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Phylogenetic and Population Analyses on P1, HC-Pro, and P3 of Potato Virus Y Isolates Infecting Potato in the Central Region of Türkiye

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Abstract
Potato virus Y (PVY) has been reported as one of the pathogens hampering production of potato (*Solanum tuberosum* L.) in Türkiye, but the genetic diversity of Turkish isolates is still largely unknown. Four hundred and forty-seven samples from Afyon, Bolu, Nevşehir, and Tokat Provinces in the central region of Türkiye, where most of the country's cultivation is performed, were molecularly tested for PVY in this study. Sequences of partial P1, complete HC-Pro, and complete P3 gene regions (concatenated length of 3258 nt) were obtained for 25 of 197 (44.07%) positive tested isolates, randomly selected to represent each province. Twenty-two Turkish isolates were positioned in N group while three other Turkish isolates, including two recombinant isolates (S9-10 and Sa12-8), were included in NTN group in the constructed phylogenetic tree. Members of the recombinant NTN group were shown to have high diversity among them according to population analysis using DnaSP software. However, negative values given by neutrality tests demonstrated that the NTN group is undergoing balancing selection. The consistently large fixation index values ($F_{ST} > 0.33$) calculated for comparisons between different groups confirmed their distinction from each other. This research contributed to our understanding of the genome variations among Turkish isolates and the evolution of PVY.

Keywords Gene flow · Genetic differentiation · Plant viral disease · Population structure · RT-PCR

Introduction

Potato (*Solanum tuberosum* L.) is one of the most economically valuable solanaceous crops in Türkiye, with a total production of 5.2 million tons in 150,000 ha during 2020. No less than 60% of the cultivation is in the provinces in the central region of Türkiye (TÜİK 2020). Like in other countries, viral diseases, for example,

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the one caused by potato virus Y (PVY, *Potyvirus yituberosi*, genus *Potyvirus*), are causing significant potato yield losses in Türkiye.

As the reference species of its genus, PVY genome is single-stranded RNA, and the particle is elongated and flexible in shape and 800 × 20 nm in size (García et al. 2014). The about 10,000 nucleotide (nt) long viral genome is translated into a large 'polyprotein precursor', which is then split by P1, HC-Pro, and NIa-Pro self-encoded proteases to generate at least 10 short viral proteins (García et al. 2014).

The virus is categorized into O, C, and Z strains based on the interactions between isolates with three potato genes: *Ny*, *Nc*, and *Nz*, respectively, which each lead to a hypersensitive resistance (HR) response. On the contrary, E and N strains do not produce HR due to no compatibility with any of the three genes, but the E isolates induce mosaic and vein clearing while the N isolates cause vein necrosis on infected tobacco (*Nicotiana tabacum* L.) (Green et al. 2017).

Different PVY strains: O and N (Bostan and Haliloglu 2004) and NTN (Bostan and Dumlupinar 2006) had been found in tubers imported from European countries to Türkiye as planting material, but the obtained isolates were not sequenced. Güner and Yorgancı (2006) applied the DAS-ELISA test to survey a high rate of PVY infection in potato plant and tuber samples collected from Afyon, Bolu, Niğde, and Nevşehir Provinces. Using ELISA test, PVY, along with potato virus X (PVX, *Potexvirus*), potato virus S (PVS, *Carlavirus*), potato virus A (PVA, *Potyvirus*), and potato leafroll virus (PLRV, *Polerovirus*), was detected in potato grown in Afyon (Yardımcı et al. 2015).

Only recently, partial genome of PVY isolates from tobacco (Randa-Zelyüt et al. 2022) and potato (Engür and Topkaya 2023, 2024; Korkmaz et al. 2025) were sequenced. Therefore, very little is known about the distribution and genetic diversity of PVY, which is needed to clarify the demography of the virus in the country (Akbaş et al. 2023; Çelik et al. 2023). Molecular and population analyses conducted on multiple genome regions in this study on the PVY potato isolates collected in four Turkish Provinces in the central region of the country could provide a clear map of the spread, strains, and genome variation of Turkish isolates that may direct effective and sustainable management strategies.

Materials and Methods

Samples Collection

Leaf samples of potato plants showing viral symptoms were sampled in cultivation centres of Afyon, Bolu, Nevşehir, and Tokat Provinces of Türkiye. Samples were put inside separate plastic bags, then kept at 4 °C in a refrigerator for 1 day, and at − 20 °C in a deep freezer for long-term storage.

