MOLECULAR CHARACTERIZATION OF CZECH AND CHINESE LEEK YELLOW STRIPE VIRUS ISOLATES FROM GARLIC

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Summary. – Using an RT-PCR specific for nuclear inclusion b (NIb) and coat protein (CP) genes of Leek yellow stripe virus (LYSV) we detected two Czech (LYSV-5CZ and LYSV-22CZ) and one Chinese (LYSV-16) isolate of LYSV in garlic plants. The RT-PCR products were cloned and sequenced. Phylogenetic analysis based on deduced amino acid sequence of NIb-CP region placed the Czech isolates in the group I and the Chinese isolate in the group II of LYSV.

Key words: coat protein gene; Leek yellow stripe virus; nuclear inclusion b gene; phylogenetic analysis; RT-PCR; sequence analysis

Introduction

LYSV (the species *Leek yellow stripe virus*, the genus *Potyvirusi)* has flexuous, filamentous particles of about 820 nm in length, which contain positive-sense single-stranded RNA. The virus is transmitted by aphids in non-persistent manner and by inoculation with the sap.

Viral diseases of garlic (*Allium sativum* L.) are spread world-wide and cause crop losses as well as deterioration of quality of garlic. LYSV, Onion yellow dwarf virus (OYDV, the genus *Potyvirus*), Garlic common latent virus (GarCLV, the genus *Carlavirus*), Shallot latent virus (SLV, the genus *Carlavirus*), and Garlic mite-borne filamentous virus (GarMbFV, the genus *Allexivirus*) are most frequently occurring viruses of *Allium* species. LYSV infects garlic, leek (*Allium porum*) and other *Allium* species, and represents a virus of great economic importance. A number of reports have described the incidence and epidemiology of LYSV in Greece (Dovas *et al.*, 2001), Italy (Dovas and Volvas, 2003), Brazil (Daniels, 1999; Fajardo *et al.*, 2001), Argentina (Conci *et al.*, 2002), Japan (Takaichi *et al.*, 1998, 2001), and the USA (Pappu *et al.*, 2005).

Only a few data concerning the genomic variability of LYSV isolates of different geographical origin have been reported so far (Chen *et al.*, 2002; Takaki *et al.*, 2005; Tsuneyoshi *et al.*, 1998). In contrast to the Asian LYSV isolates originating mainly from China and Japan, only a limited data on European LYSV isolates are available in databases.

LYSV isolates are serologically related to OYDV, Shallot yellow stripe virus (SYSV), and WOYSV (van Dijk, 1993; Tsuneyoshi *et al.*, 1997, 1998; van der Vlugth *et al.*, 1999). Also Garlic yellow streak virus (GYSV), reported from New Zealand (Mohamed and Young, 1981), is distantly related to LYSV. Based on amino acid sequence of CP, various LYSV isolates show a 78–100% identity (Chen *et al.*, 2001, 2002; Lunello *et al.*, 2002). Phylogenetically, based on amino acid sequences, LYSV isolates cluster in two groups (Chen *et al.*, 2002). As the import of Chinese garlic and 'Russian' garlic (*Allium sativum* ssp. *longicuspis)* represents a risk for spread of new LYSV strains in Czech Republic, it is necessary to identify and characterize them.

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Abbreviations: CP = coat protein; DAS-ELISA = double-antibody sandwich ELISA; GarCLV = Garlic common latent virus; GarMbFV = Garlic mite-borne filamentous virus; GYSV = Garlic yellow streak virus; LYSV = Leek yellow stripe virus; SYSV = Shallot yellow stripe virus; NIb = nuclear inclusion b; OYDV = Onion yellow dwarf virus; SLV = Shallot latent virus; WOYSV = Welsh onion yellow stripe virus

In this report, based on the NIb-CP genes sequence, we describe the position of the two Czech and one Chinese LYSV isolate in the phylogenetic tree of LYSV isolates so far available.

Materials and Methods

Virus isolates. All three LYSV isolates were obtained from naturally infected garlic plants: LYSV-5CZ (Czech) from native Czech cv. Prim, LYSV-22CZ (Czech) from a new Czech selection K556, derived from regional Kazakhstan cv. Džambul, and LYSV-16 (Chinese) from an unknown cv. from Shan Dong Province, southeastern China, recently imported into Czech Republic. The presence of the virus in garlic plants was confirmed by DAS-ELISA using a LYSV detection kit (Bioreba, Switzerland).

