

Molecular study of turnip mosaic virus population in the Czech Republic

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Table S1. List of *Turnip mosaic virus* isolates analysed in this study

Isolate	Original host	Locality	Year of collection	Acc. No.
T10*	<i>Brassica oleracea</i> var. <i>gongylodes</i> L.	Pustějov	2019	OQ675593
T27*	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Jistebník	2019	OQ675594
T28*	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Jistebník	2019	OQ675595
T29	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Jistebník	2019	OQ675596
T30	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Jistebník	2019	OQ675597
T39	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Jistebník	2019	OQ675598
T41*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Jistebník	2019	OQ675599

Isolate	Original host	Locality	Year of collection	Acc. No.
T45*	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Sedlnice	2019	OQ675600
T47*	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Sedlnice	2019	OQ675601
T49	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Sedlnice	2019	OQ675602
T52*	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Sedlnice	2019	OQ675603
T53*	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Sedlnice	2019	OQ675604
T54*	<i>Brassica napus</i> subsp. <i>napobrassica</i> (L.) Hanelt	Sedlnice	2019	OQ675605
TGZ11*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Lednice	2019	OQ675606
TGZ20*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Lednice	2019	OQ675607
TGZ33*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Lednice	2019	OQ675608
TGZ42*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Lednice	2019	OQ675609
TGZ43*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Lednice	2019	OQ675610
TGZ44*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Lednice	2019	OQ675611
TGZ49*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Lednice	2019	OQ675612
TGZ53*	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	Lednice	2019	OQ675613
CZE2	<i>Armoracia rusticana</i> P. Gaertn. B. Mey. & Scherb.	České Budějovice	1993	LC537537
CZE4*	<i>Brassica rapa</i> subsp. <i>pekinensis</i> (Lour.) Hanelt	České Budějovice	1993	LC537538
CZE5	<i>Brassica rapa</i> var. <i>rapa</i>	České Budějovice	1993	AB252107
CZE8*	<i>Brassica rapa</i> subsp. <i>pekinensis</i> (Lour.) Hanelt	České Budějovice	1993	LC537539
CZE11	<i>Brassica oleracea</i> var. <i>acephala</i> DC.	Olomouc	1994	LC537541
CZE12	<i>Brassica oleracea</i> var. <i>acephala</i> DC.	Olomouc	1994	LC537542
CZE13	<i>Brassica oleracea</i> var. <i>acephala</i> DC.	Olomouc	1994	LC537543
CZE14	<i>Brassica oleracea</i> var. <i>botrytis</i> L.	Olomouc	1994	LC537544
CZE15	<i>Brassica oleracea</i> var. <i>botrytis</i> L.	Olomouc	1994	LC537545
CZE16	<i>Brassica oleracea</i> var. <i>botrytis</i> L.	Olomouc	1994	LC537546
CZE17	<i>Brassica oleracea</i> var. <i>botrytis</i> L.	Olomouc	1994	LC537547

