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Complete nucleotide sequences of garlic viruses A and C, members of the newly ratified genus *Allexivirus**

Brief Report

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Summary. Complete genomic sequences of garlic viruses A (GarV-A) and C (GarV-C), members of an unassigned virus group recently identified in garlic plants, were determined. Their respective genomes consist of 8 660 and 8 405 nucleotides. The genomic structure and organization of these viruses are similar to shallot virus X (ShVX) which is the type species of the newly ratified genus *Allexivirus*. Phylogenetic analysis based on the amino acid sequences of the putative proteins including RNA-dependent RNA polymerase (RdRP), DNA helicase, or viral coat protein showed that the GarV-type viruses should be included in the genus *Allexivirus*. Furthermore, the amino acid sequence in the RdRP hypervariable region is highly divergent among the viruses in the genus *Allexivirus*, suggesting that they evolved independently from a hypothetical ancestor virus(es).

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Allium plants are infected with a number of viruses, in particular potex-, carla-, poty-, and unassigned GarV-type viruses [3, 5, 6, 11, 13, 16, 23–29]. GarV-type viruses, including GarV-A, -B, -C, -D and garlic virus X (GVX), are similar to shallot virus X (ShVX) but not identical [10, 20, 21]. Moreover, these viruses are closely related to the carla- and potexviruses but are clearly distinguished by their novel genomic organization of the 3′ terminal region and the presence of an open reading frame (ORF) that encodes a putative 40 kDa protein which has no similarity to any of the other sequences in the DNA and protein sequence database [10, 20, 21].

*The nucleotide sequence data reported in this paper were deposited in the DDBJ/EMBL/GenBank nucleotide sequence database under the accession numbers AB010300 for GarV-A, AB010301 for GarV-B, AB010302 for GarV-C, and AB010303 for Gar V-D.

Many studies of GarVs have been published, and the results show these viruses commonly infect garlic plants cultivated throughout the world [9, 16, 19, 22, 30]. The biological and physical properties of GarVs, particularly those of GarV-C, have been characterized [9, 21, 22, 30]. This virus is a flexuous filamentous particle approximately 700 nm long that is transmitted by the eriophyid mite, *Aceria tulipae* Keifer [21, 22, 30]. Its host range and symptomatology, as well as its physical properties; thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro, have been described [30]. More recently, the complete nucleotide sequence of the genome of a GarV, designated garlic virus X (GVX), was reported and it was reconfirmed that GarV-type viruses and ShVX are separate members of a new group of plant viruses [20]. All of these findings support the idea that these viruses should be included in a new taxon and the genus *Allexivirus* has been recently ratified by the International Committee on Taxonomy of Viruses (ICTV) [15]. However, the detailed relationships that exist among the viruses in the genus *Allexivirus* have yet to be clarified on the basis of the nucleotide sequence of the genomic RNA.

We cloned full cDNAs of GarV-A and -C using the methods of primer extension and PCR on the basis of their partial nucleotide sequences [21]. The conserved nucleotide sequence was in the 5′ non-coding regions of GVX and shallot virus X (Fig. 1), therefore a common primer sequence (GVTOP2 in Fig. 2) was designed for the RT-PCR. The extreme 5′ end sequence of the viral genome was cloned by 5′ RACE [8] according to the manufacturer's instructions (Life Technologies Inc., Rockville, MD). Lastly, cDNA clones covering the complete nucleotide sequence of the genomes for GarV-A and GarV-C, respectively 8 660 and 8 405 nucleotides [excluding the poly(A)tail], were obtained and their nucleotide sequences were determined. The respective cDNA clones are illustrated in Fig. 2 together with the primer sequences used. The genomic organization of these viruses is similar to that of carla- and potexviruses but more specifically to ShVX (Fig. 2).

