I, II, and III) (Figure 5). Clade I encompassed all Tunisian isolates, together with isolates from Cyprus, Turkey, and the United States of America, showing high nucleotide sequence similarity with the Cypriot coriander isolate from Cyprus (OM419176). Clade II was monophyletic and contained only the French sugar beet isolate (EU022496), highlighting the distinct evolutionary lineage of Clade II. Clade III grouped with chickpea isolates from Ethiopia (MZ043725) and Uzbekistan (MW929617). Overall, Clades II and III exhibited host-associated structuring (sugar beet for Clade II and chickpea for Clade III), whereas Calades I showed low host specify, including Citrus (MZ330108), *Capsicum annuum* (MZ197861), chickpea (PQ526617), faba bean (PQ526615, PQ526614), or coriander (OM419176) (Figure 5).

BrYV/TuYV (Polerovirus TUYV) - Phylogenetic analysis of *Polerovirus TUYV* resolved the dataset into two main groups (labeled I and II), largely structured by geographic origins (Figure 6). Group I comprised isolates closely related to European isolates, whereas group II clustered with isolates associated with Asian lineages. Despite the recent taxonomic revision unifying TuYV and BrYV into a single species (*Polerovirus TUYV*), isolates previously designated as TuYV remained separated into their respective lineages. This pattern indicates that the taxonomic unification did not take consideration of underlying genetic structure, and that geographic origin remains a key factor shaping the genetic diversity of *Polerovirus TUYV* (Figure 6).

BLRV - The ML phylogenetic analysis of BLRV isolates showed limited but detectable genetic variation. The Tunisian isolates (TuCp38-19, TuCp172-19, TuFa101-18, TuCp258-19, TuCp36-19, and TuCp265-19) were interspersed among sequences from different geographic origins, rather than forming well-supported, distinct clusters (Figure 7). Most Tunisian isolates showed close relationships with European isolates (from Spain, France, Germany, and Greece) as well as isolates from Saudi Arabia, Australia, or the United States of America, indicating high genetic similarity across these regions. One isolate (TuCp265-19) showed a more divergent position, clustering closer to isolates from Libya, Ethiopia, and China. Overall, these results indicate low genetic diversity among Tunisian BLRV isolates and weak geographic structuring, consistent with the broad international distribution of BLRV (Figure 7).

Pairwise identity

A pairwise identity matrix of partial CP sequences was calculated using the Sequences Demarcation Tool (SDT) (Muhire *et al.*, 2014) to evaluate genetic relationships among the analyzed isolates.

CpCSV - Similarity values ranged from 91 to 100% among CpCSV isolates (Figure 8). The 13 Tunisian isolates showed high intra-grouped similarities (95 to 100%), indicating a genetically homogeneous local population. The closest sequences corresponded to previously reported Tunisian isolates from faba bean, chickpea, or pea, sharing 93 to 97% nucleotide similarity. In contrast, comparisons with international CpCSV isolates showed slightly lower similarity values (90 to 98%), indicating moderate genetic divergence at the international level. These results support the phylogenetic relationships inferred from the ML analysis, confirming the presence of two main CpCSV lineages, despite their occurrence in different host plants (Figure 8).

BWYV - Pairwise nucleotide similarity analysis revealed values ranging from 98 to 99% among BWYV isolates. The three Tunisian BWYV isolates (PQ526614, PQ526615, and PQ526617) showed high similarity among themselves (98 to 99%), indicating a closely related local lineage. The closest sequences corresponded to the coriander isolate from Cyprus, with high nucleotide similarity. In contrast, lower similarity values were observed with sugar beet-derived BWYV isolates from