Isolate	Original host	Locality	Year of collection	Acc. No.
CZE18*	<i>Armoracia rusticana</i> P. Gaertn.. B. Mey. & Scherb.	Olomouc	1994	LC537548
CZE19*	<i>Alliaria officinalis</i> M. Bieb.	České Budějovice	1994	LC537549
CZE10*	<i>Alliaria officinalis</i> M. Bieb.	České Budějovice	1993	LC537540
CAR37A	<i>Armoracia rusticana</i> P. Gaertn.. B. Mey. & Scherb.	Poland	2004	DQ648591
RUS1	<i>Armoracia rusticana</i> P. Gaertn.. B. Mey. & Scherb.	---	1993	AB093606
CAR39*	<i>Armoracia rusticana</i> P. Gaertn.. B. Mey. & Scherb.	Poland	2004	EF374098
HC9	<i>Papaver somniferum</i> L.	Slovakia	2018	MH469725
POL1	<i>Brassica napus</i> subsp. <i>napus</i>	Poland	<1993	AB701728
POL2	<i>Papaver somniferum</i> L.	Poland	<1993	AB701731
UKR46	<i>Brassica oleracea</i> var. <i>capitata</i> L.	Ukraine	2014	LC537590
MS15	<i>Papaver somniferum</i> L.	Slovakia	2018	MH469726
MS04	<i>Papaver somniferum</i> L.	Slovakia	2018	MH469727
CAR37*	<i>Armoracia rusticana</i> P. Gaertn.. B. Mey. & Scherb.	Poland	2004	DQ648592
PV-0054	---	---	<1987	MT663307
THA1	<i>Raphanus sativus</i> L.	Thailand	2014	LC537575
CHYK56A	<i>Brassica rapa</i> L.	China	2000	LC537521
VIET80	<i>Raphanus sativus</i> L.	Vietnam	2007	AB747293
AUST21	<i>Hirschfeldia incana</i> (L.) Lagr.-Foss.	Australia	2011	AB989637
UK1	<i>Brassica napus</i> L.	United Kingdom	1975	AF169561
GRC2	<i>Brassica oleracea</i> var. <i>capitata</i> L.	Greece	1993	AP017816
TUR10	<i>Raphanus sativus</i> L.	Turkey	2006	AP017864
CHZH36	<i>Brassica campestris</i> var. <i>communis</i>	China	2000	LC537525
PV-0104	<i>Lactuca sativa</i> L.	Germany	≤1988	MZ405633
AI	<i>Alliaria officinalis</i> M. Bieb.	Italy	1968	AB093598
GRC42	<i>Allium</i> sp.	Greece	1999	AB252117
PV-1206	<i>Alliaria petiolata</i> (M. Bieb.) Cavara & Grande	Germany	2017	ON013903
IRN S1	<i>Sisymbrium loeselii</i> L.	Iran	2003	AP017788
TUR79	<i>Raphanus raphanistrum</i> L.	Turkey	2007	AP017814
PK1	<i>Papaver rhoeas</i> L.	Slovakia	2018	MH469724
TUR42	<i>Raphanus raphanistrum</i> L.	Turkey	2006	AP017815
ASP	<i>Allium</i> sp.	Germany	1995	AB701697
IRNEAM1	<i>Mattiola</i> sp.	Iran	2005	AP017754

Isolate	Original host	Locality	Year of collection	Acc. No.
IRNTOFS3	<i>Sisymbrium loeselii</i> L.	Iran	2006	AP017800
IRNRaNi3	<i>Rapistrum rugosum</i> (L.) All.	Iran	2005	AP017780
ORM	<i>Orchis morio</i> L.	Germany	1981	AB701692

