THESIS OF DOCTORAL DISSERTATION

DISTRIBUTION, PERSISTENCE AND MOLECULAR CHARACTERIZATION OF CRYPTIC AND ENDORNAVIRUSES

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BACKGROUND AND OBJECTIVES

The majority of recognized plant viruses were discovered as pathogenic parasites that cause disease in cultivated agricultural plants. However it is now well known that many viruses cause only very mild or no disease symptoms at all in some hosts. Furthermore, recent results show that certain viruses are necessary for or can contribute to survival of their plant hosts under stress conditions, such as, for example, infection of RNA viruses can improve plant tolerance to abiotic stress (Xu *et al*., 2008).

Our group is investigating the genomic organisation and molecular features of cryptic viruses and endornaviruses. These viruses persist within the plant hosts throughout their life without causing any symptoms and have a double-stranded RNA (dsRNA) genome. Cryptic viruses belong to the *Alphacryptovirus* and *Betacryptovirus* genus of *Partitiviridae* family (Ghabrial *et al*., 2005), while endornaviruses belong to *Endornavirus* genus of *Endornaviridae* (Fukuhara *et al*., 2006). The organization of the genome of these viruses is different. While the genome of endornaviruses consists of one high molecular weight (larger than 10 kbp) dsRNA, cryptic viruses have a small bi- or tripartite genome with dsRNA-segments ranging from 1–4 kbp in size. Cryptic and endornaviruses have several further peculiar features: they can not be transferred by usual virological methods and are believed to be spread only by seed and pollen, the concentration of virus particles is usually very low, and more than one virus can coexist in the same host. These features make their study rather challenging, but very interesting from the theoretical and practical point of view.

Cryptic- and endornaviruses have been often found by chance, e.g. when researchers were looking for unknown viruses by searching for high molecular weight viral dsRNA or for virus particles. Because of the lack of a broad-range, systematic analysis of their distribution, at present it is not possible to judge how many different cryptic and endornavirus persist in Nature. Cryptic viruses are widespread in many plant genera, e.g. in *Beta* genus, and may be present in almost every member of the subspecies/cultivar without being detected (Xie *et al*., 1989). In the *Beta* genus three different *Beet cryptic viruses* (BCV1, -2, -3) have been identified, which can be distinguished serologically and on the basis of the molecular weights of their genomic RNAs (Antoniw *et al*., 1986).

The primary aim of our work was to determine the genomic sequence of two cryptic viruses, *Beet cryptic virus 1* and *-2,* and to analyse prevalence and persistence of cryptic viruses and endornaviruses in selected plant genera. Characterisation of the whole genomic

sequence is crucial for prediction of the encoded protein sequences, for identification of common structural features of 5'- and 3'-UTRs and for analysing sequence similarity to other members of *Partitiviridae* to obtain more exact data about the evolutionary origin of cryptic viruses.

Examination of the occurrence of cryptic viruses and endornaviruses helps to clarify how frequently a given cryptovirus occurs in a genus, but it may also lead to identification of new putative viruses and to better understanding of virus evolution and of host-virus relationship. It was known from the literature that endorna- and cryptoviruses can occur in *Capsicum annuum* (Valverde *et al*., 1990). To clarify whether the same - or other - symptomless dsRNA-viruses also occur in other *Capsicum* species and whether the distribution of dsRNA-pattern correlates with the *Capsicum* phylogenetic tree established on the basis of microsatellite markers (Nagy *et al*., 2007), we analysed the dsRNA-pattern of more than 60 cultivars of eight *Capsicum* species.

Long-term cultivation of *in vitro* propagated plants is a dramatic change of environment compared to the natural life cycle of the plant. Therefore, we investigated the effects of *in vitro* culture conditions and of thermotherapy on the survival of cryptic viruses in *Dianthus* species and in *Beta vulgaris*.

