SOME MOLECULAR PROPERTIES OF SEVERAL ISOLATES OF *Apple chlorotic leaf spot virus* FROM BULGARIA

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A B S T R A C T

Some molecular properties of *Apple chlorotic leaf spot virus* (ACLSV) isolates, infecting fruit trees in collection and commercial orchards in Bulgaria, were studied. Sequence analysis was performed on the coat protein (CP) and movement protein (MP) coding regions for isolates from peach (R2D43, R1D2P-l), sweet cherry (P1R9D9, P1R6D4) and apple trees (P10R1D3), in order to determine the sequence variability of ACLSV isolates. The similarity in the percentage figures of the sequence variability was between 81.1 and 99.8%; the highest being between the isolates R1D2P-l and P1R6D4, and the lowest between the isolates R2D43 and P1R6D4. Sequences of the studied isolates were also compared with those of genome fragments of ACLSV isolates published in the GenBank database. To check the electrophoretic mobility and CP of six isolates of ACLSV, the following were subjected to Western blotting analysis; P1R9D9, P1R6D4, from sweet cherry, and R2D41, R2D43, R2D49, R1D2P-l from peach. Two different migration rates were found for the CP of these isolates. The coat protein of the R1D2P-l isolate migrated slower than the CP of other isolates.

Key words: ACLSV, isolates, sequencing, Western blotting

INTRODUCTION

Apple chlorotic leaf spot virus (ACLSV), a type member of the *Trichovirus* genus (Martelli et al., 1994) family *Flexiviridae* (Adams et al., 2004), is a common virus of most fruit trees of the *Rosaceae* family, which include apple, pear, quince, sweet and sour cherry, peach, plum and

apricot (Lister, 1970; Németh, 1986). Strains and isolates of ACLSV differ in biological, serological, physical, biochemical and molecular properties. The symptom severity depends largely on plant species and virus strains. Studies were conducted on the detection, identification and properties of different strains of the ACLSV from several fruit tree species occurring in Poland, (Cieślińska et al., 1995; Cieślińska, 1998; Malinowski et al., 1998).

In this paper, we report the characterization of different ACLSV isolates from Bulgaria, based on the analysis of electrophoretic mobility and genome sequence of CP and MP fragments.

MATERIAL AND METHODS

The following numbers of ACLSV isolates were studied; four from peach (R2D41, R2D43, R2D49 and R1D2P-l), two from sweet cherry (P1R9D9 and P1R6D4) and an isolate from apple (P10R1D3). The ACLSV infection was confirmed by positive reaction in enzyme-linked immunosorbent assay (ELISA) using specific antisera (Loewe Biochemica) (data not included). The biological properties of these isolates were previously investigated (Borisova and Yordanova, 2006). The RNA was isolated from phloem tissue using a silica capture method (Boom et al., 1990), and amplified by reverse transcription – polymerase chain reaction (RT-PCR). Primer sets ACLSV-s/ACLSV-as (Menzel et al., 2002), which amplify a 677 bp fragment overlapping the movement protein and CP genes, was used for RT-PCR. The cDNA fragments of P1R9D9 and P1R6D4 isolates from sweet cherry were purified from agarose gel using the QIA quick gel extraction kit (Qiagen) and sequenced directly. cDNA fragments of the other isolates (R2D43 and R1D2P-l from peach, and P10R1D3 from apple), were cloned into bacterial vector pCR 2.1-TOPO using TOPO TA Cloning (Invitrogen) kit and sequenced using universal primers. The sequences were analysed using the Lasergene (DNASTAR) computer program. The divergence between the published sequences of ACLSV and those of the isolates from Bulgaria was investigated. Database searches were conducted using BLAST facility of NCBI. Multiple alignments were performed using CLUSTAL W (Thompson et al., 1994). The following viral sequences, published in the GenBank database, were used for comparison; AJ586629 (apricot isolate Apr 110), AJ586624 (apple isolate M 54), AJ586638 (apple isolate M139) and AJ586640 (apple isolate MP-CI).

