Molecular characterization of polish blueberry red ringspot virus isolate

E. Kalinowska · E. Paduch-Cichal ·
M. Chodorska

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Abstract In this study, we determined the complete sequence of the genomic DNA of a Polish isolate of *Blueberry red ringspot virus* (BRRSV24) and compared it with a Czech (Darrow 5), and the US isolates of the virus and those of other *Caulimoviridae* family. The genomic DNA of BRRSV24 consists of 8,265 nucleotides and encodes eight open reading frames (ORFs). The sequence homologies of the eight ORFs of BRRSV24 were from 95 to 98% in respect of Darrow 5 and from 91 to 98% in respect of the US isolates at the amino acid level. This high level of amino acid sequence identity within the coding regions among the Czech, the US and Polish BRRSV isolates is suggestive of their common origin.

Keywords BRRSV · Isolate · Poland

Introduction

Blueberry red ringspot virus (BRRSV) is a member of the *Soymovirus* genus in the *Caulimoviridae* family [1]. The virus has isometric particles 42–46 nm in diameter. Its particles occur embedded in cytoplasm inclusion bodies and are also found in the nucleus [2]. The molecule of BRRSV has a length of at least 8,265 base-pairs. This virus

genome contains eight open reading frames (ORFs). ORF A, B, and C encode putative proteins of unknown function. ORF I encodes a cell to cell movement protein. ORF IV—one of the more conserved regions among caulimoviruses—encodes the putative coat protein (CP). ORF V, the putative reverse transcriptase coding region, has many regions that are conserved among caulimoviruses. ORF VI encodes a putative translational transactivator protein that is a major constituent of the inclusion bodies. ORF VII is a poorly conserved region that encodes protein of unknown function [3].

Red ringspot disease connected with BRRSV was first reported in the United States [4]. The virus has also been identified in Japan [5], Czech Republic, [6], Slovenia [7], and Poland [8]. The symptoms of red ringspot disease appear on highbush blueberry leaves and stems in a form of reddish spots and rings, which can connect during the growing season. Fruits from infected bushes can develop red circular blotches and light areas, which tend to disappear when berries ripen [9]. However, during inspections of Polish highbush blueberry plantations, all plants infected with BRRSV did not show red ringspot symptoms on fruits.

Only two isolates of the virus (those of Czech Darrow 5 and the USA from New Jersey) have been completely sequenced. To our knowledge, this is the first article of the complete nucleotide sequence of a Polish isolate of BRRSV. The complete genome sequence of BRRSV24 has been deposited in the GenBank database under accn. no. JN205460.

Provenance of the virus material

Highbush blueberry plant martial (leaves, stems) exhibiting red ringspot symptoms from cultivar Herbert (isolate

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E. Kalinowska (✉) · E. Paduch-Cichal · M. Chodorska
Faculty of Horticulture and Landscape Architecture,
Department of Plant Pathology, Warsaw University of Life
Sciences—SGGW, Nowoursynowska 159, 02-776 Warsaw,
Poland
e-mail: elzbieta_kalinowska@sggw.pl

BRRSV24) was collected from the naturally infected field plots at commercial plantation (central part of Poland) in autumn 2010. Total DNA was extracted with DNeasy® Plant Mini Kit (Qiagen Inc., Valencia, CA, USA), in accordance with the manufacturer's protocol and quantified. Thirteen primer sets were used in PCR amplifications to determinate the complete nucleotide sequence of BRRSV24 isolate. Nine primer pairs (BRRSV1/2, BRRSV7/8, BRRSV13/14, BRRSV15/16, BRRSV17/18, BRRSV19/20, RRSV1/2, RR80/81, and BRRV29/30) were designed by Polashock et al. [10]. Remaining primers were designed on the basis of sequences received in reactions with above primer sets (Primers used in this study are listed in Table S1 in the Supplementary Material). The PCR reactions were performed using *Taq* PCR Core Kit (Qiagen Inc.). Sequence identities and alignments among members of the *Soymovirus* genus were analyzed using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>) and ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). A phylogenetic tree was constructed using the neighbor-joining method run on MEGA version 5.02 [11].

Sequence properties

The BRRSV24 genome contains 8,265 base-pairs, and the average A/T content is 69.31%. The predicted genome organization was eight ORFs, being consistent with other approved members of the genus *Soymovirus*.

ORF A encodes protein with an estimated molecular mass of 14.26 kDa (122 amino acids [aa]). ORF B potentially encodes protein with a predicted molecular mass of 21 kDa (186 aa). ORF C encodes protein with a predicted molecular mass of 23 kDa (197 aa). Product of ORF C is two aa shorter than American and Czech BRRSV sequences. These three coding regions could participate in a vector transmission, although an interaction with a presumed insect is unknown. Alignments of predicted products of ORFs A, B, and C (Ib, II, III in *Soybean chlorotic mottle virus*, respectively) among soymoviruses revealed that these three coding regions are more conserved amid BRRSV isolates, than remaining soybean chlorotic mottle-like viruses. However, Darrow 5 ORF A is 19 aa shorter than Polish and the American ORF A aa sequences.