Table 3. Lists of GenBank sequences used for phylogenetic analysis

No.	Isolate	Geographical origin	Virus[b]	Host	GenBank accession No.
Isolates used in phylogenetic analysis of CpCSV					
1	12Mo-fb-19	Morocco	CpCSV	Faba bean	EU541266
2	16Sy-fb1-03	Syria	CpCSV	Faba bean	EU541270
3	15Eg-fb-p6	Egypt	CpCSV	Faba bean	EU541269
4	Ir-ch-Ker-31	Iran	CpCSV	Chickpea	GU930838
5	CHN	China	CpCSV	Pea	JF507725
6	Et-cp-am	Ethiopia	CpCSV	Chickpea	AY956385
7	EtLe13-19	Ethiopia	CpCSV	Lentil	OQ867260
8	9Sudan-cp-31	Sudan	CpCSV	Chickpea	EU541263
9	TuV258-2010	Tunisia	CpCSV	Faba bean	HQ199310
10	TuC35-06	Tunisia	CpCSV	Chickpea	HQ324137
11	Az Cp50-07	Azerbaijan	CpCSV	Chickpea	HQ180354
Isolates used in phylogenetic analysis of BWYV					
1	21506527b	Cyprus	BWYV	Coriander	OM419176
2	866	Turkey	BWYV	Bell pepper	MZ197861
3	IV400	USA	BWYV	*Citrus medica*	MZ330108
4	W1403a_2009	United Kingdom	TuYV	*Brassica napus*	OQ474522
5	MK107	Australia	TuYV	*Brassica napus*	MT586590
6	DSMZ PV-1209	Germany	TuYV	*Physalis pubescens*	MW854285
7	No8agrosCZ	Czech Republic	TuYV	*Papaver rhoeas*	OP699039
8	NAP	Japan	BrYV	Rapeseed	LC428361
9	BrYV-lnc	China	BrYV	Rapeseed	ON804808
10	FL1	France	TuYV	lettuce	NC_003743
11	ITA1	Italy	TuYV	*Phytolacca americana*	OQ632303
12	UzCp100-13	Uzbekistan	BrYV	Chickpea	MW929616
13	EthCp16-18	Ethiopia	BWYV	Chickpea	MZ043725
14	UzCp76-13	Uzbekistan	BWYV	Chickpea	MW929617
15	N20	France	BWYV	Sugar beet	EU022496
16	G43	France	BChV	Sugar beet	EU022495
Isolates used in phylogenetic analysis of BLRV					
1	SIN4	Spain	BLRV	Alfalfa	PP333098
2	ALF1067	USA	BLRV	Alfalfa	ON669091
3	Manfredi	Argentina	BLRV	Alfalfa	KR261610
4	SA-11	Australia	BLRV	Alfalfa	MF075256
5	Salzlandkreis-2_17	Germany	BLRV	Field pea	MN412735
6	12835-Norfolk-BFS-consensus	UK	BLRV	Field pea	PP744500
7	SA-49	Australia	BLRV	Alfalfa	MF075258
8	Restinclieres/2015	France	BLRV	Alfalfa	MW676129
9	DSMZ PV-0331	Germany	BLRV	Field pea	MW961159
10	MS16	Tunisia	BLRV	Chickpea	HQ840723
11	Gr-7	Greece	BLRV	Lentil	KT382811
12	UzCp84-13	Uzbekistan	BLRV	Chickpea	MW929604
13	MS28	Libya	BLRV	Faba bean	HQ840724
14	MS31	Ethiopia	BLRV	Lentil	HQ840725
15	BLRV-43	Spain	BLRV	Faba bean	AY616761
16	Chickpea-Sierra-NCIS-21-Bozeman-MT	USA	BLRV	Chickpea	MK070175
17	AKM-1	Uzbekistan	BLRV	Chickpea	OQ123402
18	Vf-khuzestan	Iran	BLRV	Faba bean	MF780974
19	ZM1_NX3	China	BLRV	Alfalfa	OL521667
20	BLRV-Hotah	KSA	BLRV	Alfalfa	KJ847770

[a] CpCSV: chickpea chlorotic stunt virus, BWYV: beet western yellows virus, TuYV: turnip yellows virus, BrYV: brassica yellows virus, BLRV: bean leafroll virus, BChV: beet chlorosis virus.