RNA Extraction and RT-PCR

Total RNA was extracted from leaf samples using a method suggested by Astruc et al. (1996). Complementary DNA (cDNA) was synthesized from the template

RNA using 'VitaScript™ FirstStrand cDNA Synthesis Kit' (Procomcure Biotech GmbH, Bergheim Austria) in a 20-µl mixture of 4 µl of × 5 VS reaction buffer, 1 µl of VitaScript™ Enzyme Mix, 3 µl of RNA template, and 12 µl of ddH_2O which was then applied in a thermal cycle consists of 42 °C (1 h) and 80 °C (10 min).

The obtained cDNAs were molecularly tested for PVY using primer pair targeting coat protein region (Table 1). Partial P1, complete HC-Pro, and complete P3 genes of selected isolates representing each province were sequenced using primer pair targeting respective genome region (Table 1). PCR mix consisted of 2.5 µl of cDNA, 5 µl of × 5 Green GoTaq® Flexi Buffer (Promega GmbH, WI, USA), 0.25 µl of Taq polymerase enzyme (Promega GmbH), 0.2 µl of dNTP, 0.5 µl each of forward and reverse primer (10 pmol), 1.5 µl of $MgCl_2$ (25 mM), 1 µl of dimethyl sulfoxide (DMSO), and then completed with ddH_2O to a volume of 25 µl. The PCR cycle for each reaction was as mentioned in the primer reference.

PCR results were loaded into a 1.5% agarose gel stained with 10 mg/ml of ethidium bromide, then electrophorized at 100 V for 1 h. The appearance of a specific target band was observed under a UV illuminator. Successfully amplified PCR results were sent for two directions of Sanger sequencing. The obtained sequences were overlapped to determine consensus sequence of each isolate.

Recombination Analysis, Phylogenetic Trees, and Percentage Identity

In 30 January 2025, sequences of isolates of different PVY strains: eight strain N, ten NTN, five Wilga, two C, and six O listed in NCBI GenBank and formally published in scientific papers (Supplementary Information) were retrieved, aligned, and then trimmed according to the length of the sequences of 25 isolates acquired in this report using ClustalW algorithm in MEGA11 program (Tamura et al. 2021).

Recombination among the aligned sequences was detected using Recombination Detection Program 5 (RDP v5.30) with default parameters (Martin et al. 2021). Only signal detected by a minimum of five of the seven algorithms: RDP, Chimaera, MaxChi, Bootscan, Siscan, GENECONV, and 3Seq with a Bonferroni-corrected P value of < 0.05 was considered to be significantly reliable.

Four phylogenetic trees based on the respective partial P1, complete HC-Pro, complete P3, and concatenation of the three regions were constructed using the

Table 1 Primer pairs that were used to sequence different PVY genome regions in this study

Region	Band size (bp)	TM (°C)	Primer sequence 5′–3′	Reference
CP	480	52	F-ACGTCCAAAATGAGAATGCC R-TGGTGTTCGTGATGTGACCT	Nie and Singh (2002)
HC-Pro	1700	58	F-CGMARGGGYGATAGTGGAGT R-GTTCTGCYGCTGACACTCG	Glais et al. (2002)
P3	1100	58	F-CTGGYATACTKATGGCTATG R-CARTCRCTCCTTTCAGCATC	Glais et al. (2002)
P1	856	58	F-AAGCTTCCATACTCACCCGC R-CATTTGTGCCCAATTGCC	Nie and Singh (2002)

maximum-likelihood (ML) algorithm implemented in MEGA11 with Kimura 2-parameter model (Kimura 1980) as nt substitution. The tree topology was statistically supported by 1000 bootstrap replicates. Nucleotide (nt) and amino acid (aa) percentage identities among sequences of the aligned isolates were calculated by the Sequence Demarcation Tool software (SDT v1.2) (Muhire et al. 2014).