MATERIALS AND METHODS

Plant materials

The double-stranded RNA pattern, which can give information about the presence of the endogenous cryptic viruses and endornaviruses was investigated in a Hungarian collection of *Capsicum* species. The collection consisted of 63 cultivars of eight pepper species; the samples were kindly provided by Gábor Csilléry and István Nagy. To analyse the influence of long term tissue culturing on the survival of cryptic viruses, *in vitro* propagated carnation and beet plants were examined. The *in vitro* cultivated diploid *Beta vulgaris* lines were started from sterilized seeds of sugar beets or fodder beets and were aseptically grown at Beta Research Ltd. for 5-7 years. The different *Dianthus*-species were grown aseptically for 16 years at the Óbuda Nursery Ltd., Budapest, Hungary. Occurrence of BCVs were analysed in 28 sugar beet cultivars representing the sugar beet cultivars field-grown in Central Europe in 2004. Mixed leaf samples from each cultivar of this collection were screened for the presence

of BCV dsRNA by immunoblotting.

Isolation of nucleic acids

RNA was isolated using RNeasy Plant Mini Kit (Qiagen) or Plant Total RNA miniprep kit (Viogene), according to the manufacturer's instructions. Total nucleic acids were extracted with phenol/chloroform. For immunoblot analysis unfractionated extracts were used. For cDNA-synthesis and cloning dsRNA were further purified by CF-11 column chromatography, followed by digestion with RNase-free DNase I and with RNase A under high salt conditions (450 mM NaCl, 45 mM sodium citrate (pH 7.2).

Detection of dsRNAs by dsRNA-specific monoclonal antibody

Prior to blotting, 30-50 µg total nucleic acid extract was separated in non-denaturing 5 % PAA-TBE gels. Viral dsRNA was detected by immunoblotting using the dsRNA-specific monoclonal antibodies K2 or J2. Both antibodies specifically recognise dsRNA independent of its nucleotide composition and sequence and do not cross-react with short helices present in single-stranded RNA. The method allows detection of ≥60 pg dsRNA/band.

cDNA synthesis and cloning

Purified dsRNA was either used directly as template for cDNA synthesis or was tailed first on its 3' end by poly(A) polymerase according to the manufacturer's instructions. In the initial experiment random hexanucleotide primers linked to an universal oligonucleotide (5' GCCGGAGCTCTGCAGAATTCNNNNNN) were used to obtain cDNA for cloning. When dsRNA-derived sequences became available from the initial experiment, sequence-specific primers were designed for further cloning. To determine the 5'- and 3'-ends poly(A)-tailed dsRNA templates and oligonucleotide linked oligo(dT) (5' GCTCTGCAGAATTCTTTTTTT TTTT) and dsRNA-specific primers were used for cDNA-synthesis and amplification. 100 ng dsRNA was denatured at 99 ºC for 5 min in the presence of 1.5 % dimethyl-sulfoxide, 0.2 µM primers and then chilled for 3 min in ice. First strand cDNA synthesis was carried out in a volume of 20 µl containing the heat denaturated mixture, 1x first strand buffer, 200 µM dNTP, 5 mM DL-dithiothreitol (DTT) and 0.75 U Thermoscript reverse transcriptase at 50 °C 90 min. After second strand synthesis and purification cDNA was amplified by PCR. Amplification of cDNA was done in 25 µl containing 5 µl of cDNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTP, 0.2 µM forward and reverse primers and 0.04 U *Taq* polymerase. All

PCR products were cloned using the pGEM3z, pGEM7zf, pCR2.1-TOPO or pTZ57R/T plasmids.

Sequence analysis

Sequences were compiled, analysed and aligned by the Vector NTI Advance 10 software (Invitrogen) and Chromas Lite version 2.01. Database searches were performed with the NCBI Blast program. The sequence-specific primers were designed using Primer3 software. Phylogenetic analyses were conducted using MEGA4 software.