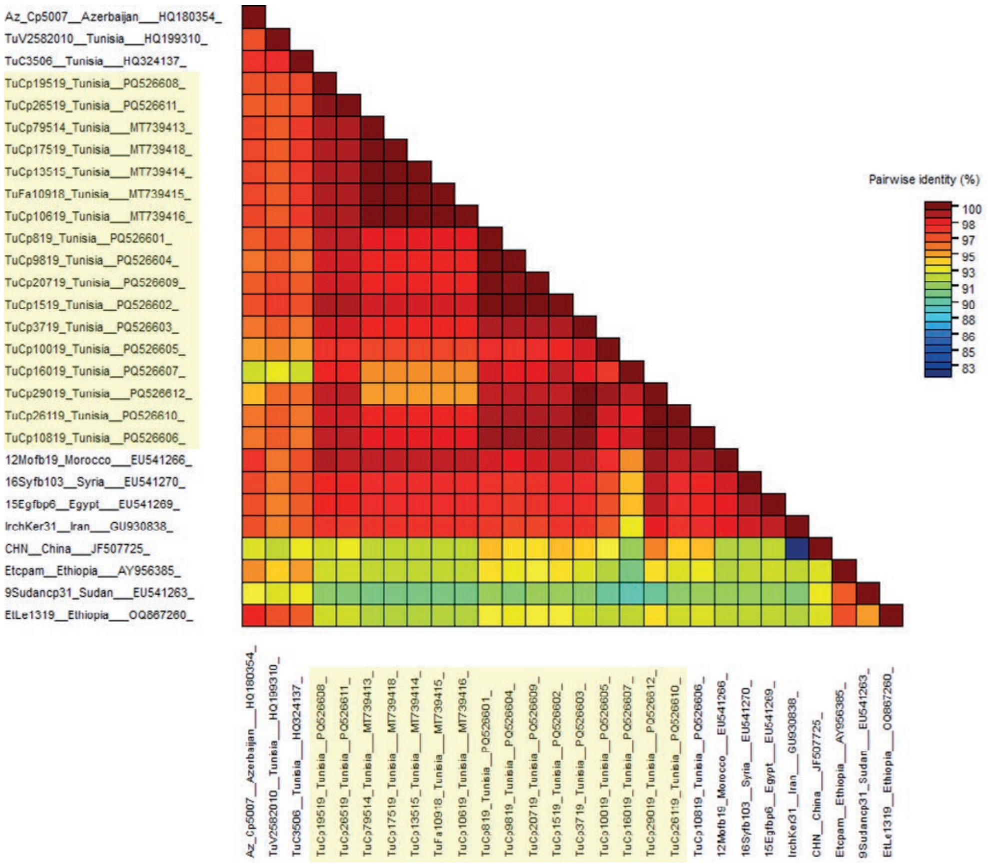

Figure 8. Pairwise nucleotide identity matrix of 24 chickpea chlorotic stunt virus (CpCSV, species *Polerovirus CPCSV*) sequences generated using SDTv1.3. Isolates characterized in this study are highlighted.

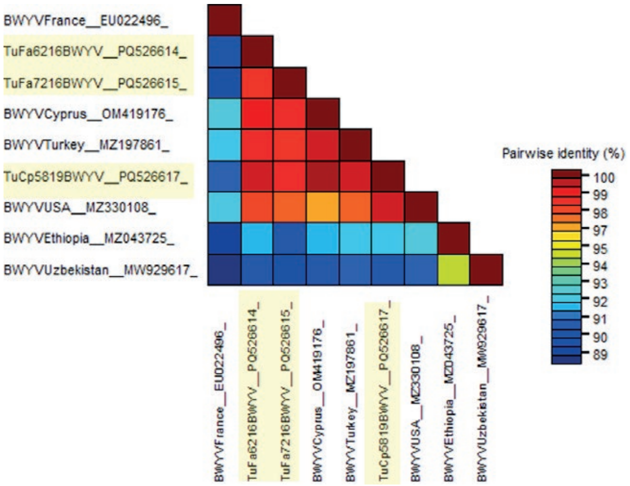

Figure 9. Pairwise nucleotide identity matrix of 9 beet western yellows virus (BWYV, species *Polerovirus BWYV*) sequences generated using SDTv1.3. Isolates characterized in this study are highlighted