* – recombinant isolates

Table S2. List of primers used in this study

Primer name	Sequence (5' > 3')	Ta [°C]	PCR product size [nt]	Position [nt] ⁴
P1-F ¹	AAAAATATAAAAACCTCAACACACATACACAAAACGA	58	1693/1564	2-37
TuMV-1F ²	ATGGCAGCAGTTACATTCGCATC			160-182
TuMV-1654R ³	GTAAATGTTTCGTGGCTTGTA			1673-1694
TuMV-622F ³	CCAATCATCCTAAAAATGGGAGC	57	983	635-656
TuMV-1607R ³	ACGTGTGGGAATGCAGC			1601-1617
TuMV-1099F ³	AATCAGGGGYGAGCAGCAAG	62	2096	1135-1154
P3-R ¹	CGCTGTATCTGCCGCCCTAAATCG			3208-3230
TuMV-3084F ³	CTTGACGARGCATGGAACGA	58	1345	3097-3117
TuMV-4429R ³	TCGACCYGGTGGTGTGGCTG			4422-4441
TuMV-4130F ³	GTACACAAGCAGTTGTCGA	59	790	4166-4185
TuMV-4900R ³	TACAACCTGGAACCTTCGCTCAACC			4932-4955
P3-F ¹	CGATTAGCGCGCAGATACAGCG	55	2413	3208-3230
CI-R ¹	TCCYTCAAGCACCTGATATGTTCTC			5597-5620
TuMV-5014F ³	GCAGGCACGAACAATGTCTG	59	915	5050-5069
TuMV-5928R ³	CCCATTGTTGTCTCGGGC			5945-5964
CI-F ¹	GAGAACATATCAGTGCCTTGARGGA	52	2060	5597-5620
NIb-R ¹	TCTTCYTTCACTCTCRGGTGTGAAGTC			7631-7656
TuMV-7325F ³	TGCATGATGAAGCAGCAGAGT	58	984	7338-7358
TuMV-8308R ³	TGTCTGCCATAGTGTCAAGGA			8301-8321
8-1F ²	ATTATTCGCTGAGTTCACACC	57	2216/1466	7620-7641
UTR-R ¹	TTTTTTTTTTTTTTTTTTTGTCCCTTGCAATCCTATCAATG			9813-9835
TuMV-9090R ³	CTTTCGCGTTGAWCGTGTGTTG			9064-9085

1 – Zhu F., Sun Y., Wang Y. et al. (2016): International Journal of Molecular Sciences 17: 888; doi:10.3390/ijms17060888; 2 – Zheng G.H., Peng D.W., Tong Q.X. et al. (2017): Journal of Plant Pathology 99 (3): 703-706; 3 – this study; 4 – on NCBI TuMV Reference Sequence: NC_002509.2