Analysis and purification of virions

Virus particles from sugar beet cultivar 'Mars' were purified as described by Kühne and Stanarius (IDÉZET). Virions were visualized after negative staining by electron microscopy. Viral nucleic acids were extracted from purified virions using proteinase K in 0.5 % SDS, 10 mM Tris-HCl (pH 8.0), 5 mM EDTA. They were separated by non-denaturing gel electrophoresis in 5 % PAA-TBE gels and visualised by dsRNA-specific immunoblotting. Virions were treated in reducing SDS-sample buffer and viral protein was visualized after separation in 12.5 % SDS-PAA gels according to Laemmli using Coomassie Brillant Blue G-250 for staining. Protein bands visible after Coomassie staining were cut out from the SDS-gel and subjected to in gel digestion. To find out whether the translated sequences of the putative viral coat protein(s) are indeed localised in viral particles, samples were analysed by mass spectrometry (LC-MS/MS and MALDI-TOF) by the Proteomic Research Group of Biological Research Center, Szeged. Virus proteins were also analysed by Western blotting, the BCV1-, BCV2-specific rabbit antiserum was provided by T. Kühne.

RESULTS AND DISCUSSION

The species-dependent occurrence of cryptic- and endornaviruses in *Capsicum* species

The presence of endornaviruses and cryptoviruses in *Capsicum annuum* has been reported previously (Boccardo *et al*., 1987). We investigated whether these endogenous dsRNA-viruses also occur in other *Capsicum* species and whether the dsRNA pattern of the putative genomic dsRNAs parallels the *Capsicum* phylogenetic tree. Samples were taken from a collection of eight pepper species (*C*apsicum *annuum*, *C. baccatum*, *C. chacoense*, *C. chinense*, *C. eximium*, *C. frutescens*, *C. praetermissum*, *C. pubescens*).

High-molecular weight dsRNA (>10 kbp), which may represent the genomic dsRNA of endornavirus was detected in every species of first the branch of the *Capsicum* phylogenetic tree (Nagy *et al*., 2007); in *C. annuum, C. chinense, C. frutescens, C. praetermissum, C. baccatum* var. *baccatum* and var. *pendulum* species. DsRNA was detected in every cultivar, except in *C. annuum*, where we found dsRNA in four cultivars, while six further cultivars were negative. The second branch of the *Capsicum* phylogenetic tree is composed of *C. eximium, C. pubescens* and *C. chacoense* species. Here we detected high-molecular weight dsRNA only in *C. chacoense* and the molecular weight of dsRNA was lower than in the first branch; no dsRNAs could be detected in cultivars of *C. eximium* and *C. pubescens*.

For cryptic viruses, a segmented genome consisting of 2-3 dsRNA-species in the size range 1-3 kbp is characteristic. The occurrence of dsRNA genomes of cryptic viruses also parallels the *Capsicum* phylogenetic tree (Nagy *et al*., 2007). DsRNA-species of 1-4 kbp were observed in almost every species of *C. chinense*, *C. frutescens*, *C. annuum* and *C. chacoense*, but no dsRNAs in this size range could be detected in the two subspecies of *C. baccatum*, *C. eximium* and *C. pubescens*. On the basis of the length of dsRNA pairs at least 3-4 different cryptoviruses may occur in these *Capsicum* species.

Taken together our results indicate that dsRNAs probably representing the genomic dsRNA of endorna- and cryptoviruses do occur in different pepper species, and the dsRNA-pattern observed in our experiments runs parallel to the *Capsicum* phylogenetic tree established on the basis of microsatellite markers. Further experiments to prove the viral origin and to detect sequence differences between the putative genomic dsRNAs are underway.

Long term survival of cryptic viruses in *in vitro* cultivated plants

Viruses have evolved to harmonise with their host plant on which they completely depend for replication and multiplication. Therefore drastic alteration of physiological conditions (e.g. long term tissue culturing) may have a strong effect on the virus (Boccardo *et al*., 1987). We therefore analysed the influence of long term tissue culturing on the survival of cryptic viruses in *in vitro* propagated collections of beet and carnation plantlets.