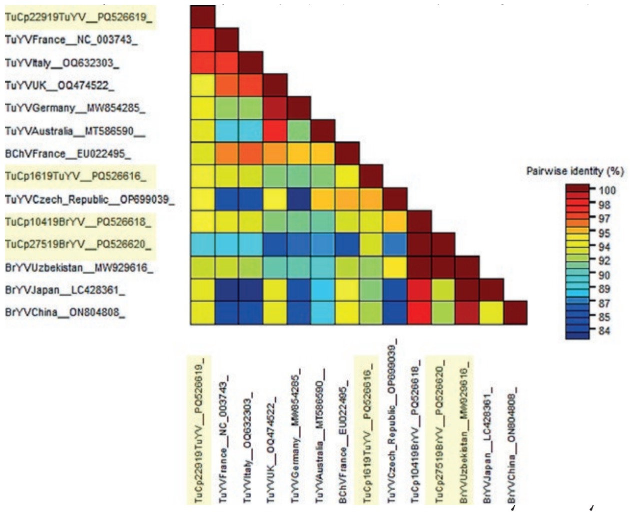

Figure 10. Pairwise nucleotide identity matrix of 14 TuYV/BrYV (species *Polerovirus TUYV*) sequences generated using SDTv1.3. Isolates characterized in this study are highlighted

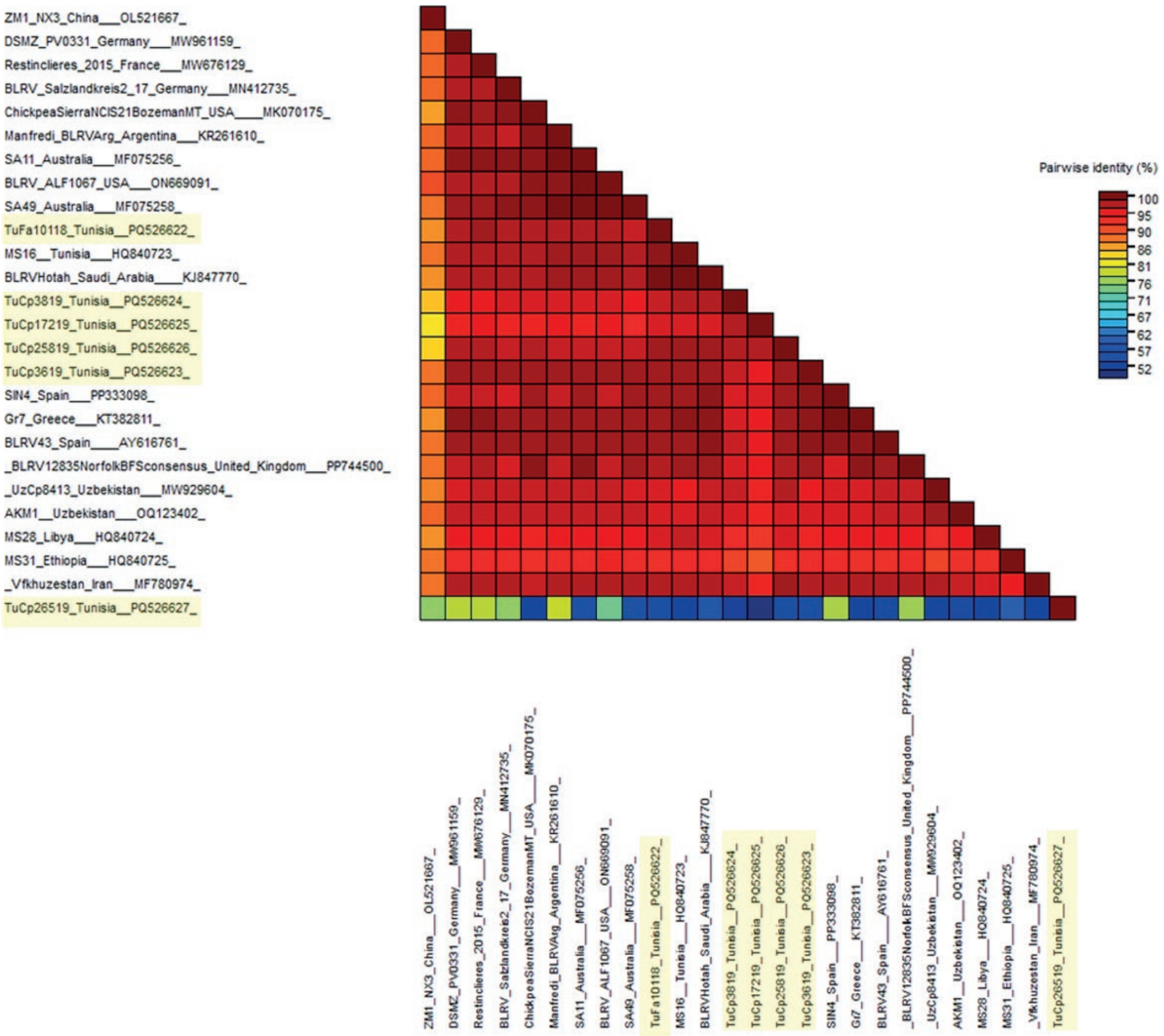

Figure 11. Pairwise nucleotide identity matrix of 26 bean leafroll virus (BLRV, species *Luteovirus phaseoli*) sequences generated using SDTv1.3. Isolates characterized in this study are highlighted

