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BRIEF REPORT

Molecular characterization of two genotypes of a new polerovirus infecting brassicas in China

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Abstract The genomic RNA sequences of two genotypes of a brassica-infecting polerovirus from China were determined. Sequence analysis revealed that the virus was closely related to but significantly different from turnip yellows virus (TuYV). This virus and other poleroviruses, including TuYV, had less than 90% amino acid sequence identity in all gene products except the coat protein. Based on the molecular criterion (>10% amino acid sequence difference) for species demarcation in the genus *Polerovirus,* the virus represents a distinct species for which the name Brassica yellows virus (BrYV) is proposed. Interestingly, there were two genotypes of BrYV, which mainly differed in the 5′-terminal half of the genome.

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Turnip yellows virus (TuYV; family *Luteoviridae*, genus *Polerovirus*) is a phloem-limited virus that is obligatorily transmitted by aphids (*Myzus persicae*) and has a wide host range [3, 17]. Since TuYV has been reported from all continents [1, 5, 6, 10, 15, 17, 20], it probably has a worldwide distribution. In the UK, TuYV is the most important viral pathogen of oilseed rape and an emerging threat to its production [17]. In Germany and Australia, yield losses of oilseed rape compared to controls amounted to about 34% and 46%, respectively [4, 11]. There was considerable confusion concerning the taxonomy of beet poleroviruses and TuYV until investigations of host range and molecular properties provided valuable data for distinguishing them [2, 8, 9, 16, 19]. The International Committee on Taxonomy of Viruses (ICTV) has assigned European isolates of beet western yellows virus (BWYV) that do not infect sugar beet to the independent species *Turnip yellows virus* in the genus *Polerovirus* [3, 16]. The complete genomic sequence of a French lettuce isolate that was previously referred to as BWYV (BWYV-FL1) is now recognized as the standard TuYV sequence (GenBank acc. no. NC003743) [3, 16, 18]. Although sequences of some open reading frames (ORF0 and ORF3) of crucifer-infecting TuYV isolates are available [8, 14], the complete genome sequences of TuYV isolates from cruciferous crops have not yet been determined.

There was only limited information on the incidence and distribution of brassica-infecting poleroviruses in China

until we first reported that TuYV is widely distributed in China, occurring on eight different cruciferous crops and in ten provinces [20]. In this report, the complete genome sequences of two genotypes of a brassica-infecting polerovirus most closely related to TuYV were determined. This revealed that the Chinese brassica-infecting polerovirus was similar to but distinct from TuYV and that the two polerovirus genotypes differed mainly in the 5′-terminal half of the genome.

An investigation of the occurrence of brassica-infecting polerovirus in mainland China was commenced in September 2006. One hundred ninety-two leaf samples showing yellowing or leafroll symptoms (Fig. 1) were collected from nine different cruciferous plant species including cabbage (*Brassica oleracea* var. *capitata*), Chinese cabbage (*B. pekinensis*), cauliflower (*B. oleracea* var. *botrytis*), flowering Chinese cabbage (*B. chinensis*), leaf mustard (*B. juncea*), oilseed rape (*B. napus* ssp. *napus*), rutabaga (*B. napobrassica*), white glabrous mustard (*B. alboglabra*) and radish (*Raphanus sativus* var. *oleifera*). Total RNA was prepared by SDS-phenol/chloroform extraction and LiCl precipitation as described previously [7]. RT-PCR detection was performed using a universal primer pair (PocoCPR/PoconF) as described previously [13, 20] (Table 1). Sixty-eight of the 192 samples tested positive, providing evidence for polerovirus infections in all of the crops tested and in 11 provinces, including Beijing, Inner Mongolia, Heilongjiang, Liaoning, Henan, Gansu, Shandong, Hubei, Jiangsu, Yunnan and Chongqing. This indicated that brassica-infecting poleroviruses are distributed widely from the northeast to the southwest in mainland China. Furthermore, some RT-PCR products from these crops and provinces were cloned and sequenced. BLAST analysis showed that these sequences shared highest identities with TuYV isolates, suggesting that a TuYV-like virus is the causal agent of crucifer yellowing disease in mainland China.