Population Structure and Neutral Selection Analysis

The genetic diversity of the nt sequences was quantitatively estimated using DnaSP v.6.12.03 software (Rozas et al. 2017) based on population genetic related parameters: the number of haplotypes (*h*), haplotype diversity (*Hd*), the number of variable sites (*S*), the total number of mutations (η), the average number of nt differences between compared sequences (*k*), and average pairwise nt diversity (per site) (π). The total number of non-synonymous sites (*dN*) per total number of synonymous sites (*dS*) was determined to estimate ω value as an indicator of transcriptional selection. The observed genome region was assessed to be experiencing positive (diversifying), neutral, and negative (purifying) selection when the $\omega > 1, = 1,$ and < 1, respectively (Rozas et al. 2017). Neutral selection tests Tajima's *D* (Tajima 1989) and Fu and Li's *D** and *F** (Fu and Li 1993) tests implemented in DnaSP v.6.12.03 were performed at a window length of 100 sites and step size of 25 sites to measure genetic divergence on the observed genome region.

Gene Flow

Using DnaSP v.6.12.03, the genetic differentiation and gene flow among viral populations reflected by K_S*, K_{ST}*, *Z**, S_{nn}, and F_{ST} (fixation index) values (Hudson 2000) were estimated. The F_{ST} value could span between 0 (totally identical populations) to 1 (strictly distinct populations) (Hudson et al. 1992; Hudson 2000). Value > 0.33 indicates a rare gene flow and expands genetic differences between the tested populations (Hudson et al. 1992; Morca et al. 2024).

Results

Samples Collection and RT-PCR

Following field surveys, leaves from 147 potato plants were collected in Afyon, 100 in Bolu, 100 in Nevşehir, and 100 in Tokat for a total of 447 samples. RT-PCR test using CP primer showed that 197 samples (44.07%) were infected by PVY. A total of 25 isolates representing the four provinces were randomly selected for sequencing of 3258 nt genome region covering partial P1, complete HC-Pro, and complete P3 encoding genes (Table 2). Obtained sequences were submitted to get GenBank accession nos. PQ821651–675. Positive samples mainly exhibited foliar symptoms of mosaic, chlorosis, mottle, and malformation; in addition, stunting was also observed.

Table 2 Disease incidence in each province and 25 PVY isolates selected for partial genome sequencing

Province	Number of samples	No. samples infected by PVY	Samples with partial P1, complete HC-Pro, and complete P3 genes sequenced
Afyon	147	57 (38%)	B3, B14, B17, B42, B51, B54, B55, S9-10, Sa11-2, Sa12-8, Sa12-12, Sa16-4
Bolu	100	78 (78%)	Bo1, Bo12, Bo18, Bo33, Bo37, Bo46, Bo62, Bo83
Nevşehir	100	27 (27%)	NP57
Tokat	100	35 (35%)	TN7, TN14, TN19, TN20
Total	447	197 (44.1%)	B3, B14, B17, B42, B51, B54, B55, S9-10, Sa11-2, Sa12-8, Sa12-12, Sa16-4, Bo1, Bo12, Bo18, Bo33, Bo37, Bo46, Bo62, Bo83, NP57, TN7, TN14, TN19, TN20

Recombination Analysis

Significant recombination signals were found in the partial genomes of 8 out of 63 PVY isolates analysed in this study, including two new Turkish isolates, following RDP5 scan (Table 3). Five Turkish isolates obtained in this study, B17, B42, B51, Bo37, and Sa11-2, were indicated to be donors to genomes of other isolates. Recombinant isolates were not included in the population analysis of this study.

Phylogenetic Trees

Phylogroups based on five PVY strains were formed in the tree constructed using concatenated sequences of partial P1, complete HC-Pro, and complete P3 regions (Fig. 1D). However, many isolates well identified belong to certain strains, mainly N and NTN, were grouped interchangeably. Twenty new Turkish isolates formed a separate subgroup within N group while two other Turkish isolates were also positioned in other subgroups of N. The remaining three isolates obtained in this study, including the two recombinant isolates (S9-10 and Sa12-8), were clustered in NTN group (Fig. 1). S9-10 and B-55 were consistently positioned within NTN group of partial P1, complete HC-Pro, and complete P3 trees, respectively (Fig. 1A–C). Meanwhile, Sa12-8 was always clustered in N group of the respective partial P1, complete HC-Pro, and complete P3 trees. This indicated that sequences of N and NTN were highly homogenous, and the recombination on Sa12-8 genome happened at the region that separated the two groups (Fig. 1).

