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Short communication

The genomic RNA1 and RNA2 sequences of the tobacco rattle virus isolates found in Polish potato fields

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ABSTRACT

Four tobacco rattle virus (TRV) isolates were identified from tobacco bait seedlings planted in soil samples from Polish potato fields. Sequence analysis of the genomic RNA1 of the isolates revealed significant similarity to the isolates Ho and AL recently found in Germany. Multiple sequence alignments of the genomic RNA2 indicated that the two isolates from northern Poland (Deb57 and Slu24) are in a cluster with the isolates PSG and PLB found in the Netherlands. The remaining two isolates, from central Poland (11r21 and Mlo7), are in a distinct group with the unique isolate SYM found in England. The RNA2 sequences of the studied isolates range from 1998 nt to 2739 nt in length, and all carry deletions of the *2b* and/or *2c* genes. The isolate Mlo7 has an atypical RNA2 structure, having its *cp* gene located in its central region.

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Tobacco rattle virus is the type species of the genus *Tobravirus* (Hull, 2009). In potato (*Solanum tuberosum*), TRV causes necrotic rings in the tuber flesh, and the disease is called spraing or corky ringspot. TRV is transmitted by the nematodes *Trichodorus* and *Paratrichodorus*; it is also mechanically transmissible and seed transmitted. The TRV genome is divided into two positive-sense, single-stranded RNA species, RNA1 and RNA2, each of which is encapsidated separately into a rod-shaped particle.

The TRV RNA1, approximately 6800 nucleotides (nt) in size, has a conserved genome organization (MacFarlane, 1999, 2010). The 5′ proximal open reading frame (ORF) of the RNA1 encodes a 134 kDa protein with methyltransferase and helicase amino acid motifs (Koonin, 1991; Rozanov et al., 1992). Translation that reads through the stop codon of this ORF produces a 194 kDa protein with amino acid motifs typical of RNA-dependent RNA polymerases (RdRp). Translation terminating at the UGA (opal) codon of the first ORF (134 kDa) can be suppressed by some chloroplast and cytoplasmic tRNAs, leading to the incorporation of either tryptophan or cysteine residues at this position (Urban et al., 1996; Zerfass and Beier, 1992). Further downstream in the RNA1 are the *1a* ORF, encoding a 29 kDa movement protein (MP), and the *1b* ORF, encoding a 16 kDa cysteine-rich silencing suppressor protein.

The TRV RNA2 encodes the virus coat protein (CP), as well as one or more additional proteins (2b and 2c) that are involved in the transmission of TRV by nematodes (MacFarlane, 1999, 2003, 2010). The RNA2 varies considerably in length (1900–3900 nt) depending on isolate, as one or both of the *2b* and *2c* genes may be missing. In addition, part or all of the 3′ region of the RNA2 that encodes these genes and the 3′ noncoding region may be replaced by recombination with the 3′ portions of the RNA1. The RNA2 of the atypical isolate SYM has been found to encode three novel ORFs upstream of the *cp* gene (Ashfaq et al., 2011). SYM is the only documented isolate in which the *cp* gene is not located at the 5′ end of the RNA2.

However, due to recombination, reassortment and deletion, more than one TRV RNA2 species may be found in an infected plant (Hernandez et al., 1996; Koenig et al., 2011, 2012; Schmidt and Koenig, 1999). In naturally infected spinach leaves, the spinach isolate SP apparently contained a second RNA2 species with a partially deleted ORF3 and an RNA1-like 3′ end (Schmidt and Koenig, 1999). The *Alstroemeria* isolate AL had one RNA1 species that was associated with seven different RNA2 species (Koenig et al., 2011). These seven RNA2 species differed considerably in size and in the types of their 3′ RNA1-related sequences. The isolate Ho, found in infected *Hosta* leaves, contained one RNA1 species (Ho-1) and two RNA2

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species (Ho-2a and Ho-2b) (Koenig et al., 2012). The RNA1-related 3′ end of Ho-2a was distinct from that of Ho-1, whereas that of Ho-2b was identical to that of Ho-1.

