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Short communication

Natural infection of wheat by the type strain of *Soil-borne wheat mosaic virus* in a field in Southern Germany

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Abstract

Molecular analyses revealed that a virus causing a severe disease of wheat in one field in Southern Germany is closely related to the Nebraska type strain of *Soil-borne wheat mosaic virus* (SBWMV) and only distantly related to *Soil-borne cereal mosaic virus* that is widely distributed in Europe. The latter virus was not found in the SBWMV-containing leaf samples. This is the first report of the occurrence of SBWMV in Germany, and perhaps in all of Europe, which has been confirmed on the molecular level.

Soil-borne wheat mosaic virus (SBWMV), the type member of the genus Furovirus, was first detected in the central parts of the USA where it causes severe damage to varieties of winter wheat (for review see Brakke and Langenberg, 1988). The complete nucleotide (nt) sequences of the two RNA species of the Nebraska type strain of SBWMV (SBWMV Neb) have been determined (Shirako and Wilson, 1993). The RNA 2 sequence of an Oklahoma isolate differed from that of the Nebraska isolate by about 2% (Chen et al., 1994). However, various genome areas analysed for a virus source from New York State (SBWMV NY) differed by c. 10% and more from the respective genome areas of the Nebraska and Oklahoma isolates. The deduced amino acid sequences of the respective gene products, nevertheless, suggested a much closer relationship between the Nebraska/Oklahoma and the New York strains of SBWMV (Koenig et al., 2002).

Furoviruses causing diseases in wheat and rye, similar to those caused by SBWMV, have been described from Japan, China and Europe. These viruses, however, differ considerably from the American SBWMV strains and between each other, not only in the nt sequences of their genomes but also in the amino acid sequences of their gene products. They have been considered as strains of SBWMV by some authors (Shirako et al., 2000; Clover et al., 2001) and as distinct viruses by others (Diao et al., 1999a,b; Koenig et al., 1999; Koenig and Huth, 2000; Yang et al., 2001). The genomes of virus sources obtained from various European countries, i.e., Italy, France, UK, Germany, Poland and Denmark, show high percentages of sequence identities to each other, but their sequences differ from those of the American strains by 25–37% depending on the genome areas compared. Koenig and Huth (2000) and Yang et al. (2001) have suggested that the name *Soil-borne cereal mosaic virus* (SBCMV) should replace the names *European wheat mosaic virus* (Diao et al., 1999b) and *Soil-borne rye mosaic virus* (Koenig et al., 1999) which were originally proposed for these European virus sources. In Germany, Poland and Denmark, SBCMV mainly infects rye, whereas in the UK, Italy and France it is wheat that becomes infected. This may be due to differences in the host preferences of the vector *Polymyxa graminis* in various regions. In 2002, severe damage of wheat due to a *Furovirus* was observed for the first time in a field in Southern Germany. Originally, we assumed that SBCMV was the causal agent, but sequence analyses revealed that the disease was caused by a virus which is

closely related to the Nebraska type strain of SBWMV. It will be referred to as SBWMV De1 in this paper.

For amplifying and analysing various regions of RNA 1 and RNA 2 of SBWMV De1 (Figure 1) we used essentially the same methods and most of the primers described by Koenig et al. (2002). The newly developed primer Sb10 corresponds to the 25 5′-terminal nts of SBWMV RNA 2 and was used together with primer sb40 for the amplification of a 5′ proximal sequence of SBWMV De1 RNA 2. The viral RNA serving as a template for reverse transciption was obtained by means of the Qiagen RNeasy Plant Mini Kit (Cat. No. 74904).

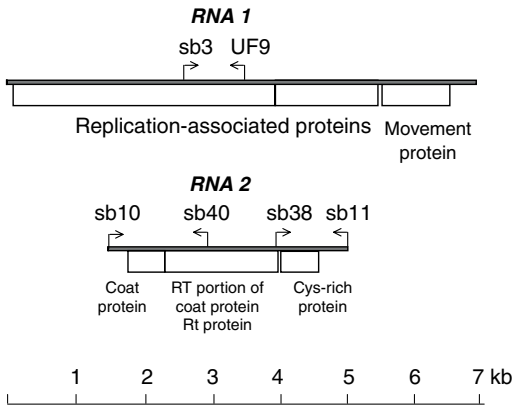

Figure 1. Schematic representation of the genome areas amplified for the German SBWMV source De1.

The 610 nt sb3/UF9 PCR product of SBWMV De1 covers the coding sequence for the C-terminal portion of the variable region and the N-terminal portion of the helicase domain of a replication-associated protein. It differed in only eight positions from the corresponding sequence of SBWMV Neb which amounts to a percentage of sequence identity of 98.7% (Figure 2a). Only three of the nt exchanges caused an amino acid change and four others occurred also in a second population of SBWMV Neb RNA 1 (Shirako and Wilson, 1993). With the corresponding genome areas of the NY strain of SBCMV and of the three German SBCMV sources De-G, De-O and De-C (Koenig and Huth, 2000), the sb3/UF9 PCR product of SBWMV De1 shared only 87% and 74% sequence identity, respectively (Figure 2a).