ORF IV encodes CP of a molecular mass 58 kDa (484 aa). In comparison with Darrow5 CP gene BRRSV24 and the American NJ ORFs IV have 27-nt deletion in the 5'-terminus of the ORF. Polish BRRSV24- and Darrow 5 CP-coding regions have single nt deletions resulting in frame-shift reading (aa 297–308). In addition BRRSV24

CP aa sequence has triplet deletions between aa 347 and aa 358. Although regions of high variability between the isolates are present, conserved region characteristic to caulimoviruses, a potential RNA-binding domain (CWICQEDGHYANEC; aa 401–414) was found in all BRRSV CP aa sequences.

ORF V encodes reverse transcriptase protein with an estimated molecular mass of 105 kDa (657 aa). RT gene is one of the most conservative ORFs in BRRSV genome. The aa sequence of BRRSV24 ORF V contains regions reported in other caulimoviruses (YVDDIIIF; aa 345–352). The BRRSV24 aa sequence YIDTGASLC (aa 20–28), also present in Darrow 5 and BRRSV-NJ ORF V is similar to a putative protease domain of caulimoviruses.

ORF VI encodes transcriptional activator protein with a molecular mass of 48.9 kDa (427 aa). In deduced aa product of ORF VI of the BRRSV24 characteristic conserved region was also found (GLADTIY; 226–232).

ORF VII encodes protein with a molecular mass of 17 kDa (153 aa). Darrow 5 and BRRSV24 ORFs VII were 11 aa-longer than the American. This difference is a result of two base deletions in both European sequences. Although the functional protein of ORF VII is still unknown, there is a tentative theory about connection with closely related BRRSV PCSV (*Peanut chlorotic streak virus*) ORF VII during the reaction between host and the virus.

ORF I is a movement protein gene with a predicted molecular mass of 35 kDa (312 aa). Similar to NJ MP gene product, BRRSV24 is 53 aa shorter than Darrow 5. However, the deduced ORF I aa product of all BRRSV isolates contains a putative “transport domain” (GNLKYGVKFDV; aa 143–154).

Sequencing analysis of the BRRSV24 genome revealed 95 and 96% nucleotide sequence identity with the US isolate from New Jersey and Czech Darrow 5 isolate, respectively (Table 1). The most conservative regions among BRRSV isolates are ORFs B and V (98% homology in aa sequences). BRRSV24 RT aa sequence is also closely related with other caulimoviruses (35–46% similarity). Comparison of the conserved ORF V aa sequence of BRRSV24 with that of other caulimoviruses clearly placed this isolate as a member of the genus *Soymovirus* (Fig. 1).

ORFs VII and I (MP gene) are characterized as poorly conservative regions. Homology in ORF VII aa sequences among BRRSV isolates varied from 87 to 98%. Related caulimoviruses revealed only 7–23% similarity in this region with Polish sequence. Despite the presence characteristic “transport domain” in the American and European BRRSV, ORF I homology in predicted MP products did not exceed 95%. Similarity between BRRSV24 MP gene and other caulimoviruses revealed 22–31% at aa level.

Table 1 Percent aa (ORF1–ORF VII) and nt (overall) sequence identities between the BRRSV24 isolate and selected caulimoviruses

	ORF I	ORF A	ORF B	ORF C	ORF IV	ORF V	ORF VI	ORF VII	Overall
BRRSV-NJ (NC_003138)	91	97	98	95	92	98	91	87	95
BRRSV-Czech Republic (HM159264)	95	97	98	95	96	98	97	98	96
<i>Peanut chlorotic streak virus</i> , PCSV (NC_001634)	31	30	16	17	29	46	27	25	59
<i>Soybean chlorotic mottle virus</i> , SbCMV (NC_001739)	27	11	18	22	26	51	24	23	59
<i>Cauliflower mosaic virus</i> , CaMV-Xinjiang (AF140604)	22	–*	–	–	23	36	18	7	54
<i>Carnation etched ring virus</i> , CERV (X04658)	23	–*	–	–	17	34	14	–**	55
<i>Figwort mosaic virus</i> , FMV (X06166)	24	–*	–	–	20	35	16	13	56

* There are no reported ORFs A, B, and C in CaMV-Xinjiang, CERV, and FMV genomes

** There is no reported ORF VII in CERV genome

Fig. 1 Phylogenetic tree illustrating the relationships in ORF V amino acid (aa) sequences of selected caulimoviruses (for isolate designations and accession numbers, see Table 1). The sequence of *Cassava vein mosaic virus* (CsVMV) was used as an outgroup. Values at the nodes indicate the percentage of trees in which this grouping occurred after bootstrapping the data with 1,000 replicates

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