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Comparison of wheat spindle streak mosaic virus (WSSMV) and barley yellow mosaic virus (BaYMV): 2 closely related bymoviruses

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Summary — In order to better understand the evolutionary development that leads to different host ranges, we made a comparison of the closely related barley yellow mosaic bymovirus (BaYMV) and wheat spindle streak mosaic bymovirus (WSSMV). Using different methods both viruses were found to be closely related. Leaves of BaYMV-infected winter barley and WSSMV-infected wheat typically showed similar symptoms. Electron microscopy studies revealed that both viruses lead to formation of 2 types of cytoplasmic inclusions bodies (CI, 'pinwheel' structures; crystal-like respectively membranous) in infected cells that showed structural differences. This close relationship is also reflected by the high degree of amino acid sequence homology (74% in CP-, 83% in NIb-, 68% in NIa-region). In spite of the remarkably high similarities both viruses have separate hosts. We demonstrated that, independently of the host range, a reliable distinction between WSSMV and BaYMV is possible using Northern blot hybridisation, reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis. Antisera raised against BaYMV proteins also detected WSSMV proteins in Western blot analyses. Even though corresponding proteins of both viruses were usually similar in size, the BaYMV-CI antiserum cross-reacted with an additional protein about 8 kDa larger in wheat extracts and the BaYMV 28 kDa antiserum detected a protein of about 30 kDa in wheat extracts.

bymovirus / barley yellow mosaic virus / wheat spindle streak mosaic virus / *Polymyxa graminis* / host range

Résumé — Comparaison du virus de la mosaïque striée du blé (WSSMV) et du virus de la mosaïque jaune de l'orge (BaYMV) : 2 bymovirus très proches. *Dans le but de mieux comprendre l'évolution qui mène à différentes gammes d'hôtes de virus transmis par des champignons, nous avons comparé le virus de la mosaïque jaune de l'orge (BaYMV) et le virus de la mosaïque striée du blé (WSSMV). En utilisant différentes méthodes, nous avons remarqué que les 2 virus étaient très proches. Les feuilles de l'orge d'hiver infecté par le BaYMV et du blé infecté par le WSSMV ont révélé des symptômes similaires. Des études au microscope électronique ont montré que les 2 virus entraînaient la formation d'inclusions cytoplasmiques cylindriques (CI ; structure* «pinwheel»*) dans les cellules infectées et ont souligné quelques différences structurelles. Ce lien de parenté est également révélé par la forte homologie des séquences d'acides aminés et de cADN (74% en CP, 83% en NIb et 68% en NIa). Jusqu'à présent, le BaYMV et le WSSMV avaient été distingués sur le seul critère des plantes hôtes. Nous avons montré que, indépendamment de la gamme d'hôtes, des distinctions reproductibles entre WSSMV et BaYMV sont possibles en Northern RT-PCR (transcription* reverse *associée à la réaction de polymérase en chaîne) et Western blot. Bien que les protéines correspondantes des virus aient généralement une taille similaire, l'antisérum dirigé contre la protéine CI du BaYMV a réagi chez le blé avec*

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une protéine supplémentaire d'une taille de 8 kDa supérieure à celle de la protéine homologue, tandis que l'antisérum dirigé contre la protéine «28 kDa» du BaYMV reconnaît chez le blé une protéine de 30 kDa.

bymovirus / virus de la mosaïque jaune de l'orge / virus de la mosaïque striée du blé / Polymyxa graminis / hôte

Yellow mosaic diseases of barley and wheat caused by *Polymyxa graminis* transmitted RNA viruses lead to considerable damage and yield losses in many European countries (Signoret, 1977; Rubies-Antonell *et al*, 1987). Bymoviruses, such as barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) infecting winter barley and wheat spindle streak mosaic virus (WSSMV) and wheat soilborne mosaic virus (WSBMV) infecting wheat, give rise to heavy infections (Huth, 1981; Miller *et al,* 1992). In this paper we present common features and differences of BaYMV and WSSMV by using several molecular biology and electron microscopy techniques.