Partial cDNA sequences of GarV-B and -D, respectively 5 077 and 4 497 nucleotides, that harbour genes for a truncated RNA-dependent RNA polymerase (RdRP) C-terminal region, as well as ORF 2 to ORF 6 were determined. All of the sequences determined in this experiment were deposited in the DDBJ/EMBL/GenBank DNA database with accession numbers AB010300 to AB010303. Analysis of the nucleotide sequences of the GarV-A and -C genomic RNAs revealed the presence of six potential ORFs (Fig. 2). ORF 1 encoded a 174.9–182.6 kDa RdRP and ORF 2 encoded a putative NTP-dependent DNA helicase approximately 27 kDa in molecular mass. ORF 3 encoded an 11 kDa protein homologous to the second of the triple gene block proteins of the carla- and potexviruses [4]. A 40 kDa protein encoded in ORF 4 had no significant similarity to any known sequence. The presence of this unusual ORF is the hallmark of GarV-type viruses [10, 20, 21]. ORF 5 and 6, respectively, encoded viral coat protein (CP) and a putative nucleic acid binding protein (NABP). Overall amino acid sequence similarities of the respective ORFs were observed among the probable allexiviruses reported so far, including ShVX, GVX and GarV-A to -D. The putative ORFs, expect for the ORF 4 encoding the novel 40 kDa protein, share significant sequence

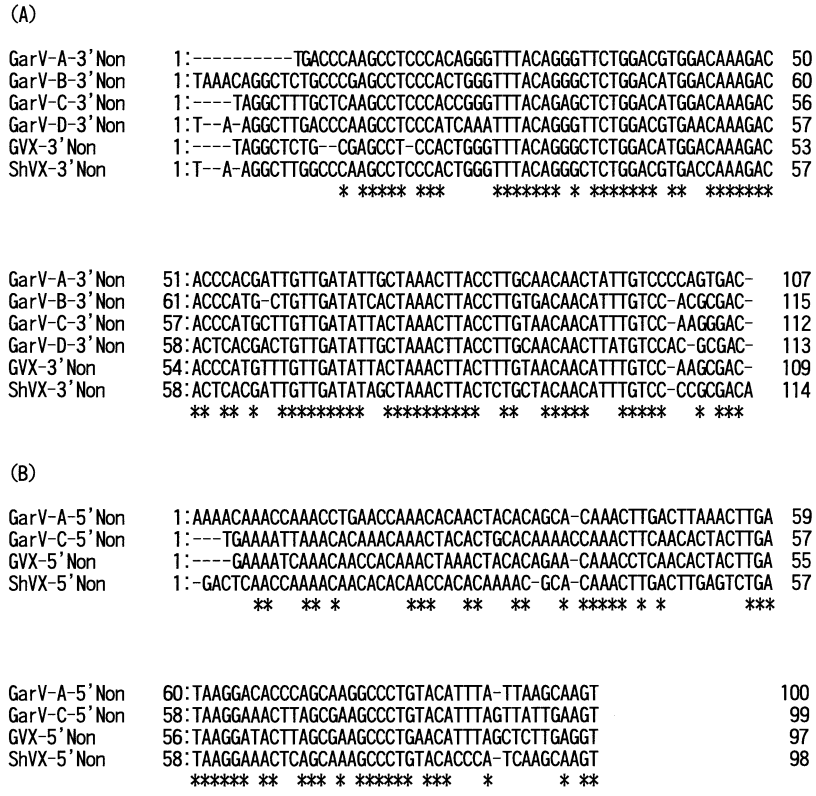

Fig. 1. Conserved nucleotide sequences in the 3′ non-coding (**A**) and 5′ non-coding (**B**) regions of GarV-type virus genomes. The nucleotides conserved are shown by asterisks

similarities from 47.2 to 84.4% among the viruses. For the 40 kDa protein, the homologies between the viruses ranged from 28.4 to 55.5%.

