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Molecular characterization of a new recombinant brassica yellows virus infecting tobacco in China

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Abstract

Brassica yellows virus (BrYV), prevalently distributed throughout mainland China and South Korea while triggering serious diseases in cruciferous crops, is proposed to be a new species in the genus *Polerovirus* within the family *Luteoviridae*. There are three distinct genotypes (BrYV-A, BrYV-B and BrYV-C) reported in cabbage and radish. Here, we describe a new BrYV isolate infecting tobacco plants in the field, which was named BrYV-NtabQJ. The complete genome sequence of BrYV-NtabQJ is 5741 nt in length, and 89% of the sequence shares higher sequence identities (about 90%) with different BrYV isolates. However, it possesses a quite divergent region within ORF5, which is more close to *Beet western yellows virus* (BWYV), *Beet mild yellowing virus* (BMYV) and *Beet chlorosis virus* (BChV). A significant recombination event was then detected among BrYV-NtabQJ, BrYV-B Beijng isolate (BrYV-BBJ) and BWYV *Leonurus sibiricus* isolate (BWYV-LS). It is proposed that BrYV-NtabQJ might be an interspecific recombinant between BrYV-BBJ and BWYV-LS, and the recombination might result in the successful aphid transmission of BrYV from cruciferous crops to tobacco. And it also poses new challenges for BrYV diagnosis and the vegetable production.

Keywords Brassica yellows virus · Recombination · Tobacco · Polerovirus · Phylogenic analysis

RNA recombination is one of the strongest forces creating the genome variability of plant RNA viruses, and it plays an important role in the viral evolution, emergence and epidemiology. Formation of some new, often dangerous viral strains or species always occur through this powerful mechanism [1, 2]. *Luteoviridae*, which is divided into three genera: *Luteovirus, Polerovirus* and *Enamovirus*, is reported to be a family with "rampant" combination [2]. Phylogenetic analysis indicated that the genomes of different poleroviruses have evolved by recombination events between *Polerovirus–Polerovirus* and *Luteovirus–Polerovirus* ancestors [3–5]. And the genus extends to contain 19 virus species based on the most recent ICTV report (December 20, 2018).

Brassica yellows virus (BrYV) is a newly identified species in *Polerovirus*. The virus was first identified in

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cruciferous crops in China, and prevalently distributed throughout Mainland China and South Korea [6–8]. Generally, BrYV genome is composed of a single-strand positive-sense RNA of 5.7 kb. It contains six open-reading frames (ORFs), a short 5′ untranslated region (UTR), an intergenic non-coding region (NCR) between ORF2 and ORF3, and a 3′-UTR without tRNA-like structure or poly(A) tail. The genomic RNA is encapsidated into a spherical virion with a diameter of 25–30 nm. Up to now, three distinct genotypes (BrYV-A, BrYV-B and BrYV-C) were identified in cabbage and radish [7, 8], and a recombinant BrYV (BrYV-CS) was reported in Chinese cabbage in South Korea [6]. And cruciferous plants are their common natural hosts in the field. In 2015, BrYV was detected in tobacco plants in Yunnan province of China, which means that the virus might have a wider host range [9]. However, the complete genome sequence of tobacco isolate was not obtained. Here, the whole genome of BrYV isolate from tobacco plants in Yunnan was obtained, and it is showed that the BrYV tobacco isolate is a new recombinant brassica yellows virus between BrYV and *Beet western yellows virus* (BWYV).

Samples of tobacco leaves with severe yellowing symptoms were collected in the summer and autumn of 2014. Total RNA extraction, cDNA synthesis, PCR, cloning and sequencing were performed as described previously [7, 10]. Several primer sets were designed for subsequent amplification (supplementary Table S1), based on the previously reported genome sequences of BrYV, BWYV, *Beet mild yellowing virus* (BMYV) and *Turnip yellows virus* (TuYV). The genome sequence was then assembled from overlapping viral segments. New primers (Br3397F and Br4988R anneal at the up and downstream of the overlapping position, respectively) were then designed and RT-PCR was performed to confirm the whole sequence. The nearly full-length genomic sequence of BrYV isolates from tobacco (BrYV-NtabQJ) was then determined and given the GenBank accession number (GenBank Accession No. MK057527).

The genomic sequence of BrYV-NtabQJ is 5741 nt in length, slightly longer than those of BrYV-A (5666 nt), BrYV-B (5666 nt), BrYV-C (5678 nt) and BrYV-CS (5666 nt). It has a typical BrYV genome organization, containing six ORFs (ORF0-ORF5), a 31 nt 5′-UTR, an intergenic NCR of 203 nt (nt 3281–3483) and a 185 nt 3′-UTR. ORF0 starts from nt 32 to 781, predicted to encode the P0 suppressor. ORF1 begins at nt 174 and ends at nt 1997, encoding P1 protein with a molecular mass of 66.1 kDa. ORF2 (nt 1629–3280) is assumed to be translated by a -1 ribosomal frameshift to produce the P1–P2 fusion protein with a predicted mass of 114.7 kDa. The shifty sequence is GGGAAA G at nt 1629 to 1635. ORF3 starts at nt 3484 to 4092, encoding a putative 22.6-kDa coat protein (CP). ORF4, embedded in ORF3, starts at nt 3515 to 4042 and codes for a putative 19.6-kDa P4 protein by a leaky scanning mechanism. Via a read-through strategy, ORF5 (from nt 4093 to 5556) is translated to produce the P3–P5 fusion protein with a mass of 76.4 kDa, which is thought to play an important role in virus transmission [8, 11].