Both viruses contain a bipartite positive-sense, single-stranded RNA genome. The slightly flexous, rod-shaped particles are of length about 350 and 650 nm (Slykhuis and Polak, 1971; Usugi and Saito, 1979; Huth *et al,* 1984). Particles are often found in aggregation. In the cell they appear as clusters and are rarely found as single virus particles in infected cells.

Electron microscopy studies of infected cells showed cytoplasmatic cylindrical inclusion bodies (CI, 'pinwheel' structures) which are typical for viruses of the family *Potyviridae* (Hooper and Wiese, 1972; Huth *et al,* 1984). In comparison to BaYMV the pinwheel inclusions of WSSMV infected cells consisted of more curved 'arms' and were accompanied more obviously by membranous invaginations between the 'arms' which appeared in cross-sections as vesicle-like structures (Hooper and Wiese, 1972). The other predominant feature in BaYMV-infected cells were crystal-like cytoplasmic inclusion bodies. Immunogold labeling studies with antisera raised against BaYMV-RNA2 encoded proteins (28 kDa, 70 kDa) revealed that the proteins are part of this structure (Schenk *et al,* 1993). The same antisera also detected cytoplasmatic inclusions in WSSMV infected cells but of a more membranous type (fig 1).

Taken together the leaf symptoms and cellular alterations were similar except that the morphology of the cytoplasmatic inclusion bodies was different. These structures contained the same viral proteins as could be shown in immunogold-labeling studies. It is unknown whether the differences of the corresponding viral proteins are responsible for the differences in morphology or whether plant proteins play a role.

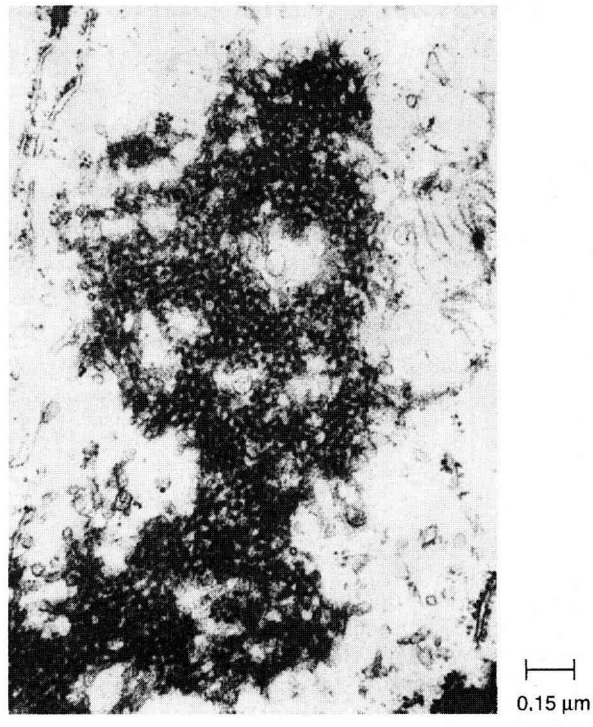

Fig 1. Electron microscopy of cytoplasmic inclusion bodies in cells of WSSMV-infected wheat leaves.

The high degree of relationship is also displayed by comparison of the accessible viral sequences (Sohn *et al,* 1994). Each viral RNA codes for an autoproteolytically processed polyprotein as is known from the analysis of the complete sequenced BaYMV genome (Kashiwasaki *et al,* 1990; Davidson *et al,* 1991; Peerenboom *et al,* 1992; Schenk *et al,* 1993) and as can be concluded from the known RNA1 sequence of WSSMV. Sequence comparison of the capsid protein (CP) region, which was often successfully used as a taxonomic parameter, revealed a very high overall CP identity (74%) and a homology in the CP core regions of WSSMV and BaYMV of 81%. This is in the reported range of 75–88% for very closely related viruses, which are regarded as distinct viruses (Sohn *et al,* 1994).