Percentage Identity

Turkish N isolates shared > 98% nt and > 99% aa identities with other members of N group. Meanwhile, 88–97% and 92–97% identities with other NTN isolates at

Table 3 Putative recombination events detected in the P1, HC-Pro, and P3 genes of PVY analysed in this study using RDP v5.30

Recombinant isolate	Major/minor parents	Beginning/ending breakpoint[1]	RDP implemented method (*p* value)
W13 (MT350288) strain O	MAF-VOY (JQ924286) strain N group N/ New Zealand (AM268435) strain N	254/2078	R (6.459×10^{-23}) G (3.626×10^{-18}) B (4.622×10^{-25}) M (6.192×10^{-25}) C (1.760×10^{-16}) S (2.052×10^{-28}) 3S (5.741×10^{-59})
W13 (MT350288) strain O	Gr99 (AJ890343) strain NTN/ PVY-3411 (KX353070) strain N-Wi group N	1/253 & 2241/3259	R (3.813×10^{-2}) B (3.864×10^{-2}) M (1.758×10^{-3}) S (5.644×10^{-4}) 3S (3.061×10^{-5})
New Zealand (AM268435) strain N	PO7 (U09509) strain O group O/ B42 (PQ821662) strain N group N	254/2146	R (2.702×10^{-60}) G (1.297×10^{-48}) B (1.321×10^{-59}) M (3.540×10^{-35}) C (1.929×10^{-2}) S (6.285×10^{-48}) 3S (5.687×10^{-21})
AF522296 strain N	605 (X97895) strain N group NTN/ B51 (PQ821663) strain N group N	254/2074	R (6.459×10^{-23}) G (3.626×10^{-18}) B (4.622×10^{-25}) M (6.192×10^{-25}) C (1.760×10^{-16}) S (2.052×10^{-28}) 3S (5.741×10^{-59})
S9-10 (PQ821666) strain N group NTN	605 (X97895) strain N group NTN/ B51 (PQ821663) strain N group N	2170/3251	R (2.133×10^{-16}) G (3.949×10^{-11}) B (2.021×10^{-4}) M (1.521×10^{-16}) C (2.469×10^{-4}) S (2.524×10^{-31}) 3S (1.483×10^{-44})
Linda (AJ890345) strain NTN	605 (X97895) strain N group NTN/ Bo37 (PQ821655) strain N group N	2170/3251	R (1.250×10^{-13}) G (6.682×10^{-9}) B (4.724×10^{-6}) M (1.056×10^{-15}) C (2.613×10^{-7}) S (4.773×10^{-26}) 3S (1.730×10^{-41})

Table 3 (continued)

Recombinant isolate	Major/minor parents	Beginning/ending breakpoint[1]	RDP implemented method (*p* value)
261–4 (AM113988) strain Wilga	YO-ANT25 (JQ924285) strain O group O/ Sa11-2 (PQ821674) strain N group N	400/2074	G (2.391×10^{-2}) B (2.108×10^{-2}) M (2.177×10^{-5}) C (2.085×10^{-5}) S (1.758×10^{-50}) 3S (1.584×10^{-30})
261–4 (AM113988) strain Wilga	B14 (PQ821660) strain N group N/ Guiding-3 (HM590405) strain N group NTN	254/399 and 2260/2747	R (2.283×10^{-15}) G (2.016×10^{-12}) M (8.009×10^{-11}) S (7.887×10^{-13}) 3S (5.002×10^{-25})
Sa12-18 (PQ821669) strain N group NTN	Tu 660 (AY166866) strain NTN group NTN/ B17 (PQ821661) strain N group N	255/2142	R (1.643×10^{-9}) G (2.339×10^{-8}) B (4.651×10^{-5}) M (6.298×10^{-14}) C (5.482×10^{-11}) S (1.036×10^{-19}) 3S (5.159×10^{-23})
Gr99 (AJ890343) strain NTN	MV175 (HE608964) strain NTN group N/ YO-ANT25 (JQ924285) strain O group O	1928/2074	R (7.962×10^{-18}) G (1.956×10^{-15}) M (1.690×10^{-4}) C (6.257×10^{-4}) S (4.337×10^{-5}) 3S (1.062×10^{-10})

R RDP, *G* GENECOV, *B* BootScan, *M* MaxChi, *C* Chimaera, *S* Siscan, *3S* 3Seq

[1]Position in alignment

nt and aa levels, respectively, were estimated by SDT analysis for the three Turkish NTN isolates (Fig. 2).