The N-terminal, central, and C-terminal parts of the putative RdRP of GarV-A and -C had sequence motifs typical, respectively, of the methyltransferase, helicase and polymerase domains, as was reported in the allexiviruses GVX and ShVX [10, 20]. Although there is overall amino acid sequence homology of RdRP among these viruses (58.4–79.0%), the sequence in the region between the putative methyltransferase and helicase domains varied markedly, having less than 33% identity. The significant sequence divergence in this hypervariable region is mainly the result of the insertion or deletion of residues. It also should be noted that acidic amino acids D and E were much more abundant in these variable regions (21.4 to 24.6% of the total amino acids) than in the other regions (approximately 11%) of the putative RdRP, except for ShVX. In ShVX, the acidic amino acid content was only 11%, but, interestingly, there was significant similarity with the corresponding region of the potexvirus, clover yellow mosaic virus (CYMV) [18] (Fig. 3). No such sequence similarity in the hypervariable region of RdRP was observed between the GarVs and any potexviruses. Phylogentic analyses based on the amino acid sequence of the putative RdRP showed the GarVs were included in the same domain as ShVX and GVX and they were clearly separated

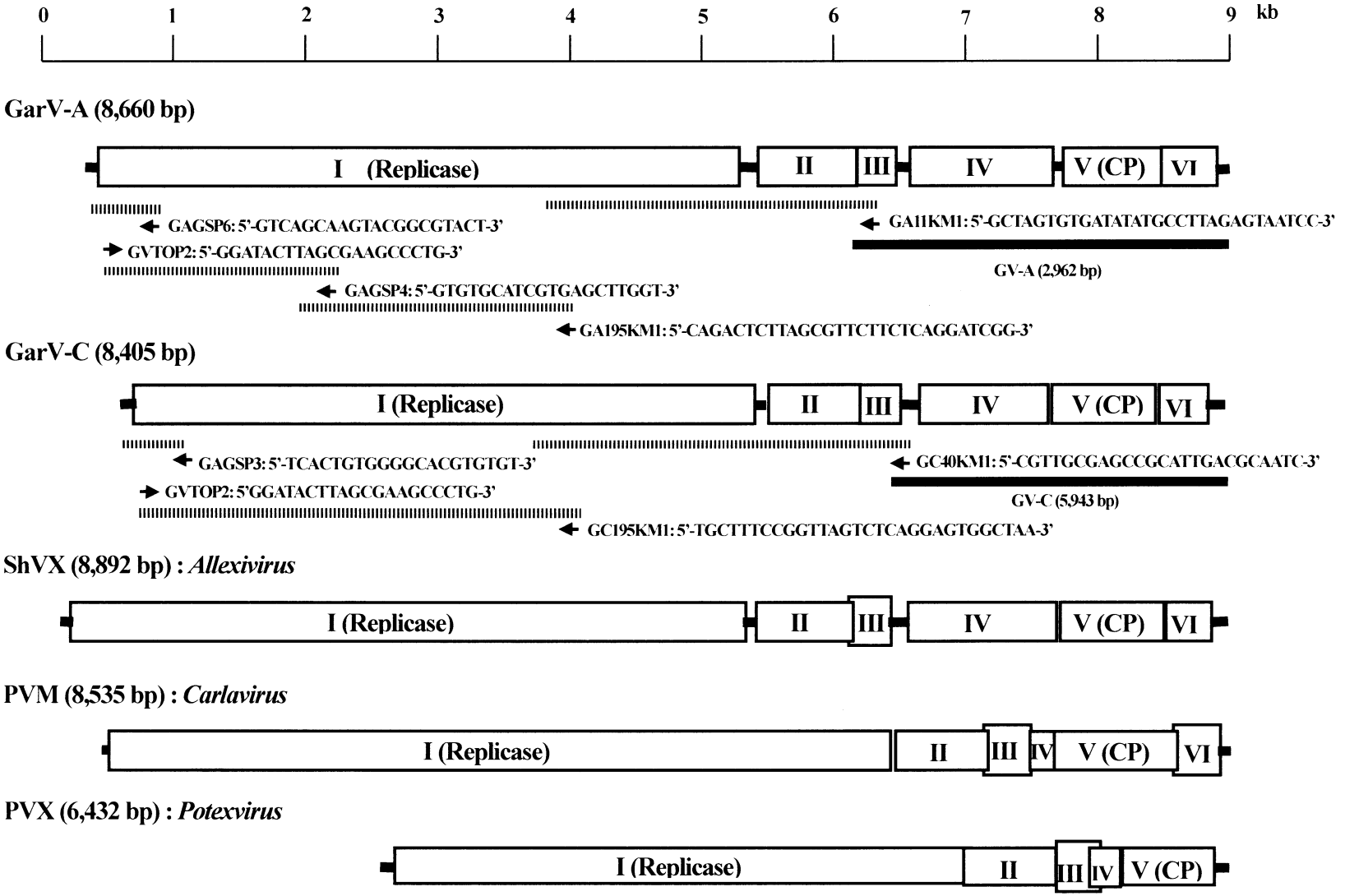

Fig. 2. Genomic organization of GarV-A and -C compared with allexivirus ShVX [10], carlavirus potato virus M (PVM) [31] and potexvirus potato virus X [14]. The respective cDNAs, arranged by size and location of the genomes, are shown with the primer sequences used for cloning. GV-A and GV-C are cDNA clones of the 3′ terminal parts of the GarV-A and -C genomic RNAs [21]