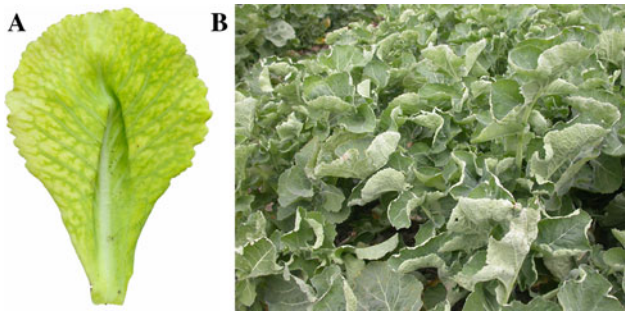

Fig. 1 Yellowing and leafroll symptoms of the brassica-infecting polerovirus. **A.** Interveinal yellowing in Chinese cabbage collected from Beijing on 3 October 2006; **B.** Leafroll symptoms on oilseed rape from Jiangsu on 28 March 2007

Since no complete genomic sequence of a brassica-infecting polerovirus had been reported, we decided to analyze the genomic sequence of a Chinese polerovirus isolate from a rutabaga plant showing leaf yellowing in the Haidian district of Beijing in September 2008. Primers for RT-PCR amplification of the genome sequences were derived from the TuYV-FL1 sequence (GenBank acc. no. NC003743) and the sequences that we obtained (Table 1). Two overlapping fragments (Tu5-31F/Tu2750R and Tu1738F/TYR3) of the genome were obtained by RT-PCR and cloned, and at least three independent positive PCR clones were sequenced. Based on genome sequence analysis, we concluded that the rutabaga-infecting polerovirus is a distinct virus, referred to as brassica yellows virus (BrYV). For the fragment (Tu5-31F/Tu2750R), sequence analysis showed there were two distinct types of clones, which shared only 90.6% nucleotide sequence identity. This suggested that there were two genotypes infecting rutabaga in Beijing, named BrYV-A^{BJ} and BrYV-B^{BJ}. Primer TuB1433F was subsequently designed specifically for BrYV-B and a specific fragment (TuB1433F/TYR3) was amplified, cloned and sequenced. For the 5′ and 3′

Table 1 Primers used for RT-PCR

Primer designation[a]	Primer sequence[b]	Position[c]
Pocon5F (+)	5′-ACAAAAGAN-3′	1–9
Tu5-31F (+)	5′-ACAAAAGAAACCAGGAGGGAATCCTAAGTTG-3′	1–31
Tu862R (−)	5′-CTTGCGTGGGTGATGGGAATC-3′	842–862
TuB1433F (+)	5′-CTATTCCAAAGCCAGACAAATGATCG-3′	1433–1458
Tu1738F (+)	5′-GACGAAGAGCCCCAAAGAAACAGCCGG-3′	1738–1764
Tu2750R (−)	5′-GGTTGAGGTCGATGGTAAGTC-3′	2730–2750
Tu4877F (+)	5′-CCAGTCAAAGACAAGACTCTAAAACTC-3′	4877–4903
PoconF (+)	5′-TGYTCYGGTTTTGACTGG-3′	2666–2683
PocoCPR (−)	5′-CGTCTACCTATTTSGGRTTN-3′	4068–4087
Pocon36R (−)	5′-ACACCGAARYGCCRKRRG-3′	5649–5666
TYR3 (−)	5′-ACACCGAAGTGCCGTGGGGATTTCTC-3′	5641–5666

[a] (+) matching or (−) reverse complementary to virus sequence

[b] K = G or T, R = A or G, S = C or G, Y = C or T, N = C or G or T or A

[c] The primer positions refer to the BrYV sequence

terminal regions, two fragments (Pocon5F/Tu862R and Tu4877F/Pocon36R) were acquired and sequenced. Thus, the complete genomic sequences of two types of the BrYV isolate from Beijing (BrYV-A^{BJ} and BrYV-B^{BJ}) were determined except for the short region where the primers annealed at the 5′-and 3′-termini (GenBank acc. no. HQ388348 and HQ388349, respectively). Using a similar strategy, polerovirus sequences were also obtained from leafroll-affected oilseed rape samples collected from the Yangzhou district of Jiangsu province in March 2009 and submitted to GenBank (acc. no. HQ388350 and HQ388351). Analysis of the sequences revealed that the oilseed rape samples from Jiangsu also contained two genotypes (BrYV-A^{JS} and BrYV-B^{JS}, respectively) that were similar to those found in the rutabaga sample from Beijing.

