

## REVIEW ARTICLE

# Progress and prospects of studies on *Polymyxa graminis* and its transmitted cereal viruses in China<sup>\*</sup>

CHEN Jianping<sup>\*\*</sup>

(Virology and Biotechnology Institute, Zhejiang Academy of Agricultural Sciences, Key Laboratory of Plant Virology, Ministry of Agriculture and Zhejiang Province, Hangzhou 310021, China)

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**Abstract** *Polymyxa graminis* is a eukaryotic obligate biotrophic parasite of plant roots that belongs to a poorly studied discrete taxonomic unit informally called “*plasmidiophorids*”. *P. graminis* is nonpathogenic, but has the ability to acquire and transmit nine plant viruses which belong to genera *Bymovirus* and *Furovirus* and cause serious diseases in cereal crop species and also result in significant yield reductions in China and elsewhere. Genus *Bymovirus* contains barley yellow mosaic virus (BaYMV), barley mild mosaic virus (BaMMV), wheat yellow mosaic virus (WYMV), wheat spindle streak mosaic virus (WSSMV), and oat mosaic virus (OMV), and genus *Furovirus* contains soil-borne wheat mosaic virus (SBWMV), oat golden stripe virus (OGSV), and newly identified Chinese wheat mosaic virus (CWMV) and soil-borne cereal mosaic virus (SBCMV). All these viruses have been sequenced and their worldwide distributions have been studied. The viruses are protected by the environment within *P. graminis* resting spores that may remain dormant but viable for decades (probably until a suitable host plant is encountered). Spontaneous deletion mutants of SBWMV, OGSV and OMV are detected, and these deletion mutants are not transmissible by the fungus. The persistent, soil-borne nature of these diseases makes the use of virus-resistant crop varieties currently the only practical and environmentally friendly means to control them, and a large number of disease-resistant germplasms have been screened.

**Keywords:** *Polymyxa graminis*, cereal viruses, genus *Bymovirus*, genus *Furovirus*.

As early as in the 1920s, a mosaic or rosette disease was first reported on winter wheat in America, and then it was found that the pathogen was transmitted via soil. In 1925, McKinney successfully transmitted the pathogen from the infected plants to healthy plants by mechanical inoculation and proved that the pathogen was a virus, named soil-borne wheat mosaic virus (SBWMV). In 1969, Rao and Brakke found that SBWMV was transmitted by *Polymyxa graminis* in soil<sup>[1]</sup>. In later years, similar diseases were also reported in Japan, Italy, France, Germany, Brazil and Argentina, which attracted people's attention because of their serious effects on production of cereal crops.

In China, *Polymyxa graminis* transmitted cereal viruses were first identified in the 1970s<sup>[2]</sup>. The diseases caused by these viruses have seriously occurred in estimated 200 million ha of successive barley and wheat crops in Henan, Sichuan, Hubei, Shaanxi, Shandong, Jiangsu, Zhejiang, Anhui provinces and Shanghai City and resulted in a loss of 1.5 million tons of yield. The rapid spread of the diseases and serious damage of crops are caused by the following reasons: (1) the thick-walled resting spores of the fun-

gal vector are extremely tolerant to a poor environment and chemicals, and they can survive in the soil for decades; (2) there are various species of viruses and strains, and the continuous appearance of new pathogenic types and strains breaks the resistance of the virus-resistant cultivars and creates difficulties in cereals breeding. Therefore, to understand the mechanism of interaction among the viruses, fungal vector and cereal crops at the molecular level, and to establish new systems and methods for disease control are significantly important. Since 1985, we have studied the fungal vector *P. graminis*, virus species, genomic organization, disease epidemiology, fungal transmission, as well as screening of resistant germplasms. In this review, I summarize the research progress and prospects of the fungal transmitted cereal viruses in China.

## 1 *Polymyxa graminis* transmitted cereal viruses

### 1.1 Taxonomy and genomic organization

All of the *Polymyxa graminis* transmitted cereal

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<sup>\*\*</sup> E-mail: jchen2001@163.com  
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viruses contain two single-stranded positive-sense RNA genomes, and belong to genera *Bymovirus* and *Furovirus* respectively.