Using dsRNA-specific immunoblotting we could detect the genomic dsRNA of all three *Beet cryptic viruses* in *in vitro* cultured sugar and fodder beets which had been aseptically grown for 5-7 years. Sequence-specific identification of individual dsRNA segments by RT-PCR verified the results of immunoblot analysis. Based on the BCV1- and BCV2 sequences

determined by us and on the RNA-dependent RNA polymerase sequence of BCV3 described in the literature (Xie *et al*., 1993), we developed an RT-PCR procedure, which allows sensitive and specific detection of each of these viruses. In the case of the *in vitro* grown plantlets the sequences of PCR products showed more than 99 % nucleic acid sequence identity to the "canonical" BCV1, -2, and -3 sequences.

We also analysed the occurrence of genomic dsRNA of *Carnation cryptic virus* (CarCV) in 18 different *in vitro* propagated *Dianthus* species and two members of *Dianthus caryophyllus* variety club, all originating from the Hungarian germplasm collection. The tissue cultures were initiated from sterilised seeds and the plantlets were aseptically grown for 15 years. We could detect genomic dsRNAs of CarCV in two members of *D. caryophyllus* variety club (*D. caryophyllus* 'Grenadin' and *D. caryophyllus* 'Chabaud') by dsRNA-specific immunoblotting (Lisa *et al*., 1981). In several further species dsRNA molecules of unknow origin were detected. Heat treatment is a frequently used method for eliminating viruses from plants. In case of the cryptic viruses heat treatment of shoot meristems was not sufficient to eliminate genomic dsRNA of CarCV from *D. caryophyllus* and the uncharacterized dsRNA-species from *D. gratianopolitanus*.

Our results clearly show that at least four different cryptic viruses (*Beet cryptic virus 1*, *-2*, *-3* and *Carnation cryptic virus*) are so well adapted to their hosts that they survive 7-16 years of *in vitro* culturing despite the dramatic change of the environment, i.e. they behave as true persistent plant viruses. We also showed that the coding regions of the RNA-dependent RNA polymerases genes of *Beet cryptic virus 1* and *-3* were preserved unaltered under *in vitro* conditions.

Molecular characterization of *Beet cryptic virus 1* (BCV1)

The genome of BCV1 is known to consist of two segments, dsRNA1 and dsRNA2. We have determined the complete sequence of the genome and deposited dsRNA1 (2008 bp) and dsRNA2 (1783 bp) sequences in the GenBank database under accession numbers EU489061 and EU489062.

DsRNA1 contains only one major ORF on the plus strand that encodes a predicted protein of 616 amino acid residues (72.5 kDa). The deduced amino acid sequence of the major ORF of BCV1 dsRNA1 contains characteristic sequence motifs (motifs III-VIII) found in genes of putative RNA-dependent RNA-polymerases (RdRp) of RNA viruses (Bruenn, 1993); we therefore conclude that dsRNA1 encodes the RdRp of BCV1. A comparison of the amino

acid sequence of the putative BCV1 RdRp with sequences in the NCBI database using the BLASTP algorithm revealed highly significant similarities to RdRp of viruses of the *Partitiviridae* family. The RdRp sequences of two plant cryptic viruses, namely those of *White clover cryptic virus 1* (WCCV1) and *Vicia cryptic virus* (VCV) show 81-82 % amino acid sequence identity to BCV1 without any gaps (Boccardo and Candresse, 2005a, b; Blawid *et al*., 2007). The RdRp of two further viruses, *Cherry chlorotic rusty spot associated partitivirus* (CCRSPV) and *Amasya cherry disease associated partitivirus* (ACDPV) also show more than 50 % identity (Coutts *et al*., 2004). The extremely high similarity between BCV1, WCCV1 and VCV was unexpected, because cryptic viruses are believed to be transferred only by seed and pollen, while the hosts of these viruses belong to very distantly related plant families.

The major ORF of dsRNA2 (1470 nt, corresponding to 489 amino acids) encodes the putative coat protein (CP) with an estimated molecular mass of 53.7 kDa. On the same RNA strand, two additional smaller ORFs were predicted by computer analysis, on the minus strand two further small ORFs were identified. The putative coat protein shows 55-62 % sequence identity to the putative CP of WCCV1 and VCV, respectively, and 30-36 % similarity to ACDPV, CCRSPV. The BCV1 CP sequence fits well into this group of cryptoviruses, because in addition to sequence similarity it also contains conserved amino acid stretches common to the CPs of VCV, WCCV1 ACDPV and CCRSPV.