Both BrYV-A^{BJ} and BrYV-B^{BJ} were 5666 nt in length, making them slightly larger than the TuYV genome (5641 nt), and had a similar genomic organization. They had six ORFs (ORF0-ORF5), a 5′ UTR of 31 nt, an intergenic NCR of 203 nt (from nt 3269 to 3471) between ORF2 and ORF3, and a 3′ UTR of 185 nt. The first ORF (ORF0) beginning with an AUG at nt 32 to 34 ended with an UGA at nt 779 to 781 and encoded putative proteins of 28.9 kDa (BrYV-A^{BJ}) and 28.8 kDa (BrYV-B^{BJ}). The second ORF (ORF1, 1812 nt) starting with an AUG at nt 174 to 176 was predicted to encode a 65.6-kDa protein (P1) and was 12 nt shorter than that of TuYV. The third ORF (ORF2, from nt 2156 to 3268) was presumed to be translated by a -1 frameshift and produce a putative P1-P2 fusion protein (115.3 kDa for BrYV-A^{BJ} and 115.0 kDa for BrYV-B^{BJ}). The intergenic NCR was 203 nt in length compared to 202 nt for TuYV. The fourth ORF (ORF3, 609 nt) began at nt 3472 and was deduced to encode a major coat protein (P3, 22.5 kDa for BrYV-A^{BJ} and 22.4 kDa for BrYV-B^{BJ}). The fifth ORF (ORF4, from nt 3503 to 4030), which was entirely embedded in ORF3, is proposed to be translated into a protein for cell-to-cell movement (P4, 19.5 kDa for BrYV-A^{BJ} to 19.4 kDa for BrYV-B^{BJ}) by a leaky scanning mechanism. The last ORF (ORF5, from nt 4267 to 5481) directly adjoins ORF3, with ORF3-ORF5 potentially encoding readthrough proteins of 75.1 kDa (BrYV-A^{BJ}) and 75.2 kDa (BrYV-B^{BJ}) past the amber stop codon of ORF3. ORF5 of BrYV-A^{BJ} and -B^{BJ} was 81 nt shorter than that of TuYV, resulting in a 1.8- to 1.9-kDa-smaller protein compared to the 47.3 kDa RTD of TuYV. The 3′ UTRs of both BrYV-A^{BJ} and BrYV-B^{BJ} were 29 nt longer than that of TuYV. The genomic size and structure of both BrYV -A^{JS} and BrYV -B^{JS} were identical to those of BrYV-A^{BJ} and BrYV-B^{BJ}. Taken together, BrYV isolates have a genomic organization similar to that of TuYV, except for the length of ORF1, ORF5, the intergenic NCR and the 3′ UTR.