In spite of these remarkably high similarities both viruses have separate hosts (Slykhius, 1970; Jackson *et al,* 1976). Inoculation of barley plants with WSSMV has always been unsuccessful and, to our knowledge, BaYMV has never been detected in barley planted on a WSSMV-infected field. Independent of testing the host range, it is possible to distinguish between the 2 viruses using molecular biology techniques such as Northern blot hybridization, reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis.

Poly(A) RNA from 200 mg infected leaves were isolated using oligo-d(T)25 Dynabeads (Dynal). Useful cDNA probes could be found which hybridised with RNA1 or RNA2 of both viruses (fig 2). Cross-hybridisation of RNA1 and RNA2 has never been found. Interestingly RNA2 detection showed not only the expected signal at approximately 3.5 kb but moreover a smaller RNA of approximately 1.8 kb. This RNA could be due to the isolation procedure resulting in a damaged, shorter RNA2 fragment, or it may be possible that this is a deletion containing a form of the RNA2 as was reported for BaMMV RNA2 (Dessens *et al,* 1995; Jacobi *et al,* 1995).

Using the same Northern blot conditions (a cDNA fragment corresponding to the 3' terminal NIb region and the 5' terminal CP region of WSSMV RNA1 (62% sequence homology to BaYMV) as a probe for hybridisation only the RNA1 of WSSMV-infected plants gave a signal.

The isolated poly(A) RNA from infected leaves was also tested with RT-PCR using WSSMV-specific primers. Using poly(A) RNA from WSSMV-infected leaves for RT-PCR, a 960 bp fragment could be detected after electrophoretic separation on agarose gel. Cutting the PCR fragment that corresponds to the part of the CP region with restriction enzymes (PvuII, Hind III) resulted in a fragment length pattern which was expected from the WSSMV cDNA sequence (data not shown). This ensured that the expected WSSMV fragment was amplified. Starting with BaYMV-infected leaf material or corresponding BaYMV cDNA, no fragment was amplified by RT-PCR under these conditions (fig 3) because the homology of the primers was not sufficient enough.

The described primers turned out to be useful to distinguish between WSSMV and BaYMV. They may be also useful for investigations of the Japanese wheat yellow mosaic virus (WYMV), which is thought to be a strain of WSSMV or *vice versa.* It would be interesting to see if a similar fragment could be amplified. Furthermore, the restriction sites in the primers (a: BamHI, b: NdeI) allowed easy cloning of an amplified fragment in a suitable vector for sequencing.

Serological studies have shown that all polyclonal antisera raised against proteins of BaYMV detected similar proteins of WSSMV-infected cells and *vice versa* (Usugi and Saito,

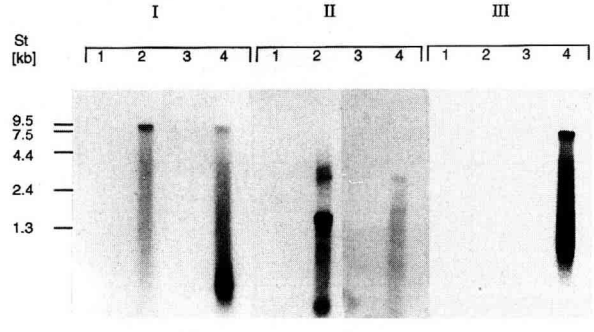

Fig 2. Northern blot analysis of poly(A) RNA isolated from BaYMV infected (2) or uninfected healthy barley leaves (1) and from WSSMV infected (4) or uninfected wheat leaves (3). The blotted filter was prehybridised with a formamide containing solution at 42°C for 4 h, hybridised overnight and was washed with 6 x, 4 x and 2 x SSC, 1% SDS at 45°C. The following cDNA fragments were used for as labeled probes for hybridization: I. A 4.4 kb SalI fragment corresponding to the 3' terminal part of the RNA1 of BaYMV; II. A 2.8 kb NruI and NsuI cut cDNA fragment corresponding to the 3' terminal part of the RNA2 of BaYMV; III. A 330 bp HincII cDNA fragment corresponding to the 3' terminal NIb region and the 5' terminal CP region of the RNA1 of WSSMV.