The RNA 2-derived PCR products sb10/sb40 and sb38/sb11 (Figure 1) also suggest that SBWMV De1

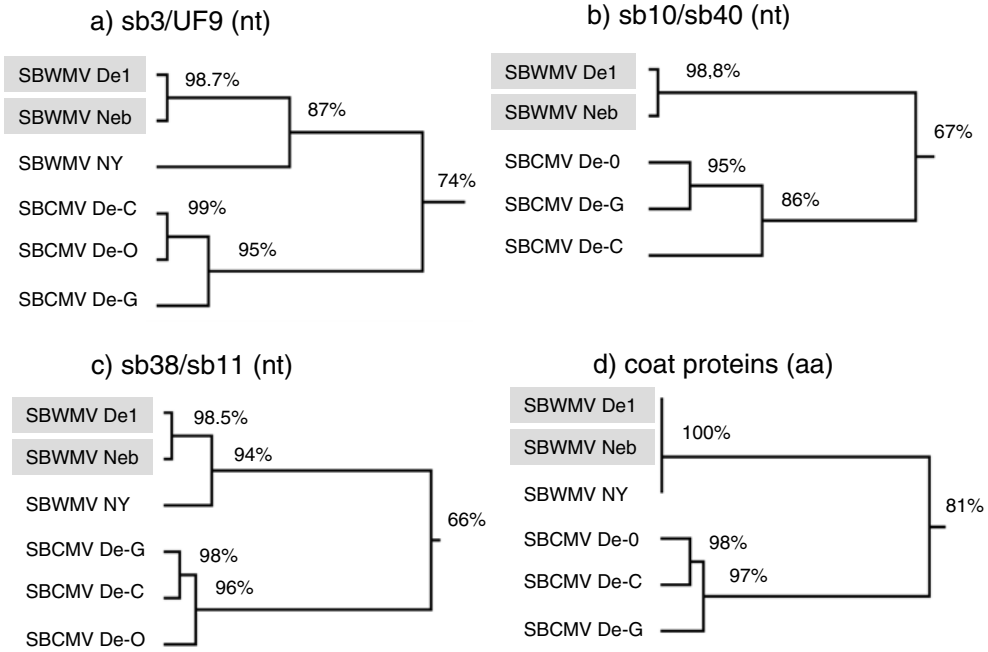

Figure 2. Percentages of sequence identities for the German SBWMV source De1, the Nebraska type strain of SBWMV Neb, the NY strain of SBWMV and three German sources of SBCMV, i.e., De-C, De-G and De-O, (a)–(c) comparison of the nucleotide sequences of the genome areas depicted in Figure 1, (d) comparison of the amino acid sequences of the coat proteins.

is very closely related to SBWMV Neb and much more distantly related to the three sources of SBCMV (Figure 2b and c). The 1415 nt sb10/sb40 and the 1029 nt sb38/sb11 PCR products of SBWMV De1 differed in 17 and 15 positions, respectively, from the corresponding sequences of SBWMV Neb. In these genome areas, the percentages of sequence identity between SBWMV De1 and SBWMV Neb, thus, amounted to 98.8% and 98.5%, respectively, whereas only 66% or 67% sequence identity were found between SBWMV De1 and the three sources of SBCMV (Figure 2b and c). Despite the fact that there were 12 nt exchanges in the coat protein genes of SBWMV De1 and SBWMV Neb, the deduced amino acid sequences of the coat proteins were identical for the two viruses and also for SBWMV NY, but greatly differed from those of SBCMV (Figure 2d). The coding regions for the cysteine-rich proteins of SBWMV Neb and De1 encoded in the 3′-terminal part of their RNAs 2 (Figure 1) differed in eight positions, but the amino acid sequences of the translations products differed in only one position (results not shown).

SBCMV was not detected in the SBWMV-containing leaf samples. No PCR products of the expected sizes were obtained with the primer combinations sb11/gsb65, sb11/gsb55 and sb11/gsb58. Gsb65, gsb55 and gsb58 were derived from sequence areas of the German De-G source of SBCMV that differ considerably from the corresponding ones of the SBWMV RNAs. Gsb65 corresponds to nts 6264–6384 and gsb55 to nts 6539–6562 of RNA 1, and gsb58 to nts 2955–2974 of RNA2, respectively, of SBCMV De-G (Accesion Numbers AF146278 and AF146282).

The results presented in Figure 2a–d clearly indicate that the virus infecting wheat in a field in Southern Germany is not SBCMV, but rather a closely related variant of the Nebraska type strain of SBWMV. There is only one previous report that viruses which on a molecular basis are closely related to SBWMV Neb occur in Europe, i.e., in the United Kingdom in oats and in France in wheat (Chen et al., 1996). This finding, however, was, at least for oats, not confirmed later; it was considered to be due to a PCR contamination (Diao et al., 1999). The present report may, therefore, be the first one on the occurrence of the type strain of SBWMV in Europe.

The genebank accession numbers for the sb3/UF9, sb10/sb40 and sb38/sb11 PCR products (Figure 1) are AF519798, AF519799 and AF519800, respectively.

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