Members of genus *Bymovirus* are mainly distributed in Asia, Europe and North America, and are barley yellow mosaic virus (BaYMV), barley mild mosaic virus (BaMMV), wheat yellow mosaic virus (WYMV), wheat spindle streak mosaic virus (WSSMV) and oat mosaic virus (OMV). These viruses seriously infect winter barley, wheat, oat and rye crops and are mostly serologically related, but have significant differences in nucleotide sequences as well as host ranges<sup>[3-8]</sup>. Recently, we have identified that those viruses that occurred in China are BaYMV and BaMMV for barley and WYMV for wheat. The genomic organization has been determined for BaYMV and WYMV and partial for BaMMV, showing a similarity among them and some homology to the insect transmitted potyviruses. BaYMV and WYMV, the well studied bymoviruses, have bipartite genomes with a larger RNA 1 (about 7.5 kb) and a smaller RNA 2 (about 3.5 kb) in the longer and shorter particles respectively. RNA1 contains one large open

reading frame (ORF) which encodes a 257—271 kD single polypeptide. From this single large polypeptide precursor, 8 mature functional proteins, P3, 7K, CI, 14K, NIa-Vpg, NIa-Pro, NIb and CP from N-terminus to C-terminus, are derived by proteolytic cleavage (Fig. 1(a)). Among these proteins, NIb is related to genome replication, CI is possibly associated to virus movement, NIa-Pro encodes main viral proteinase, NIa-Vpg encodes genome-linked protein, and CP is the only structure protein for RNA encapsidation. RNA2 also contains a large ORF and encodes a 98—101 kD polypeptide which produces two functional proteins (P1 and P2) after proteolytic cleavage. Of these two proteins, P1 is a proteinase, and P2 is possibly associated to fungal transmission. Both RNA1 and RNA2 contain a poly(A) tail at their 3'-terminus<sup>[9,10]</sup>. After infection by these bymoviruses a large number of membranous bodies connected with endoplasmic reticulum and cylindrical or pinwheel inclusion bodies form in the infected cells of the cereal crops. This cytopathological change can be used as a diagnostic character for infection of bymoviruses<sup>[4,5,11]</sup>.

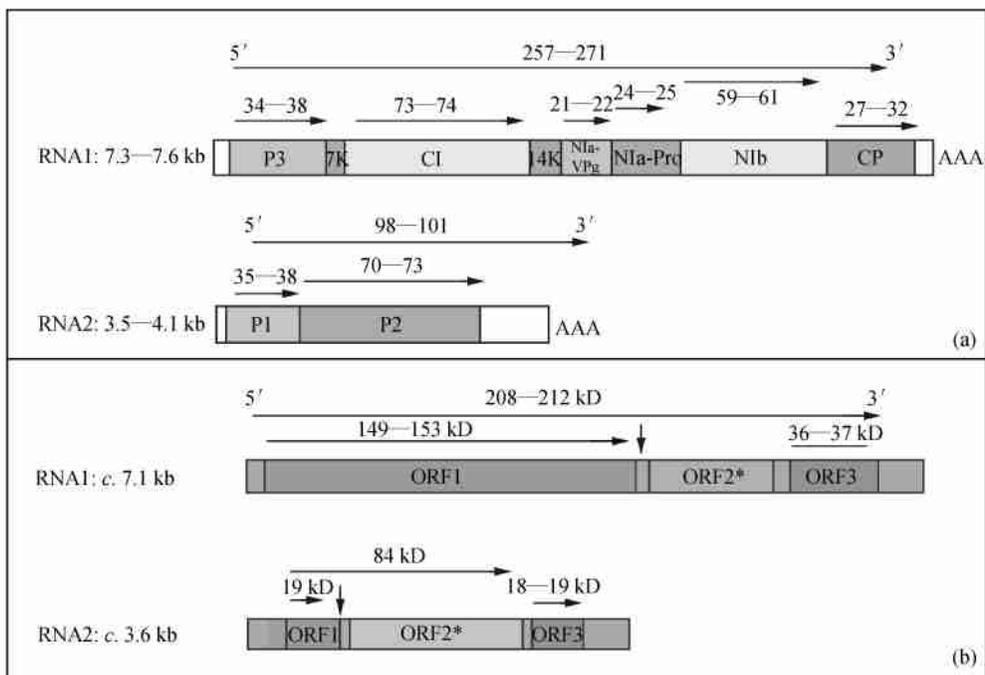


Fig. 1. Genomic organizations of genera *Bymovirus*(a) and *Furovirus*(b).