RT-PCR. Total RNA was extracted from fresh garlic leaves by means of TRI REAGENT® (Sigma). Using the 2L1 and 2R1 primers the NIb-CP LYSV gene region of about 1020 nts (nt 8507–9526, Acc. No. AJ307057) was amplified. The RT-PCR protocol of Takaichi *et al.* (1998) was modified as follows. The RT step was carried out in a reaction mixture (25 µl) consisting of 5 µl (0.5 µg) of total RNA, 0.5 µl of oligo $(dT)_{18}$ primer (20 pmoles/µl), 5.0 µl of AMV RT 5x buffer, 2.0 µl of 10 mmol/l dNTPs, 5 U of AMV reverse transcriptase, 0.5 µl of 40 U/µl RNasin® (RNase inhibitor) (Promega), and 11.5 µl of deionized water. The reaction ran at 42°C for 60 mins. The PCR step consisted of initial denaturation at 92°C for 5 mins and 40 cycles of 92°C/30 secs, 62°C/30 secs, and 72°C/60 secs. The PCR products were analyzed by 2% agarose gel electrophoresis in standard manner.

Cloning, sequencing, and phylogenetic analysis. The obtained fragments were cloned into pGEM-T plasmid (Promega). Three clones of each isolate were sequenced using the BIG DYE Sequencing Terminator kit and ABI PRISM 310 sequencer (both from Applied Biosystems). Each insert was sequenced from both directions. The obtained sequences were checked for homologous sequences in GenBank using the BLAST program (Altschul *et al*., 1990) and were aligned with corresponding sequences of other LYSV isolates available in GenBank using the Clustal W program (http://www.ddbj.nig.ac.jp). OYDV, SYSV, and WOYSV were used as the outgroup. Based on deduced amino acid sequences of NIb and CP of LYSV isolates, a phylogenetic tree was constructed using the neighbor-joining method; the bootstrap option was run with 1000 resamplings. The tree was visualized with the TreeView program ver. 1.6.1 (Page *et al.*, 1996).

Results and Discussion

Sequence analysis of LYSV isolates

The NIb-CP gene region of two Czech (1023 nts) and one Chinese (1020 nts) LYSV isolate was amplified by RT-PCR, cloned and sequenced. The obtained sequences were deposited in GenBank under Acc. Nos. DQ299380 (LYSV-5CZ, Czech), DQ299381 (LYSV-22CZ, Czech), and DQ299382 (LYSV-16, Chinese). By comparing these sequences with those of other LYSV isolates, the three isolates were identified as LYSV. A high identity (91–94%) was found between the two Czech isolates and the LYSV isolates of phylogenetic group I. On the other hand, the Chinese isolate showed a 84–88% identity with the LYSV isolates of phylogenetic group II. The two Czech isolates were highly identical (91.5%), but clearly different from the Chinese isolate, showing identities of 76% and 77.3%, respectively.

Furthermore, multiple alignment of the NIb-CP sequences of the two Czech and other LYSV isolates showed a high DNA polymorphism, the highest at the 5'-end and the lowest at the 3'-end of CP gene (data not shown).

Phylogenetic analysis of LYSV isolates

A phylogenetic tree, based on the deduced amino acid sequences of the NIb-CP region of LYSV isolates available in GenBank, showed that the two Czech isolates clustered within group I, while the Chinese isolate within group II (Fig. 1).

The two Czech isolates were identical to 94%, but, compared to the Chinese isolate (LYSV-16), they were identical only to 80.5% and 84%, respectively. In considering the identity within whole group I, it was 93.7–96.6% for the two Czech isolates. Similarly, in considering the identity within whole group II, it was 83.3–88.4% for the Chinese isolate.

In general, a high sequence variability among different LYSV isolates is well known (Fajardo *et al.*, 2001; Chen *et al*., 2002; Tsuneyoshi *et al.*, 1998). A comparison of nucleotide and/or amino acid sequences of CP has led to the partition of LYSV isolates into two phylogenetic groups (Chen *et al.*, 2002; Tsuneyoshi *et al.*, 1998). Recently, Takaki *et al.* (2005) have proposed a distinct (third) group for the isolates from northern Japan that are closely related to LYSV isolates from leek. Our findings presented here could represent independent evolution of geographically distant virus isolates and/or selection pressure; implying adaptation of the virus (Fajardo *et al.*, 2001; Tsuneyoshi *et al.*, 1998).

In the phylogenetic tree presented in this study, the group I includes 9 garlic or leek LYSV isolates originating from Europe, Japan, Argentina and New Zealand. These isolates show nucleotide and amino acid identities of 76.2–99 % and 82.6–99.1%, respectively. The group II includes 12 garlic LYSV isolates originating from China, Japan, and Taiwan. These isolates display similar identities, namely 73.1–100% for nucleotides and 82.7–100% for amino acids.