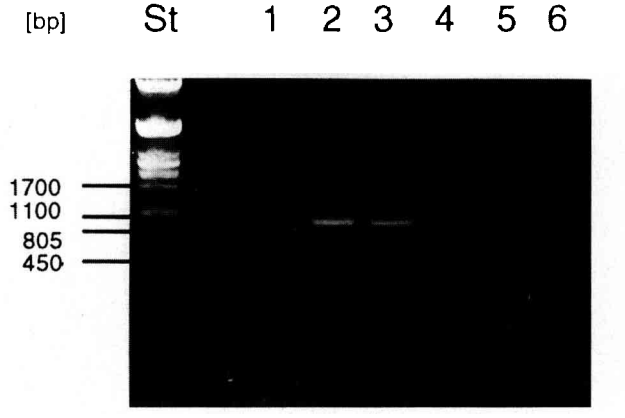

Fig 3. Agarose gel electrophoresis of RT-PCR products. Reverse transcribed poly(A) RNA isolated from healthy (1), WSSMV infected wheat leaves (2,3), BaYMV infected barley (4) and as controls cDNA corresponding to BaYMV RNA1 (5) and to WSSMV RNA1 (6) were used for PCR amplification with primer a (5'CCA CAC GGG ATC CGC) complementary to the 3' and 5' part of the WSSMV CP region. A quarter of the isolated RNA and 120 pmol primer a was used for the first strand synthesis with 1 µl 'superscript' reverse transcriptase (BRL), 10 mM NTPs, buffer, 1 µl RNasin, 0.01 M DTT for 1 h at 37°C; 1/20 of this reaction was then used for PCR with 50 pmol of each primer (a,b), 2.5 mM dNTPs, buffer and 2.5 units Taq-polymerase (Amersham). The following conditions were suitable: 30 cycles 2 min at 92°C and 2 min at 72°C. Probes containing sequences of WSSMV have lead to the amplification of the expected 960 bp fragment.