In purified virus preparations, the major virion protein had an apparent molecular mass of ~55 kDa which corresponds well to the mass of 53.4 kDa, deduced from the nucleic acid sequence and also to the size of BCV1 CP determined earlier. Peptide sequences isolated from the 55 kDa protein band were determined by LC-MS/MS. They corresponded to the deduced amino acid sequence of the putative CP and covered 32.5 % of the whole CP sequence. These polypeptides reacted strongly with the BCV1-specific antiserum as well. Taken together our results definitively prove that dsRNA2 encodes the coat protein of BCV1 which is present as full-length protein in the virions. To our knowledge this is the first time that mass spectrometric analysis has been used to show definitively that the nucleocapsid protein of a cryptovirus and the virus-encoded sequence are identical and that the whole encoded polypeptide chain is present in the virion.

Analysis of the 5'- and 3'-untranslated regions (UTRs) revealed a very high degree of sequence similarity of BCV1 to cryptic viruses residing in very distantly related host plant species, i.e. to WCCV1 and VCV. The identity of RdRp and 5' UTR sequences is particularly

high, exceeding 80 %, but even the amino acid sequences of the more rapidly evolving CP are ~60 % identical (Strauss *et al*., 2000).

Molecular characterization of *Beet cryptic virus 2* (BCV2)

On the basis of earlier publications we also expected the *Beet cryptic virus 2* genome to consist of two segments (MW $0.94 \cdot 10^6$ and $0.87 \cdot 10^6$; Natsuaki *et a1*., 1986). However, in the course of our experiments 3 genomic dsRNA segments were identified, each of which encodes a relevant virus protein. The cause of the apparent discrepancy is the co-migration of two genomic dsRNAs during conventional gel electrophoresis. The genome of BCV2 consists of three monocistronic dsRNAs. BCV2 dsRNAs are 1598 bp (dsRNA1), 1575 bp (dsRNA2) and 1522 bp (dsRNA3) long, respectively.

DsRNA2 contains one major ORF on the plus strand that encodes a predicted protein of 475 amino acid residues (54.2 kDa). On the minus strand two further small ORFs were identified. The deduced amino acid sequence of the major ORF of BCV2 dsRNA2 contains amino acid sequence motifs conserved in the RdRp of members of the *Partitiviridae* family (Bruenn, 1993); we therefore conclude that dsRNA2 encodes the RdRp of BCV2. Multiple RdRp alignments show highly significant similarities to RdRp of other cryptoviruses (e.g. *Beet cryptic virus 3*).

DsRNA1 contains a 1281-nt long major ORF on the plus strand that encodes a predicted protein of 426 amino acid residues (49.1 kDa). The major ORF on dsRNA3 is 1182-nt long and encodes a predicted protein of 393 amino acid residues (45 kDa). However, when we estimated the molecular mass of CP by denaturing gel electrophoresis of purified BCV2 virions, in good agreement with earlier published data (Kühne *et al*., 1987) we identified two dominant protein bands with considerably lower molecular mass (~36 and ~33 kDa). Both proteins reacted strongly with BCV2-specific antisera.

To find out which one of the genomic segments encodes the virion proteins peptide sequences from both virion proteins were determined by LC-MS/MS proved. The results clearly showed that dsRNA1 encodes the ~36 kDa protein and dsRNA3 encodes the ~33 kDa protein. The peptide sequences determined from the 36 kDa protein covered 30 % of protein sequence deduced from the sequence of BCV2 dsRNA1. In case of the ~33 kDa protein the peptide sequence coverage corresponded to 35 % of the protein sequence deduced from the largest ORF of BCV2 dsRNA3. On the basis of this evidence we believe that both polypeptide chains (~36 and ~33 kDa) are present in the BCV2 virion and the CPs are

encoded by dsRNS1 and dsRNA3. On the basis of our present results we cannot account for discrepancy between the observed and deduced masses of BCV2 coat proteins.