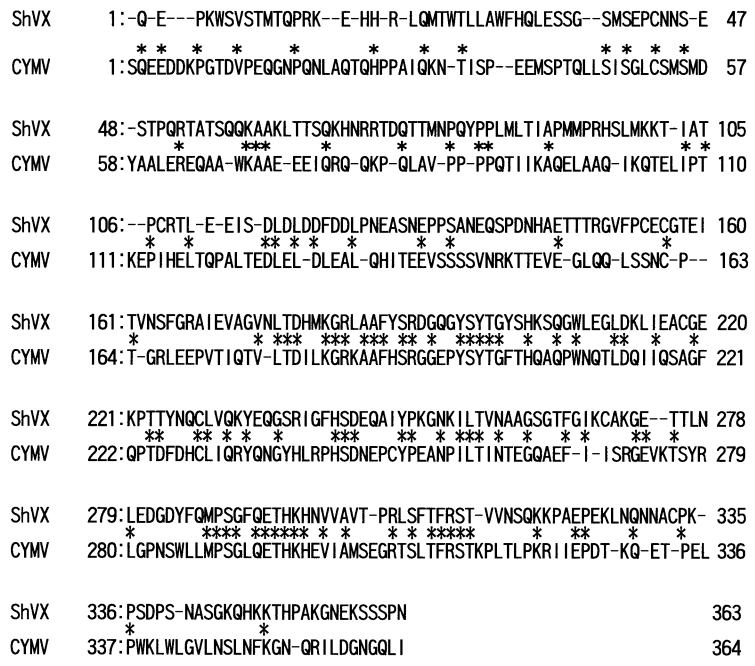

Fig. 3. Homology of the hypervariable regions in the RNA-dependent RNA polymerase (RdRP) of ShVX [10] and CYMV [18]. Deduced amino acid sequences of the hypervariable region between the putative methyltransferase and helicase domains in the RdRP gene are shown. Common amino acids are indicated by asterisks

from the domains of potexviruses or carlaviruses (Fig. 4). The analyses based on the putative DNA helicase and viral coat protein sequence gave the same result (data not shown). These findings confirm that the GarVs A to D and GVX are members of the genus *Allexivirus*, but suggest that in terms of evolution, they are independent of ShVX. The GarVs, at least GarV-A and -C, and ShVX may have evolved independently from a hypothetical common ancestor virus(es) through various evolutionary events such as gene exchange, gene and module shuffling, and gene duplication [12].

The homology of the 40 kDa proteins, as well as the hypervariable region of RdRP, is considerably lower than the values for the other viral proteins. The degree of the homology (36.9% in average), however, is higher by approximately 10% than that of the hypervariable region of RdRP (22.9% in average). The number of amino acid residues constituting the 40 kDa protein is relatively constant (356 to 386 residues), except for GVX (288 residues). In the GVX 40 kDa protein, the N-terminal sequence probably was deleted. These facts are suggestive that an evolutionary event(s) in RdRP gene occurred prior to that in 40 kDa protein gene.