revealed values ranging from 50 to 98% among BLRV isolates. Most isolates showed high similarity (92 to 98%), consistent with the limited genetic diversity observed in the phylogenetic analysis. However, isolate TuCp265-19 displayed a markedly lower similarity (50 to 57%) compared with other isolates, indicating substantial sequence divergence within the analyzed region. The closest reference sequence to the main BLRV cluster was the Spanish isolate SIN4 (PP333098; from *Medicago sativa*), sharing 95 to 99% similarities with most isolates, but only 77% similarity with TuCp265-19 (Figure 11). These results suggest that TuCp265-19 is a highly divergent variant, although additional genomic regions would be required to clarify its precise taxonomic position.

DISCUSSION

This study has shown that CpCSV and BWYV (*Polerovirus*) were the most frequently detected viruses in faba bean and chickpea samples exhibiting yellowing and stunting symptoms. The survey was geographically extensive and was carried out during multiple growing seasons for these legumes. The field surveys also showed

Table 4. Tissue blot immunoassay (TBIA) reaction with Luteo- and polerovirus monoclonal antibodies (MAbs) of faba bean and chickpea samples collected from Tunisia between 2013-2019. All the plants tested were selected on the basis of reaction with broad-spectrum legume luteovirus MAb 5G4

Year	Crop	No. of samples reacted with 5G4 MAb	No. of plants with single infection	No. of samples reacted positively to MAbs[a]					Total of mixed infection
				CpCSV + BWYV	BWYV + BLRV	CpCSV + BLRV	CpCSV + BWYV + BLRV	CpCSV + BWYV + FBNYV	
2013/2014	Faba bean	100	77	21	1	0	1	0	23
2014/2015	Faba bean	186	174	12	0	0	0	0	12
	Chickpea	480	455	25	0	0	0	0	25
2015/2016	Chickpea	148	142	6	0	0	0	0	6
2016/2017	Faba bean	56	51	3	1	1	0	0	5
	Chickpea	104	72	13	5	11	3	0	32
2017/2018	Faba bean	221	140	78	1	0	1	1	81
2018/2019	Faba bean	31	29	0	2	0	0	0	2
	Chickpea	76	68	6	0	2	0	0	8
Total	Faba bean	594	471	114	5	1	2	1	123
	Chickpea	808	737	50	5	13	3	0	71

[a] CpCSV: chickpea chlorotic stunt virus (mixture of 5-2B8, 5-3D5 and 5-5B8 MAbs); BWYV: beet western yellows virus (A5977 MAb from Agdia); BLRV: bean leafroll virus (4B10 MAb); FBNYV: faba bean necrotic yellows virus (3-2E9 MAb).

the frequent presence of aphid colonies, particularly *Aphis craccivora* and *Acyrthosiphon pisum,* on faba bean plants. These aphid species are recognized as efficient vectors of CpCSV (Abraham *et al*., 2006; Asaad *et al*., 2009), and their abundance in the surveyed fields likely contributed to the high incidence of CpCSV detected in this study.

CpCSV has previously been reported on faba bean and chickpea in Tunisia, based on molecular characterization (Najar *et al*., 2011), whereas BWYV has been reported on faba bean using serological analyses (Najar *el al*., 2000b). Since first reported in Ethiopia (Abraham *et al*., 2006), CpCSV has been identified in several food legume crops, including chickpea, faba bean, lentil and pea, across many countries of the WANA region (Abraham *et al*., 2006; Kumari *et al*., 2008; Abraham *et al*., 2009; Asaad *et al*., 2009, Kumari *et al*., 2018). The virus has also been reported infecting several leguminous weeds and four non-legume wild plant species (Asaad *et al*., 2009). Although epidemics of CpCSV have been documented in Syria, Tunisia, and Ethiopia (Abraham *et al*., 2006; Kumari *et al*., 2007; Asaad *et al*., 2009; Kumari *et al*., 2009; Najar *et al*., 2011), quantitative data on yield losses due to this virus remain limited (Abraham and Vetten, 2022). This highlights the need for further studies to better assess the impacts of CpCSV on legume crop productivity.