Further sequence comparisons were done using DNAMAN software (Version 6.0, Lynnon Biosoft, Quebec, Canada) and Bioedit software (Version 7.0.4.1). The nucleotide sequences of the two genotypes of BrYV were compared to those of TuYV and other poleroviruses. The entire genome of BrYV-A^{BJ} shared 94.6% nucleotide sequence identity with BrYV-B^{BJ}, where the 5′ terminal ORFs displayed more divergence (88.8–92.5%) and the 3′ terminal ORFs showed higher nucleotide sequence identities of 99.2–99.8%. The nucleotide sequence identity between BrYV-A^{JS} and BrYV-B^{JS} was similar to that between BrYV-A^{BJ} and BrYV-B^{BJ}. Nucleotide sequence identities for the full-length sequence and each gene of BrYV-A^{BJ} and BrYV-A^{JS}/BrYV-B^{BJ} and BrYV-B^{JS} were in the range of 96.0% to 99.6%, and their gene products shared amino acid sequence identities ranging from 94.8% to 99.4%. These results indicated that BrYV-A^{BJ} and BrYV-A^{JS}/BrYV-B^{BJ} and BrYV-B^{JS} are different isolates of the same species. However, the full-length sequence of the two BrYV genotypes shared only 80.6-80.7% nucleotide sequence identity with that of TuYV. Sequence identities between the two BrYV genotypes and TuYV were 87.6–88.7% for ORF0, 87.0–87.3% for ORF1, 89.0–89.1% for ORF1-2, 90.6–91.1% for NCR, 94.1–94.6% for ORF3, 94.3–94.7% for ORF4, 67.0–67.3% for ORF3-5, 50.5–50.8% for ORF5 and 56.8–57.3% for the 3′ UTR. At the amino acid level, the sequence identities were 81.1–83.5%, 84.4–84.8%, 89.1–90.1%, 94.1–94.6%, 89.1%, 59.9–60.8% and 39.2–40.0% for P0, P1, P1-2, P3, P4, P3-5 and P5, respectively. Therefore, the gene products of the two BrYV genotypes shared less than 90% amino acid sequence identity with those of TuYV, with exception of the highly conserved P3 (coat) protein. In order to determine the taxonomic status of BrYV, detailed sequence comparisons between BrYV and other reported poleroviruses were also conducted. The results showed that the sequence divergence of all of the gene products of BrYV from those of other poleroviruses was greater than 10%, except for the coat proteins of BrYV and beet poleroviruses, which differed by 7.9–9.4%. Based on the differences in genome length and sequence comparisons, we propose that BrYV represents a distinct species whose members infect cruciferous crops.

To better understand the relationships of BrYV to other poleroviruses, including TuYV, phylogenetic trees were generated by the Clustal W method and visualized with MEGA (Version 4.1) (Fig. 2) [12]. Phylogenetic analysis showed that the 5′-terminal ORFs (ORF0, ORF1, ORF1-2) and their gene products were closely related to, but distinct from, those of TuYV (Fig. 2a and b). However, they differed in their 3′-proximal ORFs (ORF3, ORF4 and ORF5) and their gene products (P3, P4, P3-P5 and P5), which was consistent with the sequence comparison results (Fig. 2c and d). Surprisingly, the BrYV P5 showed less sequence