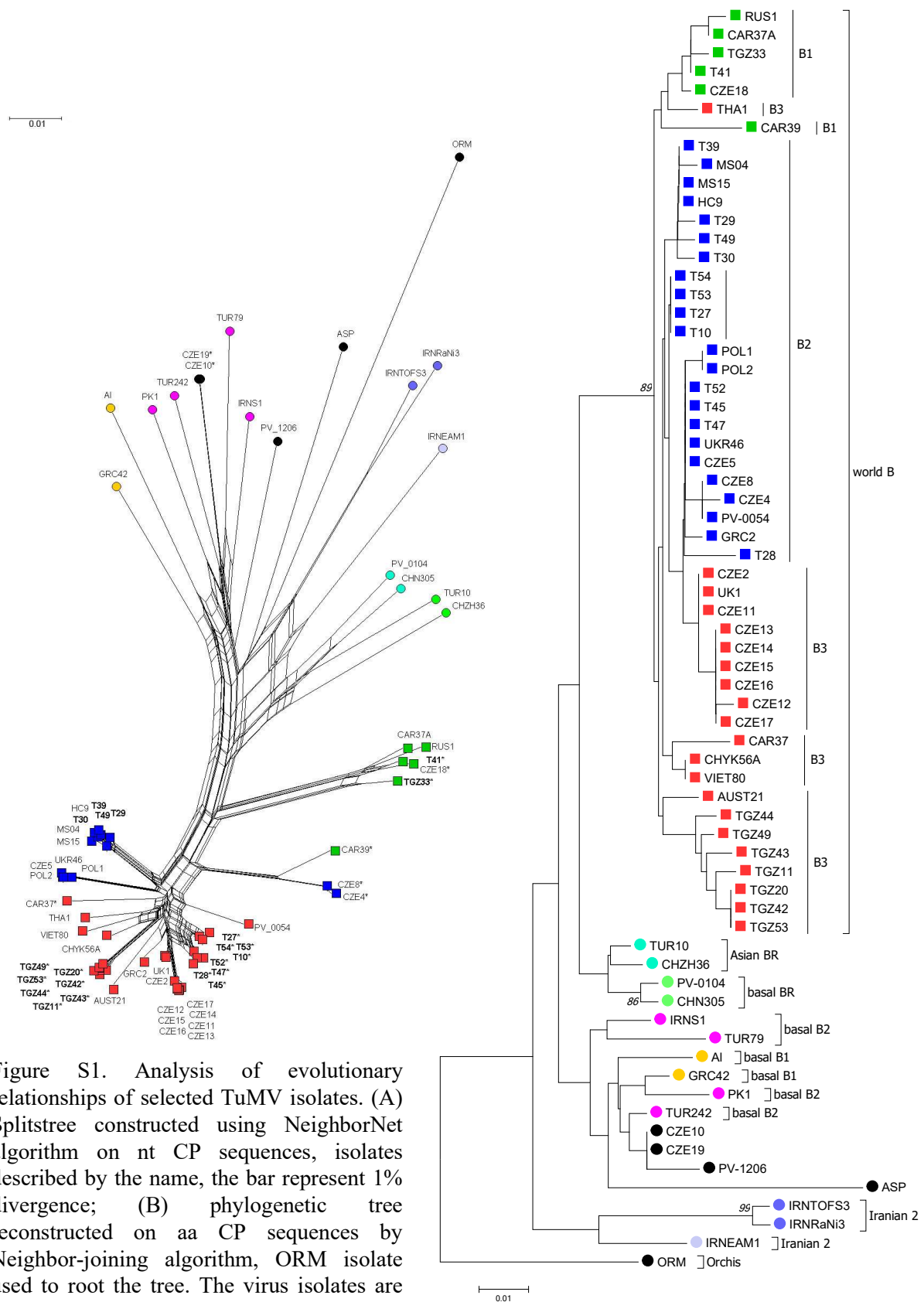


Figure S1. Analysis of evolutionary relationships of selected TuMV isolates. (A) Splitstree constructed using NeighborNet algorithm on nt CP sequences, isolates described by the name, the bar represent 1% divergence; (B) phylogenetic tree reconstructed on aa CP sequences by Neighbor-joining algorithm, ORM isolate used to root the tree. The virus isolates are described by the name; bootstrap values $\geq 70\%$ obtained from 1,000 replicates are shown; bar represents 1% divergence.



Table S3. Intra/inter group identities (in percentage) estimated on the complete nucleotide and amino acid sequences of studied new and old Czech TuMV groups

Genomic region	Intra group identities [%]				Inter group identities [%]		Scale
	NEW		OLD		NEW/OLD		
	nt	aa	nt	aa	nt	aa	
ORF1	92.6	96.6	93.4	97.3	90.9	95.9	88%
P1	94.3	94.2	91.7	91.7	88.2	88.3	90%
HC-Pro	91	96.6	91.9	98.7	88.6	97.1	92%
P3	88.1	91.2	90.2	93.8	86.6	90.5	94%
6K1	92.1	98.9	92	98.1	90.2	98.2	96%
CI	91	98	92.5	98.5	90	97.8	98%
6K2	89.4	92.9	89.7	91.9	86.9	90.6	100%
NIa-VPg	92.4	96.2	94.1	97.6	91.9	96.2	
NIa-Pro	94	96.8	95.5	97.8	93.6	96.7	
NIb	95.1	98.6	96.4	99.3	94.9	98.7	
CP	96.9	98.3	94.4	98	95	98.2	

Table S4. Genetic characterization of turnip mosaic virus ORF1 and specific genes of new and old Czech isolates