FBNYV was detected only in faba bean during the 2016/17, 2017/18, and 2018/19 growing seasons, and at low incidence. This virus was previously reported in Tunisia by Najar *et al.* (2000a), and is considered one of the most damaging viruses of faba bean in the WANA region, where it has reached epidemic levels and caused substantial yield losses in several countries (Makkouk *et al*., 2003; Kumari and Makkouk, 2007). Such severe outbreaks of FBNYV were repeated in Egypt during the 1992/1993 and 1997/1998 growing seasons, resulting in a complete of the faba bean crop productivity (Makkouk*et al*.,1998).

Environmental conditions in the Cap Bon region of northeastern Tunisia are particularly favourable for establishment and spread of aphid-borne viruses. This coastal area is characterized by a sub-humid Mediterranean climate with mild temperatures and frequent winds (Hlaoui *et al*., 2019), factors known to strongly influence aphid survival, reproduction, and dispersal (Irwin and Thresh, 1988; Ng and Perry, 2004; Puthanveed *et al*., 2023; Roonjha *et al*., 2025). The polyphagous aphid *Myzus persicae*, which feeds on more than 400 plant species (Blackman and Eastop, 2000), is an economically important pest in Tunisia, and is an efficient vector of numerous plant viruses (Boukhris-Bouhachem *et al*., 2007; Guesmi *et al*., 2010; Boukhris-Bouhachem *et al*., 2011; Mdellel and Kamel, 2014; Charaabi *et al*., 2016).

The complex life cycle of *M. persicae*, involving host alternation between primary woody and herbaceous plants and prolonged parthenogenetic reproduction, enables aphid populations to persist year-round and move readily among crops (Simon *et al*., 2002; Mdellel and Ben Halima, 2012). In the Cap Bon region, the close proximity of peach orchards, potato fields, brassica crops, cucurbit and pepper crops, and legume fields further facilitates movement of winged aphids (Stevens *et al*., 2005; Hlaoui *et al*., 2019, 2022; Roonjha *et al*., 2025), and favours the spread of persistently transmitted viruses such as BWYV. In contrast, BLRV has a more restricted host range mainly limited to legumes, so vectoring of BLRV may be less favoured under these ecological conditions.

Weeds and invasive plants may also contribute to virus epidemiology by acting as reservoir hosts ("Green Bridge") for viruses and their aphid vectors (Wisler and Norris, 2005; Kazinczi *et al*., 2007). Because many aphid-transmitted viruses infect cultivated and wild plants, they can persist in alternative hosts between cropping seasons, increasing infection pressure on nearby crops (Aguiar *et al*., 2018; Hussein Aliyu *et al*., 2021; Yazdkhasti *et al*., 2021). Poleroviruses such as BWYV and CpCSV have been reported as infecting numerous cultivated plants and weeds (Abraham *et al*., 2006; Kumari *et al*., 2008; Venkataravanappa *et al*., 2023), highlighting the roles of reservoir hosts in virus persistence and spread.

Serological analysis using TBIA showed that 54.7% of faba bean and 46.5% of chickpea symptomatic samples tested negative for the antibodies used, suggesting the presence of additional viruses not detected in the present study. These results highlight the need for expanded diagnostic approaches using additional antisera and molecular assays for detecting an increased spectrum of virus pathogens.