The virus CP of six ACLSV isolates was examined by SDS-PAGE (Laemmli, 1970), followed by Western blotting (Hammond, 1987). Phloem tissue from healthy and ACLSV infected trees were ground in a mortar with 4 volumes of 0.05 M Tris-Cl, pH 6.8 buffer containing 10% SDS; 0.1% bromophenol blue and 0.05% 2-mercaptoethanol. The homogenate was centrifuged (5 minute at 3000 rpm) and boiled for 5 minutes at 95°C. Then, samples were run on 12% SDS-PAGE, followed by electro blotting on

nitrocellulose membranes, (Immobilon P Millipore, USA), using a semi-dry transfer unit (Pharmacia). Viral CP was serologically visualised using polyclonal antibody specific to the Polish plum isolate SX/2 of ACLSV, conjugated with alkaline phosphatase. A mixture of Fast Red RC Salt (Sigma), and Naphtol AS-TR phosphate (Sigma) in 0.2 M Tris-CL buffer at a pH of 8.2, was used as a substrate.

RESULTS AND DISCUSSION

RT-PCR with the primer sets ACLSV-s/ACLSV-as, resulted in products of the expected size amplified on the matrix of RNA of all seven ACLSV isolates studied. No specific bands were observed on agarose gel for the RT-PCR product on RNA matrix isolated from a healthy peach (negative control, Fig. 1). Sequence analysis of the coat protein (CP) and movement protein (MP) coding regions of isolates from peach (R2D43, R1D2P-l), sweet cherry (P1R9D9, P1R6D4) and apple trees (P10R1D3) and corresponding regions of ACLSV isolates sequences published in GenBank database has been performed to determine the variability of ACLSV isolates (Tab. 1). The similarity in the percentage figures among the five investigated isolates was between 81.1 and 99.8%, the highest being between the isolates R1D2P-l and P1R6D4, and the lowest between isolates R2D43 and P1R6D4. Previous reports have shown that ACLSV genomes show a high variability between different isolates (Candresse et al., 1995; Pasquini et al., 1998; Krizbai et al., 2001; Al Rwahnih et al., 2004). The nucleotide sequence, of a genome fragment of P1R9D9 isolate from sweet cherry, had a similarity match of 92.1% with that of the P10R1D3 isolate from apple, but only 85.2% compared to that of the P1R6D4 isolate from sweet cherry. Similarity between R1D2P-1 and R2D43 isolates from peach was only 81.6%. The R2D43 isolate shared the lowest similarity at the nucleotide sequence level, at between 81.1 and 83.6%, compared to the sequences of corresponding regions from other investigated isolates. The similarity in the percentage figures for this particular region, between the genome fragment of the R2D43 isolate and ACLSV sequences published in the GenBank database, was highest with the apple isolate MP-CI (AJ586640) and lowest with the apricot isolate Apr 110 (AJ586629). The nucleotide sequence of the genome fragment from the apricot isolate Apr 110 showed the highest similarity, compared with that of the corresponding sequence from the peach isolate R1D2P-l, at 96%; the lowest being from the peach isolate R2D43 at 81.2%. The sequence of the CP and MP regions of the peach isolate R1D2P-l was the same at 96%, compared with that of the corresponding sequence from the apple isolate MP-CI (AJ586640), and only 81.3% with the isolate from Apr 110 (AJ586629).

Western blotting analysis revealed two CP electrophoretic mobility rates (Fig. 2). The CP of the peach isolate R1D2P-1 migrated slower than those of

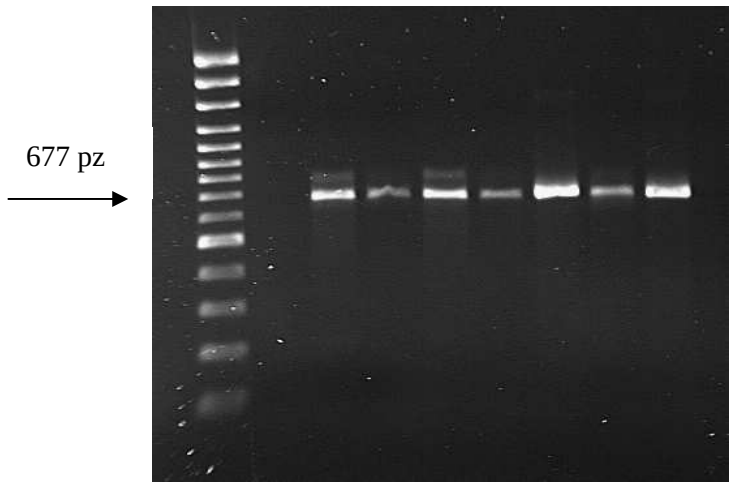

Figure 1. Reverse transcription-polymerase chain reaction on the matrix of RNA from Bulgarian ACLSV isolates using ACLSV-s /ACLSV-as primers. Lane M - 100 bp DNA ladder; 1 – healthy peach; 2 – R2D41; 3 – R2D43; 4 – R2D49; 5 – R1D2P-l; 6 – P1R9D9; 7 – P1R6D4; 8 - P10R1D3