Population Structure and Neutral Selection Analysis

Analysis on P1, HC-Pro, and P3 genes suggested low genetic diversity of N group despite having the largest members, likely due to many highly similar Turkish isolates in here. Members of NTN group were shown to be highly divergent by acquiring much higher *S* and η values than other groups. However, the highest *k* and π parameters were assigned to C group (Table 4). All of the tested groups were under strong negative selection pressure with $\omega < 0.2$ except Wilga which received high dN/dS ratio of almost 1 (Table 4).

The three neutrality tests consistently estimated positive values just for NTN group. However, only N group had sufficient data to draw statistically significant conclusions (Table 5).

Gene Flow

When comparing sequences of four genome regions of PVY isolates using DnaSP, the genetic differentiation analysis always drew maximum S_{nn} (1.0000) and high F_{ST} values (> 0.5) for the compared five phylogroups (Table 6). These results provided strong evidence that the separation of isolates analysed here into five phylogroups is justified. However, K_S, K_{ST}, and Z values for comparison of Wilga and C groups were low and not statistically significantly supported. Furthermore, K_S, K_{ST}, and Z values of Wilga vs NTN, Wilga vs O, and C vs O were also not significantly supported (Table 6). These were likely due to insufficient data on Wilga, C, and O isolates.

Discussion

Using specific antibodies, the presence of PLRV and PVS, along with PVY^O and PVY^N, was detected in potato tubers used as planting material in Türkiye. PVY^C was not detected in the same study (Bostan and Haliloglu 2004). Another survey observed $PVY^{N/NTN}$ in potato seed tubers (Bostan and Dumlupinar 2006). Involvement of different aphid species in the epidemiology of PVY, PVS, and PLRV in Erzurum province, the highest region of Türkiye, has also been studied (Bostan et al. 2006). In the latest distribution of PVA, PVX, PVY, PVS, and PLRV in different Turkish provinces was determined using DAS-ELISA (Yardımcı et al. 2015). Furthermore, population structure of Turkish PVS has been discussed in detail recently (Topkaya et al. 2023). Demographic analyses have been conducted to elucidate the population structure of numerous viruses in Türkiye, contributing to the understanding of interactions among viral populations (Coşkan et al. 2022; Çelik et al. 2022). However, due to lack of molecular testing, genetic diversity of PVY in Türkiye remains largely unknown.

Disease caused by PVY infection continues to pose a worldwide problem in potato cultivation (Dupuis et al. 2024; Santosa et al. 2025; Satoh-Cruz et al. 2025). Likewise, RT-PCR of this study clarified that PVY is still widely distributed among potato cultivated in Türkiye, with incidence of > 25% in all four surveyed provinces, and even reached 78% among the tested samples from Bolu. This could be an indication that the virus is causing considerable damage to potato production in the country. Although attempts had been made to improve resistance (Ozkaynak et al. 2018; Ahmed and Elci 2019), the clear and, most of the time, severe symptoms suggested that the currently planted cultivars possessed only little tolerance to PVY infection. Furthermore, symptomatic samples that tested negative in this study might be infected with other viruses. These should alert relevant stakeholders to prepare effective management strategies.