The 5' untranslated regions (UTR) of all three BCV2 segments share a terminal region of high sequence homology (5' AGAATTA), which is not present in BCV1, but occurs in other plant cryptoviruses (e. g. *Beet cryptic virus 3*; Xie *et al.*, 1993). None of the BCV2 dsRNAs contain a poly(A) tail.

NEW SCIENTIFIC RESULTS

1. We have shown that dsRNAs, probably representing the genomic dsRNA of endorna- and cryptoviruses occur in different *Capsicum* species and that the dsRNA-pattern runs in parallel to the *Capsicum* phylogenetic tree established on the basis of microsatellite markers. High-molecular weight dsRNA, which may indicate the presence of an endornavirus, was detected in almost every cultivar of *C. annuum, C. chinense, C. frutescens, C. praetermissum, C. baccatum* var. *baccatum* and var. *pendulum*, but it was not present in *C. eximium* and *C. pubescens* plants, and in *C. chacoense* a smaller variant of HMW dsRNA was seen. In addition, in *C. chinense*, *C. frutescens*, *C. chacoense and C. annuum* cultivars one or two pairs of small dsRNA bands in the size range 1-3 kbp were also found. The latter dsRNAs probably represent genomic dsRNA of putative cryptic viruses and may indicate that new, not yet characterised cryptoviruses are present in *Capsicum.*

2. We have shown that at least four different cryptic viruses (*Beet cryptic* virus 1, -2 and -3 and *Carnation cryptic virus*) are able survive several years or even more than a decade under *in vitro* culturing conditions of their host plants.

3. We have demonstrated that the prevalence of BCV viruses has altered in sugar beet cultivars within last decade. BCV1 has become very rare and was detected in only one cultivar in a collection of 28 cultivars BCV1, whereas BCV2 was present in half of the cultivars.

4. We determined the sequence of the bipartite genome of *Beet cryptic virus 1* (BCV1) and proved that dsRNA1 encodes the viral replicase (RdRp) and dsRNA2 encodes the coat protein. We were first to observe that BCV1 sequences show high sequence identity to *Vicia cryptic virus* and *White clover cryptic virus 1*, which occur in phylogenetically

distant host plants. The high degree of sequence identity suggests that these cryptoviruses diverged considerably later than their host plants during evolution.

5. We have shown that the genome of *Beet cryptic virus 2* (BCV2) is tripartite and all genomic segments encode functional proteins. The virion is built up of two capsid proteins encoded by dsRNA1 and dsRNS3. The dsRNA2 encodes the RNA-dependent RNA polymerase.

6. We developed and introduced an RT-PCR procedure, which allows sensitive and specific detection of the three known *Beet cryptic viruses*.