Genus *Furovirus* has not been classified to any family of plant virus. This genus used to be considered to contain all fungus transmitted rod-shaped viruses. According to species of fungal vector, num-

bers and structures of RNA genome, Torrnce and Mayo re-classified the previous genus *Furovirus* into four new genera including *Benyvirus*, *Pomovirus*, *Pecluvirus* and *Furovirus*. The new genus

*Furovirus* is characterized by rod-shaped virus particles, bipartite RNA genomes containing CP-RT gene, but not TGB genes and poly (A) tail, and transmitted by *P. graminis*. The definite members of this genus are SBWMV and sorghum chlorotic spot virus (SrCMV), and oat golden stripe virus (OGSV) was classified as a strain of SBWMV<sup>[12]</sup>. Recently, our studies demonstrate that OGSV is an independent member<sup>[13]</sup>, and two new identified species, Chinese wheat mosaic virus (CWMV) and soil-borne cereal mosaic virus (SBCMV), are different from SBWMV, and are also members of this genus<sup>[14, 15]</sup>.

Virus particles of genus *Furovirus* consist of two single-stranded positive-sense RNA genomes (Fig. 1 (b)) and a coat protein. Both RNA1 and RNA2 are necessary for virus infection and replication, and contain a cap structure (M7GpppG) at their 5'-termini, but no poly(A) tail at the 3'-termini. RNA1 contains three ORFs. The first one encodes a 149–153 kD polypeptide and the opal termination codon (UGA) can be partially suppressed to extend to the second ORF which produces a 208–212 kD read-through protein. The motifs for methyl-transferase and NTP-binding helicase activity are identified in the 149–153 kD protein whereas that for RNA-dependent RNA polymerase (RdRP) in the read-through portion. The third ORF at the 3'-terminal region encodes a putative protein of 36–37 kD that is believed to be a movement protein. RNA2 also has three predicted ORFs potentially encoding proteins of 19, 84 and 18–19 kD. The first ORF encodes the coat protein. There is an in-frame CUG codon upstream of the first AUG, which is believed to initiate a large coat protein of 25 kD, adding 40 extra amino acids to the N-terminus of the normal coat protein. The coat protein gene finishes with an opal codon that is probably read through to generate an 84 kD product (or 88 kD if it is initiated at the earlier CUG codon) that incorporates ORF2. The final ORF encodes a putative 18–19 kD cysteine-rich protein of unknown function<sup>[13, 15, 16]</sup>.

1.1.1 Barley yellow mosaic virus and barley mild mosaic virus BaYMV was first reported in Japan in 1940 and has become a severe disease of winter wheat in Japan, China, Korea and northwest Europe. In China, BaYMV was first recorded from Zhuhai Farm of Ninghai County, Zhejiang Province in the 1950s. However, the disease only became serious in the mid-1970s as a consequence of the intensive use of a new barley cultivar, Zhaoshu 3. This cultivar

was widely adopted because its short growing period and high yield made it particularly suitable for use in a cropping system having three crops each year (rice from May–August and again August–November, followed by either barley, wheat or oilseed rape from November–May). This system became widely established during the 1960s in the middle and lower regions of the Yangtze River basin and other parts of Eastern China. However, Zhaoshu 3 proved to be very susceptible to BaYMV, and by the mid-1970s, BaYMV had become serious in all the areas where the cultivar was regularly grown<sup>[17, 18]</sup>.

We have determined the complete sequence of BaYMV Yancheng isolate<sup>[9]</sup>. Sequence comparison among Chinese, German and Japanese BaYMV isolates indicated that the 5'-UTR regions have the most significant variation, and P1, P3, CL, N1a, 5'-part of CP and 3'-terminus of RNA1 also present different variations. In general, RNA2 shows a greater variation than RNA1. The P2 fragment was more variable than the CP and phylogenetic analysis of both regions showed that Asian and European isolates form distinct clusters, indicating that molecular evolution of BaYMV isolates is linked to their geographical distribution<sup>[9]</sup>.