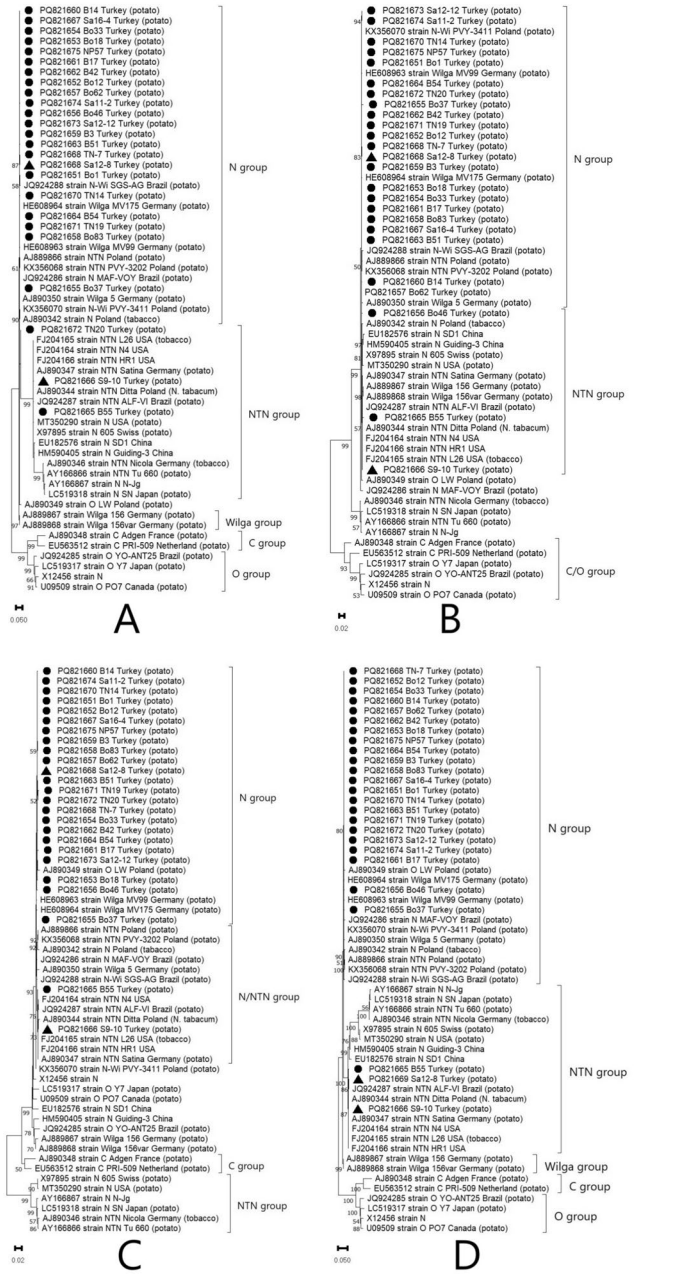

Fig. 1 Maximum-likelihood phylogenetic analysis on the sequences of (**A**) partial P1 (798 nt), **B** complete HC-Pro (1365 nt), **C** complete P3 region (1095 nt), and (**D**) the three regions concatenated (3258 nt) of PVY genome. The trees were generated using the Kimura 2-parameter model in MEGA11 software, with uniform rates among sites and 1000 bootstrap replicates (only values > 50% were shown). Twenty-three non-recombinant Turkish isolates obtained in this study were highlighted with black dots while two recombinant Turkish isolates were highlighted with black triangles