The results of this study have shown clear predominance of single virus infections in chickpea and faba bean crops in Tunisia, as determined by TBIA. Chickpea crops had the greatest proportion of singly infected plants (77%), whereas faba bean crops had a relatively greater proportion of mixed virus infections (21%). This difference may be related to host plant traits influencing aphid feeding behaviour and virus transmission. Faba bean plants have large canopies and soft tissues, that attract aphid vectors and promote repeated feeding events, increasing the likelihood of acquiring and transmitting multiple viruses. In contrast, chickpea plants generally have small and rigid leaves, that may reduce aphid settling and feeding duration, favouring single virus infections. Similar patterns have been reported in other legume cropping systems where plant morphology and nutritional traits influence aphid colonization and dynamics of virus transmission (Ghorbani *et al*., 2010; Chiquito Almanza *et al*., 2017; Mulenga *et al*., 2022; Ademe *et al*., 2025). These results underscore the importance of considering vector-mediated processes alongside host susceptibility when developing strategies to manage virus spread in legume cropping systems (Lei *et al*., 2025).

Mixed infections were predominantly associated with *Polerovirus* (*Solemoviridae*) viruses, particularly CpCSV and BWYV, accounting for 95% of mixed infections in faba bean and 69% in chickpea. Virus-virus interactions in mixed infections may influence virus accumulation, host range, symptom severity, and vector transmission efficiency (Moreno and López-Moya, 2020; Sánchez-Tovar *et al*., 2025). The frequent association of CpCSV and BWYV has been previously reported in legume crops (Abraham *et al*., 2009; Moukahel *et al*., 2021; Maina and Jones, 2023), although the nature of the interaction between these two viruses remains unclear.

While serological assays (TBIA) frequently suggested co-infections by CpCSV and BWYV, molecular analyses detected only single *Polerovirus* species per sample. This discrepancy probably reflects the limitations of serological diagnostics for closely related viruses, as CpCSV and BWYV share conserved coat protein epitopes that may lead to antibody cross-reactivity (Martin and D'Arcy, 1990; Oshima *et al*., 1990; Fortass *et al*., 1997; Kidanemariam and Abraham, 2023). Consequently, molecular analyses occasionally reveal mixed infections not detected by TBIA, highlighting the complementary nature of serological and molecular diagnostic approaches. While TBIA remains a rapid and cost-effective tool for large-scale virus surveillance (Makkouk and Kumari, 1996), molecular techniques such as MP-PCR provide increased sensitivity and specificity for detecting closely related viruses and mixed infections (Abraham *et al.,* 2008; Deb and Anderson, 2008; Abraham and Vetten, 2022). The present study results also showed that CpCSV was the most prevalent virus involved in mixed infections in chickpea and faba bean crops. This predominance may be explained by the broader host range of CpCSV than of BWYV host range, and efficient persistent transmission by *A. craccivora*, which was frequently observed during field surveys (Abraham *et al*., 2006; Asaad *et al*., 2009; Abraham and Vetten, 2022). In the Bizerte region where winters are mild, CpCSV and BWYV co-infections were particularly common, representing 59% of mixed infections in faba bean crops and 52% in chickpea. A similar pattern was observed in Cap Bon, where these co-infections accounted for 37% of mixed infections in faba bean and 26% of mixed infections in chickpea. These results highlight the ecological adaptability and transmission efficiency of CpCSV in cool-season legume cropping systems.

The present study observations are consistent with previous studies demonstrating that climate change, agricultural history, and the invasion or persistence of alternative host plants strongly influence vector dynamics and virus emergence at regional scales (Robert and Lemire, 1999; Jones and Barbetti, 2012; Jones, 2016; Trebicki, 2020). They also emphasize the importance of weed and reservoir host management as key components of integrated strategies aimed at reducing virus inoculum and limiting epidemic development (Wisler and Norris, 2005).

The use of the partial *CP* gene in the present study was a strategic choice to combine practical feasibility with phylogenetic relevance. Virus *CP* remains a reliable and accessible molecular marker for initial identification and diagnosis, because of its structural role and its conservation among related viruses. Despite its conserved nature, the partial *CP* gene has also been shown to contain sufficient variability to discriminate between isolates, and to reveal phylogeographic structuring in several *Polerovirus* species, including CpCSV.