In this study, we report the genomic sequences of the RNA1 and RNA2 of four TRV isolates. The evaluated TRV isolates in this study were identified from tobacco bait seedlings planted in soil samples from Polish potato fields. The isolates Deb57 and Slu24 were obtained from Dębnica and Słupsk in northern Poland in 2009, respectively. The isolates 11r21 and Mlo7 were collected from Młochow in central Poland in 2010. The isolates were maintained in the tobacco cv. Samsun by mechanical inoculation. In tobacco, all isolates caused systemic distortions, necrotic lesions and line patterns on the leaves and lesions on the stems, with the isolate 11r21 causing the most severe symptoms. In *Chenopodium amaranticolor*, the isolates Deb57, Slu24 and Mlo7 caused only local lesions, whereas 11r21 induced severe systemic leaf distortions. In *C. quinoa*, Deb57 and Slu24 caused only local lesions. In contrast, 11r21 and Mlo7 induced severe and mild systemic leaf distortions and necroses on *C. quinoa*, respectively. All isolates showed a positive reaction to the TRV PRN polyclonal antibody (SASA, UK).

The TRV RNA1 and RNA2 cDNAs were amplified using the sense and antisense primers specific for the 5′ and 3′ ends of RNA1 and RNA2, which have been described by Crosslin et al. (2010). For each isolate, the RT-PCR products used in the sequencing were amplified using the RNA extraction from a single selected plant. The RT-PCR products were resolved by electrophoresis in 1.0% agarose gel and visualized by ethidium bromide staining. The corresponding bands were purified by a QIAquick Gel Extraction Kit (Qiagen) and sequenced directly. Sequencing was conducted by a commercial company (DNA Sequencing and Oligonucleotide Synthesis Laboratory, IBB, PAS, Poland) using the Sanger dideoxy chain termination method and primer walking. Sequences were assembled using the software package DNASTAR SeqMan Pro. Version 9.1.0 (109) 418. Gene structure annotation was predicted by two software packages, GenMark (Besemer and Borodovsky, 1999, http://exon.gatech.edu/) and fgenesV0 (http://linuxl.softberry.com). The models of gene prediction were consolidated, and the consensus sequences were established for each protein. Functional annotation was conducted using Blast P (http://www.ncbi.nlm.nih.gov/blast). Protein weight prediction was conducted according to Science Gateway (http://www.sciencegateway.org). Multiple sequence alignments were obtained using ClustalW & MEGA version 5 (http://www.megasoftware.net).

The 194 kDa (134 kDa) protein and the movement protein encoded by the RNA1 of Deb57, Slu24, 11r21 or Mlo7 share 99% to 100% identity with those of the isolates Ho and AL from Germany, respectively. Similarly, multiple sequence alignments of the RNA1 nucleotide sequences of the four studied isolates with those of the 14 isolates from GenBank revealed significant similarity, indicating 99% or 100% identity to the RNA1 sequences of the isolates Ho (JQ235203) and AL (HM195288). Our data confirm that the Ho- and AL-type TRV RNA1 is detected in potato fields in northern and central Poland. The short sequence portions that shared 99.9–100% identity with Ho RNA1 and AL RNA1 have also been found in TRV-infected garlic and potatoes (Koenig et al., 2012; Koeing, unpublished). The 16 kDa protein encoded by the ORF *1b* is more variable than the other viral proteins encoded by the RNA1 of the studied isolates. The 16 kDa protein encoded by the RNA1 of Slu24 and Deb57 shares 100% identity with that of the isolate Ho (AFN02873) and is composed of 136 amino acid (aa) residues, with a predicted molecular mass of 15.72 kDa. The 16 kDa protein encoded by the RNA1 of Mlo7 shares 92% identity with that of the isolate Ho and contains 124 aa residues, with a predicted molecular mass of 14.15 kDa. The 16 kDa protein encoded by the RNA1 of 11r21 shares 96% identity with that of the isolate Ho and has 140 aa residues, with a predicted molecular mass of 16.24 kDa. The NCBI GenBank accession numbers for the RNA1 sequences of the isolates Deb57 (6776 nt), Slu24 (6776 nt), 11r21 (6788 nt) and Mlo7 (6784 nt) are KF758791, KF758793, KF758790 and KF758792, respectively.

