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FIRST DETECTION AND PARTIAL CHARACTERIZATION OF A FUROVIRUS ISOLATED FROM WINTER BARLEY IN GERMANY

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Summary

The yellow mosaic virus complex of winter barley which is caused by the two bymoviruses Barley mild mosaic virus (BaMMV) and Barley yellow mosaic virus (BaYMV), is one of the most severe threats to the crop growing of winter barley in Germany. Other soil-borne cereal viruses like Soil-borne cereal mosaic virus (SBCMV) or Soil-borne wheat mosaic virus (SBWMV) also transmitted by Polymyxa graminis and infecting wheat, rye and triticale have never been observed up to now on winter barley under field conditions in Germany. However field examinations of yellow mosaic virus resistant barley genotypes has shown that plants carrying mosaic symptoms contained rod-shaped virus particles. Several isolates were obtained by mechanical inoculation of test plants and one isolate originating from variety 'Jorinde' (harbouring the rym5 resistance gene) was designated as Jorinde-1 and was further characterized. The biological, serological and molecular data obtained so far indicate that the German isolate Jorinde-1 belongs to the genus Furovirus and is closely related to Japanese SBWMV (JT) and the French Soil-borne barley mosaic virus (SBBMV, later renamed as SBWMV-Mar). Phylogenetic analysis by means of sequencing data for RNA2 coding for cistrons of coat protein, read through protein and cysteine rich protein revealed that Jorinde-1, SBBMV and SBWMV (JT) form a separate cluster and may represent a new species in the genus Furovirus within the family Virgaviridae.

Introduction

Besides virus diseases transmitted by insects, the soil-borne viruses of cereals represent the greatest danger potential for the production of cereals in Germany. In recent years, yield losses in winter barley caused by the barley yellow mosaic virus complex have been reduced by growing new varieties carrying different resistance genes. In order to assess further barley accessions for virus resistance, annual field trials were carried out at different locations in cooperation with the Federal Plant Variety Office (Bundessortenamt). The
resistant varieties ‘Jorinde’ and ‘Nerz’ e.g. first released in 2005 and 2008, respectively remained virus free in all preceding examinations (Rabenstein et al., 2008).

However, during field inspections in spring 2010 unexpected yellowing and mosaic symptoms were observed in both control varieties ‘Jorinde’ and ‘Nerz’ harbouring resistance allele rym5 that confers resistance to bymovirus isolates including to the pathotype *Barley yellow mosaic virus* 2 (BaYMV-2) (Hofinger et al., 2011; Kanyuka et al., 2005; Stein et al., 2005). Therefore, it was initially assumed that a new resistance-breaking pathotype had emerged. However, the plants contained, instead of bymoviruses, rod-shaped furovirus-like particles which could be transmitted mechanically. A discrete isolate originating from the variety ’Jorinde’, designated as Jorinde-1, was further investigated and characterized.

**Materials and Methods**

The field trials were carried out at one site near Braunschweig (JKI) where the soil was contaminated with both BaYMV-1 and BaMMV and at two further locations (Bornum and Schladen) additionally infested with the resistance breaking pathotype BaYMV-2. All field trials were inspected for virus symptoms at three different times in the early spring. Plants from varieties with or without visible symptoms were tested serologically using polyclonal antisera to BaMMV and BaYMV in DAS-ELISA. Serologically negative tested plants were further assayed by RT-PCR using virus specific primer combinations (Steyer et al., 2005; Götz & Huth, 2005). Additionally, plants with suspect symptoms were tested by TAS-ELISA using specific monoclonal antibodies (Rabenstein et al., 2005) for the presence of other cereal infecting viruses like *Soil-borne wheat mosaic virus* (SBCMV) and *Soil-borne cereal mosaic virus* (SBWMV). IC-RT-PCR and electron microscopy including immunogold labelling were performed as described by Rabenstein et al. (2011). Phylogenetic analysis was performed as described by Shirako et al. (2000).