Previous studies have demonstrated clear phylogeographic structuring in CpCSV populations. Abraham *et al.* (2009) identified two major strains associated with geographic origins, Strain I (from Ethiopia and Sudan) and Strain II (from Morocco, Syria, and Egypt), separated by approx. 8 to 10% nucleotide divergence in the CP. Similarly, Najar *et al.* (2011) reported that Tunisian isolates of CpCSV clustered into these two groups. Consistent with these findings, the present study phylogenetic analysis showed that ten newly characterized Tunisian isolates clustered with Ethiopian and Sudanese isolates (Strain I), whereas three isolates grouped with isolates from Morocco, Egypt, Syria, and Iran (Strain II). These results confirm the coexistence of at least two CpCSV lineages in Tunisia, and indicate that geographic origin rather than host species drives CpCSV genetic differentiation. Presence of distinct strains may also have epidemiological and host resistance breeding implications, as different isolates may vary in symptom severity and resistance-breaking potential (Abraham *et al.*, 2009).

BWYV is widely distributed and infects chickpea and numerous other plant species, with reported yield losses due to BWYV infections ranging from 8 to 90% (Abraham, 2025). Despite this broad host range, previous studies have reported limited sequence variability among BWYV isolates (Yoshiba and Tamada, 2019). In agreement with these observations, SDT pairwise similarity analysis revealed high nucleotide conservation among the Tunisian BWYV isolates (98 to 99%), indicating circulation of a genetically homogeneous lineage. However, these isolates were clearly differentiated from sugar beet derived BWYV variants (89 to 90%), and from chickpea isolates from Ethiopia and Uzbekistan (89 to 92%), suggesting existence of host- or geography-associated lineages.

Sequencing of amplicons obtained with BWYV-specific primers also showed presence of TuYV/BrYV in addition to BWYV. This is consistent with recent studies showing that many poleroviruses previously identified as BWYV by serology correspond to TuYV or related species (Abraham, 2025). The discrepancy between PCR detection and sequencing probably results from primer annealing in conserved genomic regions shared among poleroviruses, which may result in cross-detection of closely related species (Hauser *et al.*, 2000; 2002; D'Arcy and Domier, 2005). These results highlight the limitations of PCR targeting conserved regions, and emphasize the need for additional genomic markers for accurate species identification.

TuYV, formerly known as BWYV, belongs to *Polerovirus* within *Solemoviridae*. BrYV shares approx. 80% nucleotide similarity with TuYV and has been subdivided into several strains associated with different brassica hosts. Phylogenetic analyses indicate that TuYV and BrYV share a common evolutionary origin, and recombination has been recognized as an important driver of their diversification (Filardo *et al.*, 2021; Peng *et al.*, 2023). In some cases, whole-genome analyses have indicated that BrYV may represent a divergent lineage within the TuYV species complex, rather than being a distinct species (Filardo *et al.*, 2021). These findings highlight the taxonomic complexity within the TuYV/BrYV group, and the importance of highly specific molecular diagnostic tools. More recently, high-throughput sequencing studies detected BWYV-related sequences in Citrus, including an isolate associated with citrus yellow vein-associated virus and "fatal yellows" disease (Keremane *et al.*, 2024). Consistent with these observations, Pierre *et al.* (2026) developed and validated a real-time RT-PCR assay for specific detection of BWYV and related poleroviruses, which demonstrated improved sensitivity and specificity compared with generic ELISA and RT-PCR assays.

Phylogenetic and SDT analysis further showed that Tunisian TuYV isolates form a genetically homogeneous cluster closely related to international TuYV populations, with clustering largely associated with geographic origin. In contrast, BLRV isolates showed both high conservation and notable divergence. Most Tunisian isolates clustered with sequences from Europe, the United States, Australia, and Spain, sharing 95 to 99% nucleotide similarity, suggesting a cosmopolitan lineage. However, one isolate (TuCp265-19) exhibited marked divergence (50 to 57% similarity) compared with other Tunisian isolates, indicating the possible presence of a highly divergent variant or distinct lineage. Similar patterns of divergence within BLRV populations have been reported previously; for example, Hajiyusef *et al.* (2017) showed that an

Iranian BLRV isolate formed two subgroups with other international isolates, while maintaining high similarity within each subgroup.

The present study has highlighted the diversity of viruses infecting chickpea and faba bean in Tunisia, with poleroviruses (CpCSV and BWYV) as the predominant pathogens. Phylogenetic analyses revealed two CpCSV strains, and high conservation among BWYV and TuYV isolates. The detection of TuYV/BrYV among BWYV-positive samples underscores the importance of molecular diagnostics for virus pathogens. These results provide essential baseline data for improving virus surveillance and management in Tunisian legume crops.

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