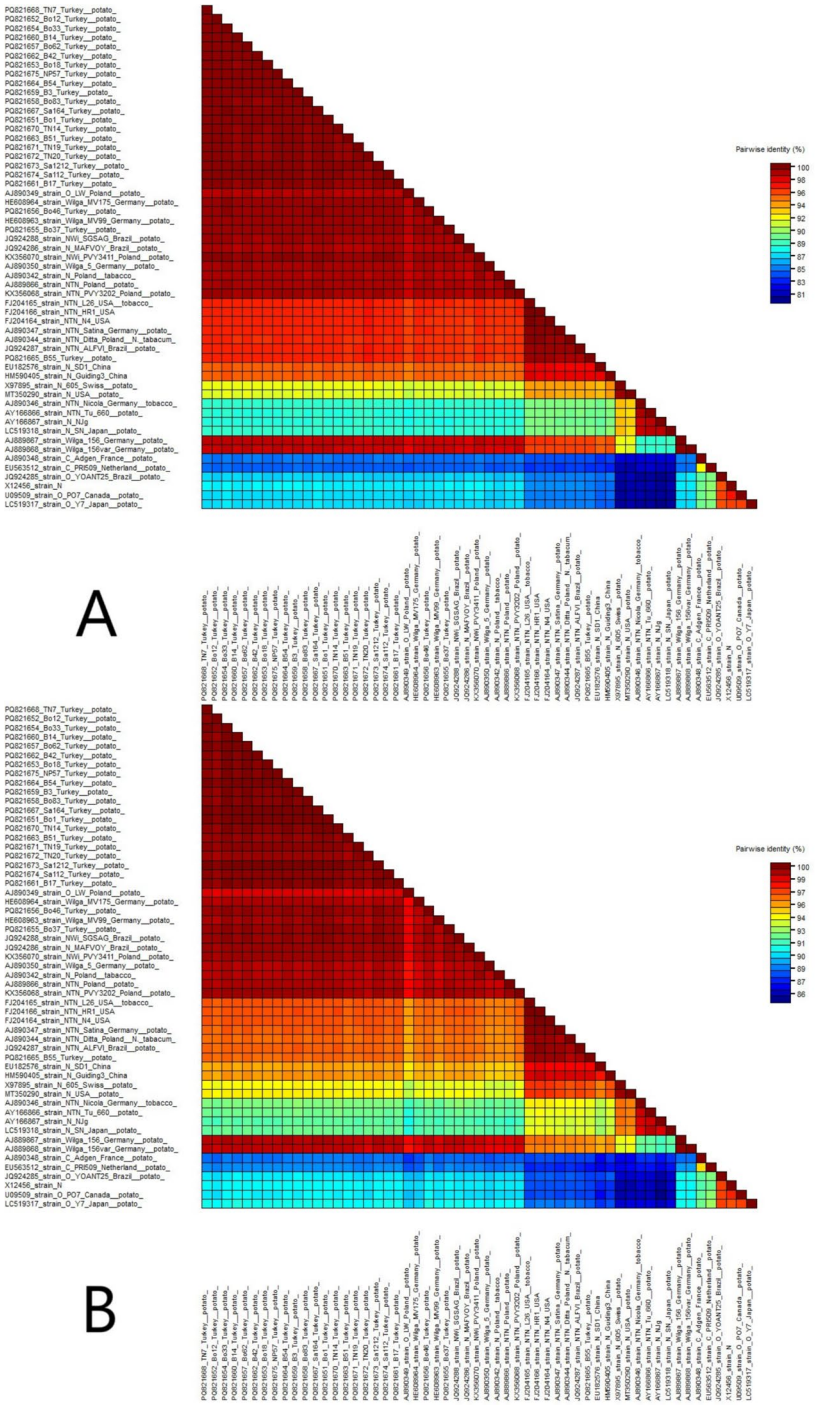

Fig. 2 Percentage identities at the partial P1, complete HC-Pro, and complete P3 regions (3258 nt) among 23 non-recombinant Turkish PVY isolates and 22 isolates in the NCBI GenBank. **A** Nucleotide. **B** Amino acid

Table 4 Summary of population genetic and polymorphism analyses for P1, HC-Pro, and P3 genes of PVY isolates tested in this study

Group	*N*	*h*	*Hd*	*S*	η	*k*	π	*dS*	*dN*	ω
All	55	55	1.000	1134	1291	190.180	0.058	0.179	0.022	0.123
N	32	32	1.000	274	280	21.677	0.007	0.021	0.003	0.143
NTN	15	15	1.000	488	506	177.781	0.055	0.186	0.016	0.086
Wilga	2	2	1.000	10	10	10.000	0.003	0.011	0.001	0.909
C	2	2	1.000	249	249	249.000	0.076	0.231	0.031	0.134
O	4	4	1.000	232	235	120.001	0.037	0.119	0.013	0.109

N number of isolates, *h* number of haplotypes, *Hd* haplotype diversity, *S* number of variable sites, *η* total number of mutations, *k* average number of nucleotide differences between sequences, *π* nucleotide diversity (per site), *dN* non-synonymous nucleotide diversity, *dS* synonymous nucleotide diversity, *ω* dN/dS

Table 5 Summary of neutrality tests estimated for P1, HC-Pro, and P3 genes of PVY isolates tested in this study

Species	Fu and Li's *D**	Fu and Li's *F**	Tajima's *D*
All	− 0.75773 ns	− 1.10629 ns	− 1.17968 ns
N	− 4.43820**	− 4.53824**	− 2.64753***
NTN	0.32103 ns	0.47182 ns	0.63347 ns
Wilga	nd	nd	nd
C	nd	nd	nd
O	− 0.62886 ns	− 0.68527 ns	− 0.66979 ns

ns not significant

nd not determined, at least four isolates needed for analysis

***P* value < 0.02; ****P* value < 0.001

Analyses on the region that covers around a third of PVY genome showed that most Turkish isolates belong to N group and share high percentage identities at both nt and aa levels with other isolates listed in GenBank. Three of 12 Afyon isolates (B55, Sa12-8, and S9-10) are highly divergent and members of NTN group, showing larger genetic diversity here than in other provinces. PVY^{NTN} was observed to emerge from recombination of PVY^{O} and PVY^{N} (Glais et al. 2002; Nie and Singh 2002). In agreement, two recombinant Turkish NTN isolates, Sa12-8 and S9-10, were parented by other NTN: 605 and Tu 660, respectively, and Turkish N isolates. Therefore, it can be suggested that this recombination occurs locally.