Sequence analysis based on DNAMAN software (version 6.0, Lynnon Biosoft, Quebec, Canada) indicated that main genome of BrYV-NtabQJ (from nt 1 to 4877) shares higher sequence identities with BrYV-A (90.7–90.9%), -B (91.8–92.0%), -C (92.0–92.3%), and BrYV-CS (86.9%), but it possesses a quite divergent region within ORF5 (Fig. 1a). Subsequent alignments indicated that the P0, P1, P1–P2, P3 and P4 protein of BrYV-NtabQJ share much higher amino acid sequence identities (88.8–92.8% for P0, 85.8–89.8% for P1, 90.3–93.5% for P1-P2, 91.1–94.1% for P3, 88.0–93.7% for P4) with the corresponding proteins of BrYV isolates, respectively, while its P5 protein only shares 40.0–52.4% identities with the other BrYV P5 proteins (Figure S1; Supplementary Table S2). Interestingly, BrYV-NtabQJ P5 was found to be more closely related to the P5 protein of BWYV strains (*Leonurus sibiricus* isolate, GenBank accession No. KM076647, 67.1%; *Capsicum Annuum* isolate, LC198684, 67.1% identity; here they were designate as BMYV-LS and BMYV-CA, respectively) and BMYV (GenBank accession No. NC003491; 64.7% identity) (Figure S1; Supplementary Table S2). And further analysis demonstrated that the 3′ half of BrYV-NtabQJ ORF5 (nt 4956–5500) shows much higher sequence identities with those of BWYV-LS and BWYV-CA (80.1–81.3% identities), which suggested that a chimeric genome assembly might occur.

To detect the genome recombination events occurred in BrYV-NtabQJ, viral genome sequence alignments were performed using ClustalW alignment program on MEGA 6.06 [12], and the aligned sequences were then analyzed by recombination detection program 4 (RDP v.4.43) [13]. When the genomic sequences of different BrYVs were aligned together, one significant recombination event was detected among BrYV-NtabQJ, BrYV-BBJ and BWYV-LS, using 7 methods in the RDP4 package, including RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan and 3Seq (P value for each method was shown in supplementary Table S3) (Figure S2). And BrYV-NtabQJ was demonstrated to be a recombinant virus between BrYV-BBJ and BMYV-LS. It is different form BrYV-CS, which is the recombinant virus of BrYV (major parent) and TuYV (minor parent) with the recombinant region of positions 3531–4819 [6]. BrYV-BBJ and BMYV-LS were its potential major and minor parent, respectively. The probable beginning and ending breakpoint locate at nt 4878 and nt 5521 in the ORF5 region, respectively. The recombinant region takes up 11.2% of the genome, and it also leads to a slightly longer genome, compared to other BrYV strains (Fig. 1b).

Here, a BrYV tobacco isolate was obtained and it shows great diversity with other BrYVs at its ORF5 region. P5