Genomic region	TuMV isolates	N	H	Hd	π	ω	Tajima's D	Fu and Li's D*	Fu and Li's F*
ORF1	new	21	21	1.000	0.07969	0.051	0.44671	0.55323	0.70316
	old	12	12	1.000	0.07172	0.046	-0.21582	-0.30616	-0.33557
P1	new	21	20	0.995	0.06153	0.158	0.44671	-2.34421**	-2.33127**
	old	12	10	0.955	0.09255	0.159	-0.21852	-0.83828	-0.92895
HC-Pro	new	21	20	0.995	0.09860	0.038	0.33551	-0.47650	-0.27736
	old	12	9	0.909	0.08997	0.015	-0.26069	-0.25003	-0.28657
P3	new	21	19	0.986	0.13466	0.097	0.99104	1.20187	1.32786
	old	12	12	1.000	0.11051	0.082	-0.29984	-0.33117	-0.40294
6K1	new	21	9	0.843	0.08565	0.016	1.03284	1.19225	1.33497
	old	12	8	0.894	0.08823	0.023	0.03440	0.04424	0.06634
CI	new	20	19	0.995	0.09898	0.022	1.30798	1.07772	1.31707
	old	12	12	1.000	0.08154	0.022	0.03404	-0.08219	-0.06349
6K2	new	21	9	0.890	0.11968	0.081	1.47553	1.04959	1.37581
	old	12	8	0.894	0.11694	0.091	0.61530	0.32656	0.40611
NIa-Vpg	new	21	17	0.976	0.08319	0.053	0.44457	-0.14171	0.04452
	old	12	12	1.000	0.06245	0.044	0.18317	0.01894	0.07101
NIa-Pro	new	21	16	0.957	0.06354	0.015	0.08877	-0.90786	-0.70430
	old	12	9	0.909	0.04717	0.005	0.38886	0.04425	0.15412
NIb	new	21	18	0.971	0.05160	0.026	0.56732	0.24940	0.40618
	old	12	10	0.955	0.03702	0.020	-0.16624	-0.20219	-0.21989
CP	new	21	19	0.990	0.03230	0.062	-0.41511	0.58837	0.34847
	old	12	10	0.995	0.03502	0.023	-0.93019	-0.81079	-0.96094
	OLD	12	12	1.000	0.07172	0.046	-0.21582	-0.30616	-0.33557

** $P \leq 0.01$; N – number of samples; H – number of haplotypes; Hd – haplotype diversity; π – average pairwise nt diversity; ω – ratio of non-synonymous to synonymous nucleotide diversity

Table S5. Population differences in the ORF1 and specific genes of the new and old Czech TuMV isolates

Comparison	Kst* P value	Z* P value	Snn	Fst	Genomic fragment
new(21)-old (12)	0.06367 0.0000***	4.9807 0.0000***	0.8788 0.0000***	0.2270	ORF1
new(21)-old (12)	0.1340 0.0000***	4.8355 0.0000***	0.8990 0.0000***	0.4041	P1
new(21)-old (12)	0.1027 0.0000***	4.9843 0.0000***	0.8182 0.0000**	0.2514	HC-Pro
new(21)-old (12)	0.0829 0.0000***	5.0143 0.0000***	0.9242 0.0000***	0.1899	P3
new(21)-old (12)	0.0946 0.0010**	5.0729 0.0000***	0.9394 0.0000***	0.1844	6K1
new(20)-old (12)	0.0763 0.0010**	4.9804 0.0030**	0.8750 0.0000***	0.1721	CI
new(21)-old (12)	0.1050 0.0000***	5.0638 0.0000***	0.8485 0.0000***	0.2032	6K2
new(21)-old (12)	0.0744 0.0000***	5.0802 0.0000***	0.8788 0.0000***	0.1623	NIa-VPg
new(21)-old (12)	0.0991 0.0000***	5.0190 0.0010**	0.8788 0.0000***	0.1832	NIa-Pro
new(21)-old (12)	0.0828 0.0000***	5.0960 0.0050**	0.8788 0.0000***	0.1703	NIb
new(21)-old (12)	0.0785 0.0000***	5.0092 0.0000***	0.9394 0.0000***	0.1496	CP

** $P \leq 0.01$; *** $P \leq 0.001$; Kst*, Z* and Snn – statistical tests of genetic differentiation; Fst – coefficient of gene differentiation which measures inter-population diversity