![Figure 1: Symptoms on barley variety ‘Kathleen’ after inoculation with isolate Jorinde-1.](image)
Results and Discussion

Preliminary host range studies showed that the isolate Jorinde-1 was mechanically transmissible to all tested barley varieties. All recently listed new varieties as ‘Kathleen’ (Fig.1), ‘Yokohama’ and ‘Otto’ possessing resistance to viruses of the barley yellow mosaic disease complex could be infected and large amounts of virus particles arranged in bundles were observed in ultrathin sections as shown as an example in Figure 2 for the barley variety ‘Kathleen’.

In addition the isolate Jorinde-1 infected a few wheat varieties but could never be mechanically transmitted to rye. On inoculated leaves of *Nicotiana tabacum* varieties ‘Samsun - NN’ or ‘Samsun - nn’ local lesions appeared as early as 4 dpi. In contrast to a German isolate of SBWMV originally obtained from naturally infested wheat plants in the south if Germany (Heddesheim) isolate Jorinde-1 also infected *N. benthamiana* systemically.

Initial ELISA and Western blotting experiments using rabbit antisera and specific monoclonal antibodies (MABs) to SBCMV or SBWMV (Rabenstein et al., 2005) were confirmed by electron microscopic decoration tests and immunogold labeling. By using a SBCMV specific MAB (4G11) all tested SBCMV isolates and Jorinde-1 were labeled similarly as shown in Figure 3. In contrast MAB 4G4 specific for SBWMV gave no labeling and reacted only with the type strain of SBWMV (ATCC) and the German SBWMV isolate Heddesheim (data not shown). The new furovirus isolated from barley variety ‘Jorinde’ was in this way serologically more closely related to SBCMV than to SBWMV, which occurs so far only in wheat, rye and triticale in Germany.

![Figure 2: Virus particles arranged in bundles in the cytoplasm in ultrathin sections of barley variety ‘Kathleen’ after inoculation with isolate Jorinde-1, bar 200 nm.](image)

Using movement protein-specific primers for RNA1 of SBCMV or SBWMV in RT-PCR only amplification products for the homologous furovirus isolates from wheat, rye and triticale were obtained, but not for the Jorinde-1 isolate, indicating differences in the movement protein gene sequence. So far, for the German barley furovirus the complete genome sequence was obtained only for RNA2. Blast analysis revealed close homology to the isolate Marne (F), first described as ‘French barley mosaic virus’ or ‘Soil-borne barley mosaic virus’ (SBBMV’) in France in 2001 (Hariri, 2004), and to a virus designed as SBWMV-JT, which was originally isolated from barley in Japan (Shirako & Ehara, 1986). On the nucleotide level, the Jorinde-1 sequence showed 99% identity to the
incomplete sequence of the French SBBMV and 94% identity to the complete RNA2 sequence of SBWMV-JT. The amino acid sequence of the CP of isolate Jorinde-1 was nearly identical to that of SBBMV with only phenylalanine at position 63 replaced by leucine.

The CP and the sequences for cystein rich protein of SBWMV-JT and SBBMV formed a single clade with Jorinde-1 in phylogenetic analyses that was well supported by bootstrapping as shown in Figure 4A using the example of CP amino acid sequences.

A slightly divergent cluster was formed using the read through protein amino acid sequences of several members of the genus Furovirus (Fig. 4B). Isolate Jorinde-1 formed together with SBWMV-JT, SBBMV and SBCMV isolate G a single well-supported clade. SBCMV-G isolated from rye was the most divergent from other sources of SBCMV found in Germany (Koenig et al., 1999).

In conclusion, the biological, serological and phylogenetic data obtained so far indicate that the German furovirus from barley is closely related to Japanese SBWMV (JT) and the French SBBMV, later renamed as SBWMV-Mar (Hariri & Meyer, 2007). Future investigations will focus on economic relevance and the distribution of this virus in German fields.

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References