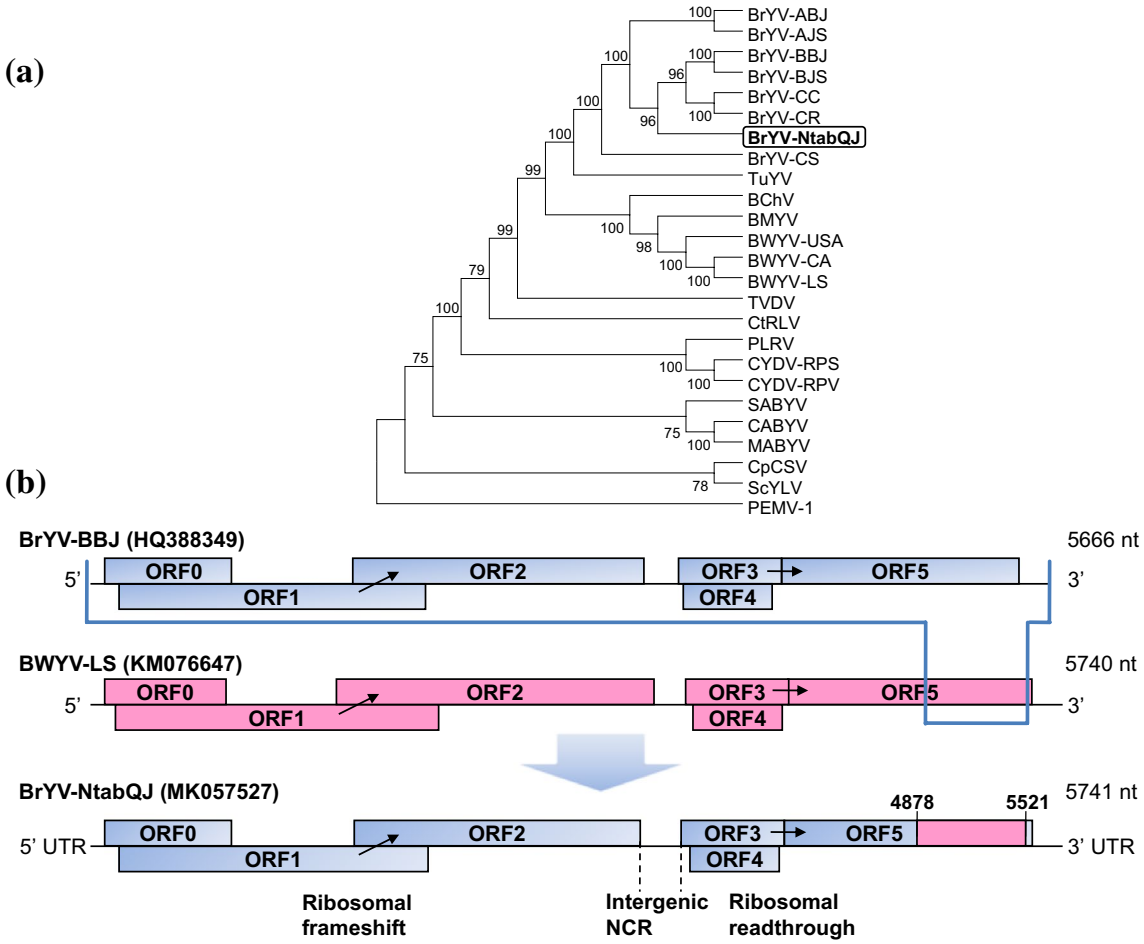

Fig. 1 Genome phylogenetic trees and representation of the recombinant region of BrYV-NtabQJ genome. **a** The genome phylogeny was analyzed by the neighbor-joining method and visualized with MEGA6.0. Bootstrap values shown at the nodes indicate the percentage of 1000 replications supporting the branching patterns shown. The virus sequences used in this study are listed as follows: Brassica yellows virus (BrYV-ABJ, NC016038; BrYV-AJS, HQ388350; BrYV-BBJ, HQ388349; BrYV-BJS, HQ388351; BrYV-CC, KF015269; BrYV-CR, JN015068; *Turnip yellows virus* (TuYV, NC003743); *Beet chlorosis virus* (BChV, NC002766); *Beet mild yellowing virus* (BMYV, NC003491), *Beet western yellows virus* (BWYV-USA, NC004756; BWYV-CA, LC198684; BWYV-LS, KM076647), *Tobacco vein-distorting virus* (TVDV, EF529624); *Carrot red leaf virus* (CtRLV, AY695933); *Potato leafroll virus* (PLRV, D00530); *Cereal yellow dwarf virus* (CYDV-RPS, AF235168; CYDV-RPV, L25299); *Suakwa aphid-borne yellows virus* (SABYV, JQ700308); *Cucurbit aphid-borne yellows virus* (CABYV, X76931); *Melon aphid-borne yellows virus* (MABYV, EU000534); *Sugarcane yellow leaf virus* (ScYLV, AF157029); *Chickpea chlorotic stunt virus* (CpCSV, AY956384). *Pea enation mosaic virus*-1 (PEMV-1, genus *Enamovirus*, NC003629) is used as an outgroup sequence. **b** The genome organization of BrYV-NtabQJ and the recombinant region. The recombinant region is shown in pink at nt 4878–5521. The ribosomal frameshift and read-through strategies are shown by arrows

protein was previously found to function in aphid transmission and vector specificity [11, 14]. As most BrYV isolates were originally identified in cruciferous crops in the field, the recombination might result in the successful aphid transmission of BrYV from cruciferous crops to tobacco. Whether the recombinant P5 of BrYV-NtabQJ correlates with aphid transmission on tobacco needs more detailed research. Both inter- and intraspecific recombination have been thought to be a common and important way in the process of polerovirus evolution, and there are some examples described in this species, including BrYV [6], *Cucurbit aphid-borne yellows virus* (CABYV) [15, 16], *Sugarcane yellow leaf virus* (ScYLV) [4, 17] and *Cotton leafroll dwarf virus* (CLRDV) [18]. As being detected in cruciferous and solanaceous plants, BrYV might be easy to evolve to different recombinant strains or isolates. This may pose new challenges for diagnostic testing and agricultural production.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Research involving human participants or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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