All the three LYSV isolates under study contain a DAG motif in CP. In contrast, a variability was found in the sequence of the cleavage site between NIb and CP. FVFQ/AN was found for the Czech isolates, while FEYQ/AG for the Chinese

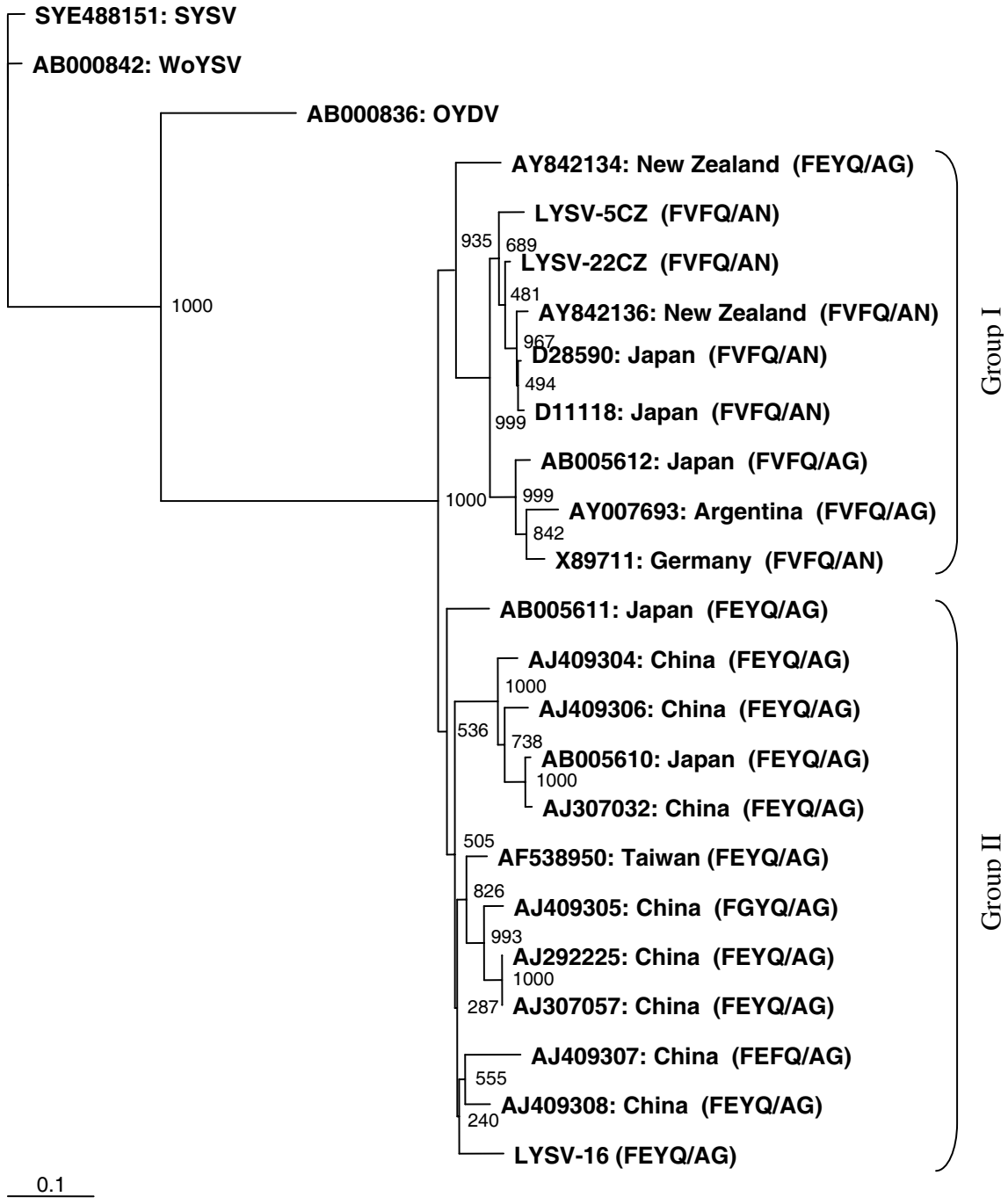

Fig. 1

Phylogenetic tree of LYSV isolates based on amino acid sequence of of NIb-CP region

The NIb-CP region consisted of 319 aa. NIb/CP protease cleavage site sequences are in brackets. The scale bar represents a phylogenetic distance of 1%. The numbers on the branches are bootstrap values.

isolate. This finding is roughly consistent with known differences in these sites in two groups of LYSV isolates (Fig. 1). FVFQ/AN and FVFQ/AG cleavage sites predominate in group I. FVFQ/AN is typical for isolates from garlic, while FVFQ/AG is characteristic for isolates from leek with one exception (Acc. No. AY842134). FEYQ/AG is typical for group II with two exceptions (Acc. No. AJ409305 with FGYQ/AG and Acc. No. AJ409307 with FEFQ/AG).

Summing up, (i) the partial genome sequences of two Czech LYSV isolates represent first sequences of European LYSV isolates from garlic, (ii) the Czech isolates clustered within group I, while the Chinese isolate within group II of LYSV in the phylogenetic tree, and (iii) the Czech isolates clearly differed from typical LYSV isolates from China and Japan.

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