Recently, Arshava et al. [1] reported that the 40 kDa protein is expressed in plants infected with ShVX, indicating that this novel protein has a biological function. The finding suggests that the central region, which includes the conserved sequence, LHALHXNS/TLEWLTH(X can be Q, K, R, or L), is involved in the putative biological function of the 40 kDa protein.

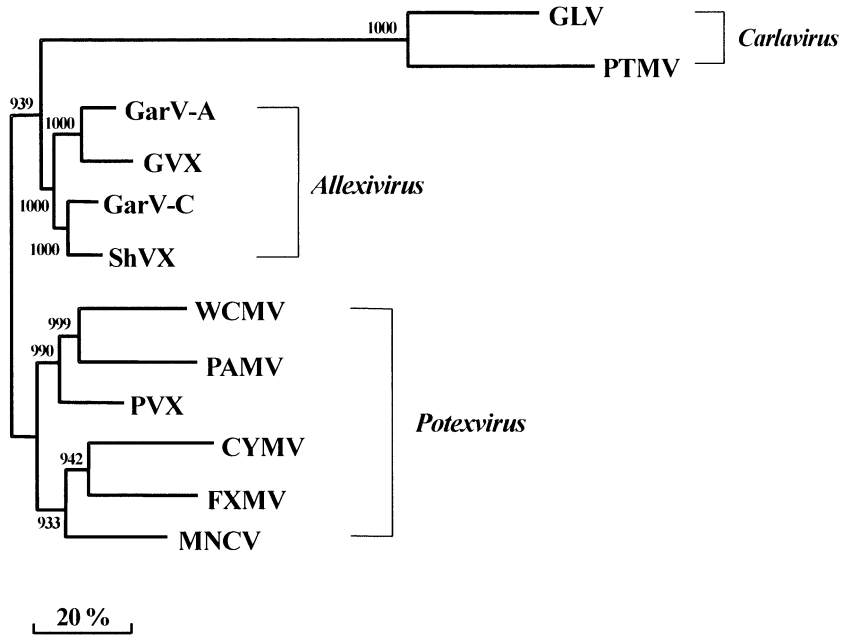

Fig. 4. Cluster dendrogram showing relationships among *Allexivirus*, *Carlavirus* and *Potexvirus* deduced from comparison of the predicted amino acid sequences of RdRP. Phylogenetic analyses were done with CLUSTAL W, then relationship dendrograms were calculated with bootstrapping (1 000 replicates) using the neighbor-joining option in CUSTAL W. Garlic latent virus (GLV) [Choi YD unpublished: DDBJ/EMBL/GenBank database accession number Z68502] and potato virus M (PVM) [31] belong to *Carlavirus*. White clover mosaic virus (WCMV) [7], papaya mosaic virus (PAMV) [17], potato virus X (PVX) [14], clover yellow mosaic virus (CYMV) [18], Foxtail mosaic virus (FXMV) [2] and narcissus mosaic virus (NMCV) [32] are members of *Potexvirus*. All horizontal distances are proportional to sequence differences indicated by inset scale, but vertical distances are arbitary. Numbers adjacent to nodes are bootstrap scores (out of 1 000 replicates)

Highly conserved regions were detected in the 5′ and 3′ non-coding sequences (Fig. 1). Microcomputer software GENETYX-WIN (Software Development Co., Tokyo, Japan) predicted that these regions possibly form stem-loop structures (data not shown). Such secondary structures would be important in the replication of viral genomic RNA as well as in the translation of the viral replicase gene.

Lastly, we conclude that the GarV-type viruses are members of a new virus genus, *Allexivirus*. The molecular data presented in this report together with recent findings on the physical, biological properties of the viruses support this conclusion.

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