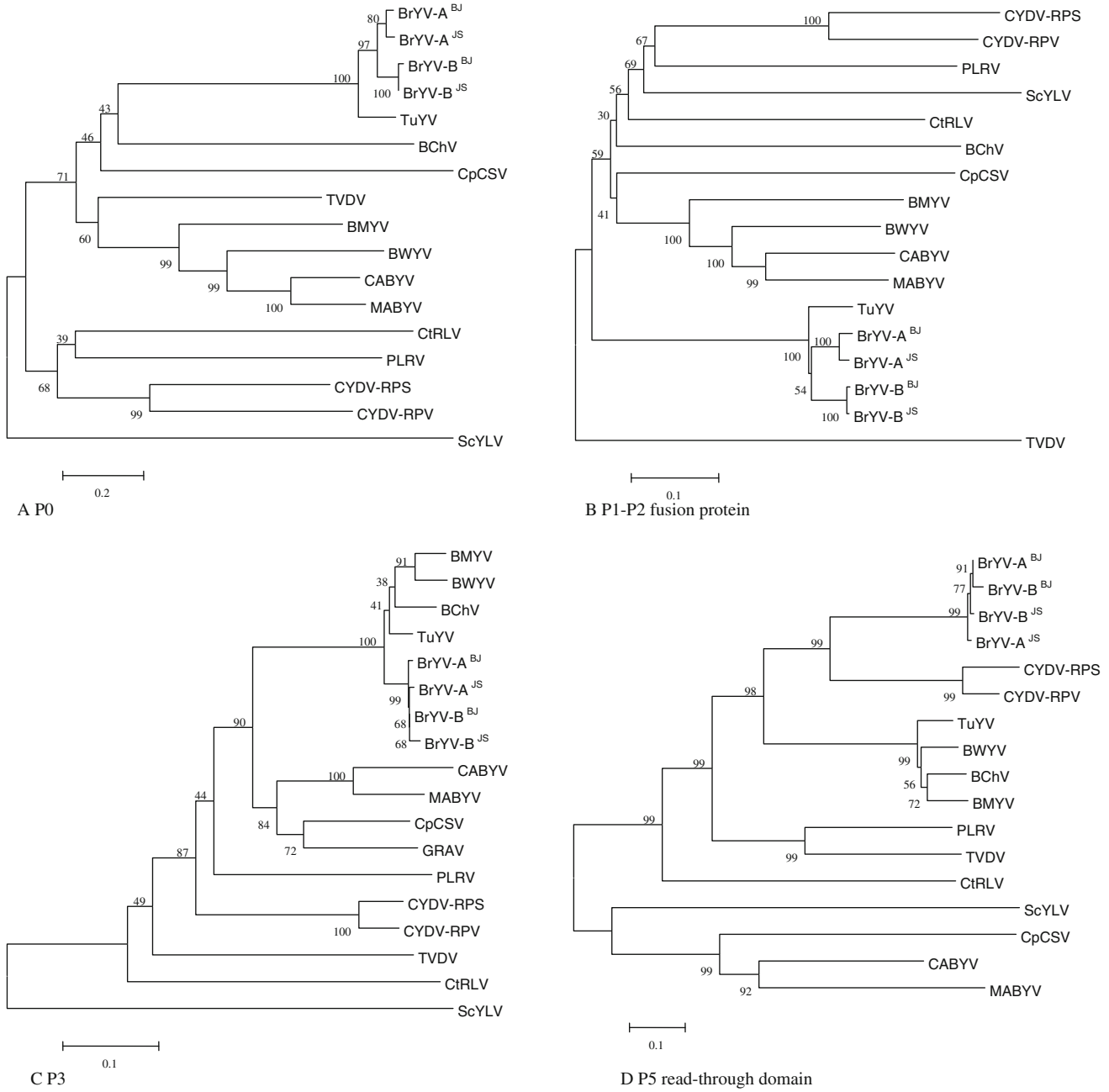

Fig. 2 Phylogenetic trees generated from aligned amino acid sequences of poleroviruses. The phylogeny reconstruction was carried out via the N-J method and visualized by using the MEGA4.1 program, where the bootstrap value was for 500 replicates. **A.** P0; **B.** P1-P2 fusion protein; **C.** P3, coat protein; **D.** P5 readthrough domain. The following virus sequences (virus abbreviations and accession numbers in parentheses) were obtained from the GenBank database: beet chlorosis virus (BChV, NC002766), beet mild yellowing virus (BMYV, NC003491), beet western yellows virus (BWYV, NC004756), cucurbit aphid-borne yellows virus (CABYV, NC003688), chickpea chlorotic stunt virus (CpCSV, NC008249), carrot red leaf virus (CtRLV, NC 006265), melon aphid-borne yellows virus (MABYV, NC010809), potato leafroll virus (PLRV, NC001747), turnip yellows virus (TuYV, NC003743), cereal yellow dwarf virus-RPS (CYDV-RPS, NC002198), CYDV-RPV (NC004751), sugarcane yellow leaf virus (SCYLV, NC000874), BrYV-A^{BJ} (HQ388348), BrYV-B ^{BJ}(HQ388349), BrYV-A^{JS} (HQ388350) and BrYV-B^{JS} (HQ388351)

identity to that of TuYV but was more similar to those of cereal poleroviruses (cereal yellow dwarf virus-RPS and -RPV), suggesting that the difference in P5 sequence between the BrYV and the TuYV isolates could be linked to different aphid transmission specificity or efficiency [16]. Taken together, the phylogenetic analysis results strongly indicated that BrYV was distinct from TuYV and other members of the genus *Polerovirus*.

In conclusion, this article describes the first BrYV genome sequences isolated from *Brassica* crops and provides evidence for the presence of two distinct genotypes infecting rutabaga in Beijing and oilseed rape in Jiangsu. With the exception of the highly conserved P3 protein, the amino acid sequence identities of all of the gene products were lower than 90%, thus meeting the molecular criteria for species demarcation in the genus *Polerovirus* [3]. However, since there is no information on the pathogenicity and aphid transmission specificity and efficiency of the BrYV genotypes, our future investigations will emphasize these aspects. Moreover, the determination of complete genomic sequences of virus isolates from different crucifer crops and geographical regions is essential for elucidating the genetic diversity and epidemiology of crucifer poleroviruses in China and elsewhere.