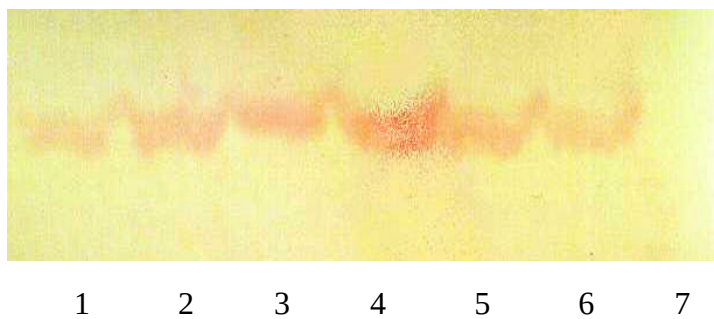

Figure 2. Western blotting analysis of phloem tissue extracts from plants infected with various ACLSV isolates. Lane 1 – R2D41; 2 – R2D43; 3 – R1D2P-l; 4 – R2D49; 5 – P1R9D9; 6 – P1R6D4; 7 – healthy peach

the other ACLSV isolates. The various mobility rates of the CP of different ACLSV isolates were described previously (Malinowski et al., 1998; Pasquini et al., 1998; Al Rwahnih et al., 2004). Studies of Italian strains led these authors to classify them into three main groups and suggested that the differences in their electrophoretic mobility were not a result of the size of CP but of the variation in amino acid composition (Pasquini et al., 1998).

Table 1. Nucleotide sequence homology of RT-PCR amplified fragments from Bulgarian ACLSV isolates compared with other known sequences

Isolate	Similarity [%]								
	R2D43	R1D2P-l	P1R9D9	P1R6D4	P10R1D3	AJ586624	AJ586638	AJ586640	AJ586629
R2D43	100	81.6	83.6	81.1	82.7	83.8	84.1	85.4	81.2
R1D2P-l		100	85.7	99.8	84.9	84.7	83.4	81.3	96.0
P1R9D9			100	85.2	92.1	92.1	93.4	85.4	85.1
P1R6D4				100	84.4	84.2	82.9	80.7	95.8
P10R1D3					100	92.7	91.1	86.3	84.6
AJ586624						100	93.0	87.3	84.2
AJ586638							100	86.7	83.7
AJ586640								100	81.3
AJ586629									100

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NIEKTÓRE WŁAŚCIWOŚCI MOLEKULARNE KILKU IZOLATÓW WIRUSA CHLOROTYCZNEJ PLAMISTOŚCI LIŚCI JABŁONI POCHODZĄCYCH Z BUŁGARII

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S T R E S Z C Z E N I E

Badano właściwości molekularne izolatów ACLSV z Bułgarii pochodzących z brzoskwini (R2D43, R1D2P-l), czereśni (P1R9D9, P1R6D4) i jabłoni (P10R1D3). W tym celu stosowano analizę sekwencji nukleotydowej regionów kodujących genów białka płaszcza i białka transportowego. Podobieństwo sekwencji nukleotydów pomiędzy badanymi izolatami wynosiło 81,1-99,8%. Najwyższe podobieństwo wykazano pomiędzy izolatami R1D2P-l z brzoskwini i P1R6D4 z czereśni, najniższe zaś pomiędzy R2D43 i P1R6D4. Sekwencje nukleotydowe badanych izolatów ACLSV porównywano także z analogicznymi sekwencjami fragmentów genomu izolatów wirusa dostępnymi w bazie GenBank. Metodą Western blotting badano mobilność elektroforetyczną białka płaszcza (CP) izolatów P1R9D9, P1R6D4 z czereśni oraz R2D41, R2D43, R2D49 i R1D2P-l. Stwierdzono, że białko płaszcza izolatu R1D2P-l migrowało wolniej w polu elektrycznym od CP pozostałych badanych izolatów.

Słowa kluczowe: ACLSV, izolaty, sekwencjonowanie, Western blotting