LITERATURE CITED

1. JF. Antoniw, JM. Linthorst, RF. White, JF. Bol, J. Gen. Virol. **67**, 2047-2051 (1986)
2. R. Blawid, D. Stephan, E. Maiss, Arch. Virol. **152**, 1477-1488 (2007)
3. G. Boccardo, T. Candresse, Arch. Virol. **150**, 399-402 (2005a)
4. G. Boccardo, T. Candresse, Arch. Virol. **150**, 403-405 (2005b)
5. G. Boccardo, V. Lisa, E. Luisoni, RG. Milne, Adv. Virus Res. **32**, 171-214 (1987)
6. JA. Bruenn, Nucleic Acids Res. **21**, 5667-5669 (1993)
7. RH. Coutts, L. Covelli, F. DiSerio, A. Citir, S. Acikgöz, C. Hernandez, A. Ragozzino, R. Flores, J. Gen. Virol. **85**, 3399–3403 (2004)
8. T. Fukuhara, R. Koga, N. Aoki, C. Yuki, N. Yamamoto, N. Oyama, T. Udagawa, H. Hoiuchi, S. Miyazaki, Y. Higashi, M. Takashita, K. Ikeda, M. Arakawa, N. Matsumotho, H. Moriyama, Arch. Virol. **151**, 995-1002 (2006)
9. SA. Ghabrial, KW. Buck, BI. Hillman, RG. Milne, in Eigth Report of the International Committee on Taxonomy of Viruses, ed. by C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, L.A. Ball (Elsevier/Academic Press, London, 2005), pp. 580-590
10. T. Kühne, A. Stanarius, H. Kleinhempel, Arch. Phytopathol. Pflanzenschutz **23**, 95-104 (1987)
11. V. Lisa, E. Luisoni, RG. Milne, Ann. Appl. Biol. **98**, 431-437 (1981)
12. I. Nagy, A. Stágel, Zs. Sasvári, M. Röder, M. Ganal, Genome **50**, 668-688 (2007)
13. T. Natsuaki, KT. Natsuaki, S. Okuda, M. Teranaka, RG. Milne, G. Boccardo, E. Luisoni, Intervirology **25**, 69-75 (1986)
14. EE. Strauss, DK. Lakshman, SM. Tavantzis, J. Gen. Virol. **81**, 549–555 (2000)
15. RA. Valverde, DL. Gutierrez, Mexican Journal of Phytopathology **26**, 1-6 (2008)
16. RA. Valverde, S. Nameth, O. Abdallha, O. Al-Musa, P. Desjardins, JA. Dodds, Plant Science **67**, 195-201 (1990)
17. WS. Xie, JF. Antoniw, RF. White, J. Gen. Virol. **74**, 1467-1470 (1993)
18. WS. Xie, JF. Antoniw, RF. White, Plant Pathol. **38**, 527-533 (1989)
19. P. Xu, F. Chen, JP. Mannas, T. Feldman, LW. Sumner, MJ. Roossinck, New Phytol. **180**, 911-921 (2008)

MOST IMPORTANT PUBLICATIONS RELATED TO THE SUBJECT OF THESIS

Journal with impact factor

Szegő, A., Enünlü, N., Desmukh, PS., Veliceasa, D., Hunyadi-Gulyás, É., Kühne, T., Ilyés, P., Potyondi, L., Medzihradszky, K., Lukács, N. (2009). The genome of *Beet cryptic virus 1* shows high homology to certain cryptoviruses present in phylogenetically distant hosts. *Virus Genes* (DOI: 10.1007/s11262-009-0432-4). IF: 1,376 (2008)

Journal without impact factor

Ilyés, P., **Szegő, A.**, Neer, Zs., Potyondi, L., Lukács, N. (2007). A *Beta* nemzetség kriptikus vírusainak kimutatására alkalmas PCR-en alapuló eljárás kidolgozása. *Növényvédelem* **43**, 453-459.

Szegő, A., Tóth, EK., Potyondi, L., Lukács, N. (2005). Detection of high molecular weight dsRNA persisting in *Dianthus* species. *Acta Biologica Szegediensis* **49**, 17-19.

Congress full papers in English

Szegő, A., Tóth, E., Potyondi, L., Lukács, N. (2004). Long term survival of cryptic viruses in aseptically grown *in vitro* propagated plants. 5th IVCHB Conference. 12-17 September, Debrecen, Hungary. 505-511 p.

Szegő, A., Tóth, E., Potyondi, L., Lukács, N. (2004). Detection of *Carnation cryptic virus* in wild *Caryophyllaceae* and in *in vitro* cultured *Dianthus* species. International Conference on Horticulture Post-graduate. 17-19 November, Lednice, Czech Republic. 217-222 p.

Abstract in English

Szegő, A., Enünlü, N., Veliceasa, D., Desmukh, PS., Szilák, L., Lukács, N. (2008). Complete nucleotide sequence and genome characterization of two *Beet cryptic viruses*: BCV1 and -2. 9th International Congress of Plant Pathology. 24-29 August, Torino, Italy. *Journal of Plant Pathology* **90**, S2.203.

Szegő, A., Enünlü, N., Veliceasa, D., Desmukh, PS., Szilák, L., Lukács, N. (2008). Cloning and molecular characterization of double-stranded RNA of two *Beet cryptic viruses* (BCV1 and -2). First Symposium on Horticulture in Europe. 17-20 February, Vienna, Austria. 76 p.