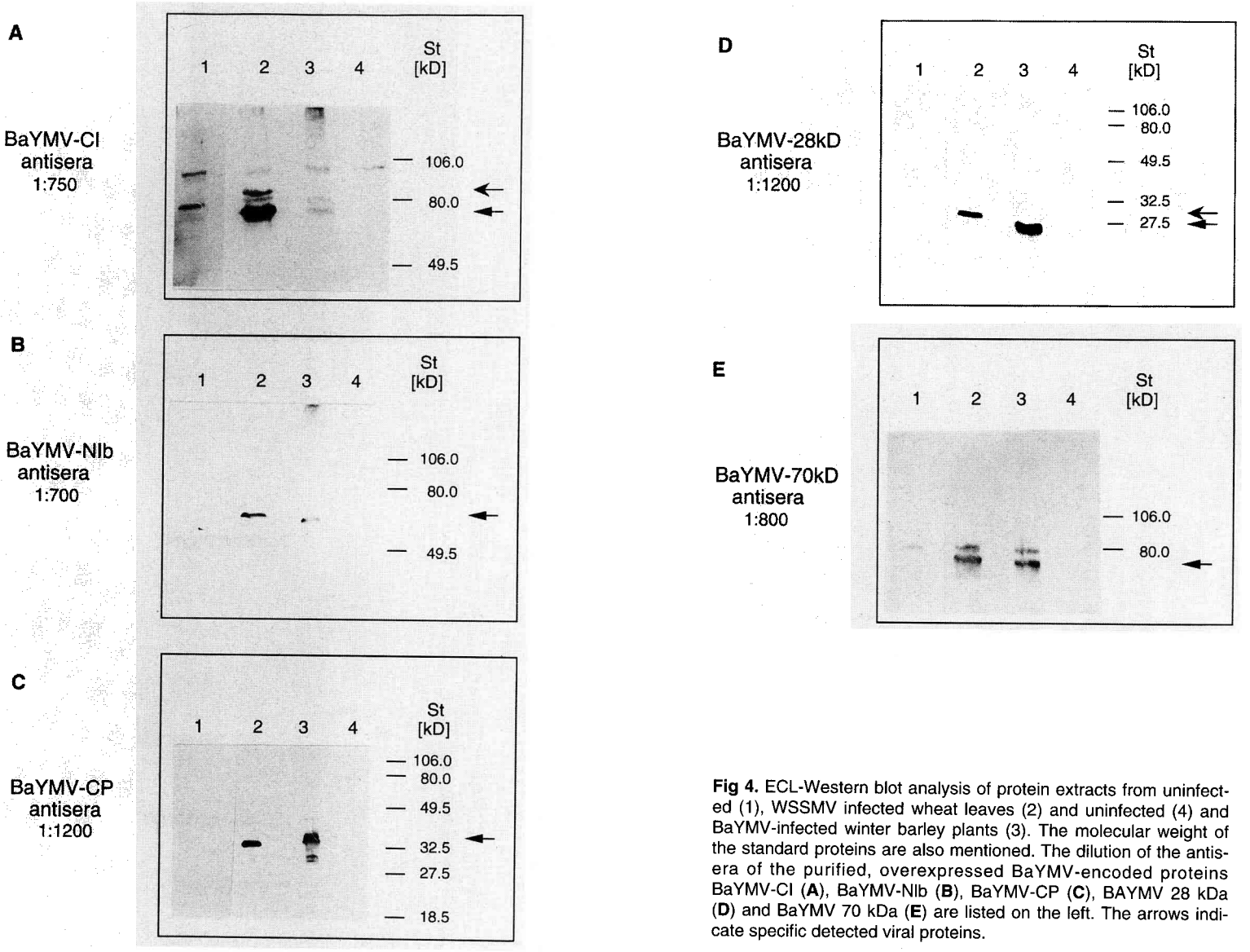

Fig 4. ECL-Western blot analysis of protein extracts from uninfected (1), WSSMV infected wheat leaves (2) and uninfected (4) and BaYMV-infected winter barley plants (3). The molecular weight of the standard proteins are also mentioned. The dilution of the antisera of the purified, overexpressed BaYMV-encoded proteins BaYMV-CI (**A**), BaYMV-NIb (**B**), BaYMV-CP (**C**), BAYMV 28 kDa (**D**) and BaYMV 70 kDa (**E**) are listed on the left. The arrows indicate specific detected viral proteins.

1976; Langenberg, 1986; Chen and Adams, 1991). A distinction by using these antisera for ELISA is not possible. In this study we have tested antisera in Western blot analyses which were raised against purified BaYMV proteins that were over-produced in *Escherichia coli*, such as the RNA1 encoded CI, NIb, CP and both the RNA2 encoded proteins (28 kDa, 70 kDa). Proteins of the same molecular weight were detected with these antisera in BaYMV- and WSSMV-infected leaf extracts (fig 4) with the following 2 exceptions. The BaYMV-CI antisera detected in WSSMV extracts an additional protein which is about 8 kDa higher. A possible explanation for this might be an inefficient processing of a putative additional NIa processing site at the N-terminal site of the CI region, which is known for potyviruses (Garcia *et al,* 1992). However, the sequence data of WSSMV for this region are still missing.

The other difference could be detected with BaYMV 28 kDa antisera which reacted in Western blot analyses of WSSMV-infected leaf extracts with a protein of 30 kDa which is slightly but significantly higher than the BaYMV RNA2 encoded 28 kDa protein. Further investigations of the RNA2 of WSSMV will be made in the future, especially with emphasis on further differences to BaYMV. To understand the virus–plant interactions that lead to different host ranges or to preference of races of *P graminis* to one of these viruses would be very helpful for the development of a resistance strategy.

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