Being a recombinant group, NTN isolates showed higher genetic diversity than other groups. However, although consisting only of two and four isolates, respectively, members of C and O groups also demonstrated considerable divergence among them according to the average number of nucleotide differences between sequences (*k*) and nucleotide diversity (per site) (π) parameters. The soaring ω value of Wilga group (0.909) indicates that the genome regions of the two observed isolates are undergoing very strong negative (purifying) constraint which is in line with their very low values of *S*, η, *k*, and π parameters.

Table 6 Genetic differentiation estimates for PVY isolates tested in this study, based on pairwise comparisons of P1, HC-Pro, and P3 gene sequences

Comparison	$^{\alpha}K_S$*	$^{\alpha}K_{ST}$*	*P* value	$^{\alpha}Z$*	*P* value	S_{nn}	*P* value	$^{\beta}F_{ST}$
N ($n=32$) vs NTN ($n=15$)	3.5332	0.1468	0.0000***	5.5789	0.0000***	1.0000	0.0000***	0.5149
N ($n=32$) vs Wilga ($n=2$)	3.0222	0.0314	0.0000***	5.2234	0.0000***	1.0000	0.0000***	0.6726
N ($n=32$) vs C ($n=2$)	3.0222	0.1089	0.0000***	5.2171	0.0000***	1.0000	0.0000***	0.7308
N ($n=32$) vs O ($n=4$)	3.1267	0.1435	0.0000***	5.2794	0.0000***	1.0000	0.0000***	0.8339
NTN ($n=15$) vs Wilga ($n=2$)	4.7123	0.0196	0.1840 ns	3.8615	0.2070 ns	1.0000	0.0160**	0.5503
NTN ($n=15$) vs C ($n=2$)	4.7123	0.0714	0.0040**	3.6889	0.0050**	1.0000	0.0140**	0.6188
NTN ($n=15$) vs O ($n=4$)	4.7173	0.1046	0.0000***	3.7474	0.0000***	1.0000	0.0000***	0.7284
Wilga ($n=2$) vs C ($n=2$)	0.0000	1.0000	1.0000 ns	0.0000	1.0000 ns	1.0000	0.3280 ns	0.7394
Wilga ($n=2$) vs O ($n=4$)	4.7886	0.0968	0.0600 ns	1.4209	0.0600 ns	1.0000	0.0600 ns	0.8464
C ($n=2$) vs O ($n=4$)	4.7886	0.1097	0.0670 ns	1.0965	0.0670 ns	1.0000	0.0670 ns	0.4415

*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$

$^{\alpha}K_S$*, K_{ST}*, Z*, and S_{nn} are test statistics of genetic differentiation

$^{\beta}F_{ST}$, coefficient of gene differentiation, which measures inter-population diversity

Results of the three neutrality tests suggested that N and O groups are evolving out of expanding or bottleneck selections by estimating negative values for both populations. Presumably, there will be further subdivision of N and O groups. On the other hand, the already highly diverse NTN group is undergoing balancing selection as shown by negative values assumed by the neutrality tests. Our calculated F_{ST} values from comparisons between all formed groups were always > 0.33, even reached > 0.5 except for C vs O which shared a basal node in the phylogenetic tree (Table 6), suggesting expanding genetic distinction and limited gene flow between groups. Therefore, the separation of 55 isolates analysed in this study into five groups was further evidenced by this.

This research provided important molecular data that helps to improve our understanding of the position of PVY Turkish isolates among isolates from other countries that may encourage strong control such as by engineering of resistant cultivars through molecular methods. Results of the population analysis also contributed additional information on the genetic diversity and evolution of PVY.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11540-025-09938-x.

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Data Availability A part of polyprotein gene sequences of 25 novel Turkish PVY isolates has been made available in GenBank, reference numbers PQ821651–675.

Declarations

Consent to Participate Not applicable.

Conflict of Interest The authors declare no competing interests.

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