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References

1. Coutts BA, Hawkes JR, Jones RAC (2006) Occurrence of Beet western yellows virus and its aphid vectors in over-summering broad-leafed weeds and volunteer crop plants in the grainbelt region of south-western Australia. Aust J Agric Res 57:975–982
2. D'Arcy C, Torrance L, Martin R (1989) Discrimination among luteoviruses and their strains by monoclonal antibodies and identification of common epitopes. Phytopathology 79:869–873
3. D'Arcy CJ, Domier LL (2005) Luteoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) Virus Taxonomy: VIIIth Report of the ICTV Elsevier Academic Press, London, pp 891–900
4. Graichen K, Schliephake E (1999) Infestation of winter oilseed rape by *Turnip yellows luteovirus* and its effect on yield in Germany. In 10th International Rapeseed Congress - New Horizons from an Old Crop, pp 131
5. Graichen K, Rabenstein F (1996) European isolates of Beet western yellows virus (BWYV) from oilseed rape (*Brassica napus* L ssp *napus*) are non-pathogenic on sugar beet (*Beta vulgaris* L var *altissima*) but represent isolates of Turnip yellows virus (TuYV). Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz 103:233–245
6. Graichen K, Rabenstein F, Kurtze E (2000) The occurrence of Turnip yellows virus in winter oilseed rape in Austria. Pflanzenschutzberichte 59:35–46
7. Han C, Li D, Xing Y, Zhu K, Tian Z, Cai Z, Yu J, Liu Y (2000) Wheat yellow mosaic virus widely occurring in wheat (*Triticum aestivum*) in China. Plant Dis 84:627–630
8. Hauser S, Stevens M, Mougel C, Smith H, Fritsch C, Herrbach E, Lemaire O (2000) Biological, serological, and molecular variability suggest three distinct polerovirus species infecting beet or rape. Phytopathology 90:460–466
9. Hauser S, Weber C, Vetter G, Stevens M, Beuve M, Lemaire O (2000) Improved detection and differentiation of poleroviruses infecting beet or rape by multiplex RT-PCR. J Virol Methods 89:11–21
10. Jasnic S, Bagi F (2007) Viral diseases of oilseed rape. Biljni Lekar (Plant Doctor) 35:458–461
11. Jones R, Coutts B, Hawkes J (2007) Yield-limiting potential of *Beet western yellows virus* in *Brassica napus*. Aust J Agric Res 58:788–801
12. Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9:299–306
13. Robertson N, French R, Gray S (1991) Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. J Gen Virol 72:1473–1477
14. Schubert J, Rabenstein F, Graichen K, Richter K (1998) Comparison of the 5′-end nucleotide sequences of luteoviruses from oilseed rape and sugar beet. Arch Phytopathol Plant Prot 31:519–530
15. Shahraeen N, Farzadfar S, Lesemann D-E (2003) Incidence of viruses infecting winter oilseed rape (Brassica napus ssp oleifera) in Iran. J Phytopathol 151:614–616
16. Stevens M, Freeman B, Liu H, Herrbach E, Lemaire O (2005) Beet poleroviruses: close friends or distant relatives? Mol Plant Pathol 6:1–9
17. Stevens M, McGrann G, Clark B, Authority H (2008) Turnip yellows virus (syn Beet western yellows virus): An emerging threat to European oilseed rape production. Research Review 69 HGCA. Available via:http://www.hgca.com/document.aspx?fn=load&media_id=4473&publicationId=4579
18. Veidt I, Lot H, Leiser M, Scheidecker D, Guilley H, Richards K, Jonard G (1988) Nucleotide sequence of beet western yellows virus RNA. Nucleic Acids Res 16:9917–9932
19. Waterhouse P, Gildow F, Johnstone G (1988) Luteovirus group. CMI/AAB Descr Plant Viruses 339:9
20. Xiang H, Shang Q, Han C, Li D, Yu J (2009) Detection of Turnip yellows virus in eight cruciferous crops in Mainland China. Phytopathology 99:S144