Szegő, A., Árvay, K., Vajda, GB., Sun, HM., Hu, S., Dalmadi, Á., Nagy, I., Csilléry, G., Lukács, N. (2008). Putative endogenous dsRNA-viruses in *Capsicum* species. First Symposium on Horticulture in Europe. 17-20 February, Vienna, Austria. 271 p.

Szegő, A., Enünlü, N., Veliceasa, D., Desmukh, PS., Sun, H., Szilák, L., Lukács, N. (2007). Sequence comparison of RNA-dependent RNA-polymerases indicates different origin of *Beet Cryptic Virus 1* and *-2*. 15th International Congress of the Hungarian Society for Microbiology. 18-20 July, Budapest, Hungary. *Acta Microbiologica et Immunologica Hungarica* **54**, 124.

Szegő, A., Vajda, G., Árvay, K., Hu, S., Nagy, I., Lukács, N. (2006). Occurrence and distribution of putative endogenous dsRNA-viruses in *Capsicum* species. Non-specific and Specific Innate and Acquired Plant Resistance Conference. 31 August-3 September, Budapest, Hungary. 71 p.

Szegő, A., Vajda, G., Hu, S., Nagy, I., Lukács, N. (2006). Occurrence of double-stranded RNAs indicating the presence of putative cryptoviruses and endornaviruses in *Capsicum* species. 8th Conference of the European Foundation for Plant Pathology and the BSPP presidential Meeting. 13–17 August, Copenhagen, Denmark. 116 p.

Congress full papers in Hungarian

Szegő, A., Vajda, G., Kiss-Bába, E., Nagy, I., Lukács, N. (2006). Putatív endogén duplaszálú RNS-vírusok paprikafajokban. XVI. Keszthelyi Növényvédelmi Fórum. Január 26-27., Keszthely. 95-99 p.

Abstract in Hungarian

Albert, Zs., **Szegő, A.**, Ladányi, M., Lukács, N. (2009). A növényi kriptikus vírusok rokonsági viszonyainak vizsgálata a kodonhasználat analízisével. 55. Növényvédelmi Tudományos Napok. Február 23-24., Budapest. 37 p.

Szegő, A., Albert, Zs., Enünlü, N., Ladányi, M., Veliceasa, D., Lukács, N. (2008). Növényi kriptovírusok molekuláris jellemzése és evolúciós eredetük vizsgálata. Fiatal agrárkutatatók az élhető Földért. November 24., Budapest. 28 p.

Szegő, A., Enünlü, N., Veliceasa, D., Desmukh, PS., Szilák, L., Lukács, N. (2008). A *Beet Cryptic Virus 1* és *–2* cukorrépa kriptikus vírusok dsRNS genomjainak molekuláris jellemzése. 54. Növényvédelmi Tudományos Napok. Február 27-28., Budapest. 30 p.

Ilyés, P., **Szegő, A.**, Lukács, N. (2006). A *Beta* nemzetség kriptikus vírusainak kimutatására alkalmas immunológiai ill. PCR-en alapuló eljárások kidolgozása. 53. Növényvédelmi Tudományos Napok. Február 13-15., Budapest. 34 p.

Szegő, A., Enünlü, N., Veliceasa, D., Kiss-Bába, E., Vajda, G., Illés, P., Desmukh, PS., Lukács, N. (2005). Cryptic viruses and enigmatic double-stranded RNAs in vegetables. Lippay János - Ormos Imre - Vas Károly Tudományos Ülésszak. Október 19-20., Budapest. 384-385 p.

Szegő, A., Tóth, E., Lukács, N. (2005). Perzisztáló kriptikus vírusok. XI. Növénynemesítési Tudományos Napok. Március 3-4., Budapest. 128 p.

Enünlü, N., Veliceasa, D., Deshmukh, PS., Morgun, B., **Szegő, A.**, Koster, S., Beuther, E., Lukács, N. (2003). Cryptic plant viruses. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. November 6-7., Budapest. 414-415 p.