The Mlo7 16 kDa protein (AHG52757) might be the smallest 16 kDa protein described so far. Compared to the 11r21 16 kDa protein, the Mlo7 16 kDa protein lacks 15 aa at its C-terminus. The region 6431–6455 nt of the Mlo7 *1b* gene contains five nucleotide deletions and one insertion compared to the corresponding region of the 11r21 *1b* gene (6431–6459 nt). This leads to the reading frame shifts in the Mlo7 *1b* gene and premature translation termination of the Mlo7 16 kDa protein at TAA (6483–6485 nt). Although the N-terminal aa 1–107 of the Mlo7 16 kDa protein shares 100% identity with that of the 11r21 16 kDa protein, these two 16 kDa proteins differ considerably in their C-terminal ends. Previous studies based on computational prediction and experimental verification indicated that the 16 kDa protein of the TRV isolate PpK20 (AAM50511) possesses two independent bipartite nuclear localization signals (NLSs) responsible for nuclear targeting in context of the C-terminal half of the protein (Ghazala et al., 2008; Robbins et al., 1991). The NLS1-Mlo7 (RKRVEARNREVWKQIQ) is identical to the NLS1-11r21, but the NLS2-Mlo7 (KNSKGEENLGHQKDF–) contains 9 substitutions and two deletions compared to the NLS2-11r21 (KKFKREREFGTPKRFLR, 111–125 aa).

In contrast to the relatively conserved genome organization of RNA1 in the four isolates studied, the corresponding RNA2 sequences differ widely in length, genome organization and the RNA1-related recombinant region (Fig. 1). The RNA2 sequences of the four studied isolates range from 1998 nt to 2739 nt in length, and all carry deletions of the *2b* and/or *2c* genes. Gene structure annotation revealed that three of the isolates, Deb57, Slu24 and 11r21, show typical genome organization, with the *cp* gene located in the 5′ proximal position of the RNA2 molecule (Fig. 1). The Deb57 *cp* gene encodes a protein of 22.82 kDa (AHG52762) that shares 99% identity with the CP of the isolates PSG (P69470) and PRN (CAA85447). The Slu24 RNA2 contains a *cp* gene identical to that of Deb57. The RNA2 molecules from isolates Slu24 and Deb57, in addition to their RNA2-specific regions, contain a region resembling that of the 3′ terminus of the TRV RNA1. The remaining isolate, Mlo7, has an atypical genome structure, having its *cp* gene located in the central region of the RNA2 (Fig. 1). The GenBank accession numbers for the RNA2 sequences of the isolates Deb57 (2002 nt), Mlo7 (2292 nt), Slu24 (1998 nt) and 11r21 (2739 nt) are KF758794, KF758795, KF758796 and KF758797, respectively. Multiple sequence alignments, based on comparisons of the RNA2 nucleotide sequences of the four studied isolates with those of 32 isolates from GenBank, revealed that the isolates from Poland are in a separate cluster with a small number of European isolates. Among the tested isolates, those from northern Poland (Deb57 and Slu24) are in a sister clade with the type strains PSG (X03686) and PLB (J04347) from the Netherlands. However, the isolates from central Poland (11r21 and Mlo7) are in a sister clade with the unique strain SYM (FR854197) from England. The Mlo7 RNA2 is a second example resembling the SYM RNA2. A detailed comparison of some features of the RNA2 sequences of the isolates Mlo7, 11r21 and SYM are shown in Table 1.

The CPs of Mlo7 (AHG52764) and 11r21 (AHG52766) are predicted to be 28 kDa and 19 kDa, respectively. Legorburu et al. (1996) demonstrated that the C-terminal domain of the TRV CP is highly immunogenic, whereas the N-terminal region and a central region are more weakly immunogenic. Six sequence motifs conserved in the CP of tobraviruses were identified (Goulden et al., 1992; Legorburu et al., 1996). The Mlo7 CP contains the motif I (WVE, aa 24–26) at its N-terminus and the motif IV (ASAFKRRADEKNAV, aa 93–106) in the central region. However, there are two substitutions in the motif VI (FEKEFGIKF, aa 175–183) of the Mlo7 CP at its C-terminus compared to the conserved motif VI (the

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