Streatley isolate of BaMMV in the UK was originally considered to be a strain of BaYMV<sup>[19]</sup>. In China, this virus was first detected in Rudong, Haian and Nantong of Jiangsu Province in 1991<sup>[6, 18]</sup>. The virus is always present in association with BaYMV in Chinese BaYMV susceptible barley cultivar, Yanfuaizhao 3 and Zhaoshu 3 but not in 16 European and Japanese cultivars tested, indicating that the pathogenicity of Chinese BaMMV differs from Japanese and European ones, and a Chinese strain of BaMMV has long been established<sup>[18]</sup>. Using the GCG Pileup program, the available BaMMV coat protein sequences fell into three distinct groups: (1) Chinese, Korean, Japanese (Nal) isolates; (2) Japanese (Kal), German isolates; and (3) UK and French isolates<sup>[8]</sup>.

1.1.2 Wheat yellow mosaic virus and wheat spindle streak mosaic virus Yellow mosaic of wheat was first described by Sawada in 1927 in Japan, and the agent was identified as wheat yellow mosaic virus (WYMV) by Inouye in 1969. It had been confused with a similar virus which was first identified in 1960 in Canada as wheat spindle streak mosaic virus (WSSMV). These viruses have also been reported in

China, India, America, Italy, France and Germany. Because of the similarities of biological and serological properties, transmissibility and particle morphology of these viruses, Usugi and Saito suggested that these viruses were, at best, strains of the same virus and in the absence of any serological differentiation, and it was probably best to treat them as synonymous<sup>[20]</sup>. Therefore, both WYMV and WSSMV were used to describe similar viruses that occurred in wheat and rye in China and Germany.

To study the variation between isolates and to resolve the uncertainties about the identity of the virus in some countries, we have collected 20 infected wheat samples from China, Europe and North America including a rye isolate from Germany, which was identified as WYMV<sup>[21]</sup> and a typical isolate of WSSMV during 1997–1999. In RT-PCR, using primers designed from a partial sequence of a French isolate (tentatively described as WSSMV), the fragments of genomic cDNA were consistently amplified from the Italian and French isolates but not from the Chinese ones. Conversely, products were consistently obtained from the Chinese isolates but not from the Italian or French ones when using primers designed from the sequence of a Japanese isolate of WYMV. Nucleotide sequences were also obtained from regions at or near the 3'-terminus of RNA1 of the 16 Chinese isolates and 4 European and North American isolates, usually including the coat protein gene. Nucleotide and amino acid sequence comparisons demonstrated that the European and North American isolates were extremely similar and were WSSMV while the Chinese isolates were close to the Japanese isolate and were thus WYMV<sup>[7, 21]</sup>. The homology between WYMV and WSSMV groups was lower than 70% and obviously they are different viruses. In addition, we have identified 2 strains (Yaan and Yangzhou) of WYMV based on their differences of pathogenicity to some wheat cultivars, and determined their complete nucleotide sequences. Sequence analysis shows that the Yangzhou strain is more similar to the Japanese isolate<sup>[10]</sup>.

**1.1.3 Oat mosaic virus** OMV was first reported in America in 1946, and now also occurs in the UK, France, Ireland and possibly New Zealand. We have determined the complete sequence of a UK isolate of OMV<sup>[22]</sup>. Sequence comparison shows that OMV proteins are almost equally similar to the homologous proteins of BaYMV, WSSMV and WYMV but more distantly related to BaMMV. Among the 10 viral

proteins, NIb protein is most conservative, and variations are mainly distributed in the N-terminus of CP, C-terminus of CI, 14 K and P1 proteins. It appears that most of the P2 region on RNA2 is deleted during repeated mechanical transmission of the isolate. The 3'-UTR of RNA2 is very long (1262 nt) and proves to have a 532 nt slightly overlapping repeat (99.1% identical nucleotides). This is similar to results with BaMMV, in which a direct imperfect repeat of 522 nt in the 3'-UTR was identified from a sub-population of an isolate with a part of the P2 coding region deleted<sup>[22]</sup>. The occurrence of both deletion and duplication in the same isolate suggests that the laboratory isolate is more competitive without P2 region needed for fungus transmission but there is some disadvantage if the whole RNA2 becomes short. Presumably both features arise from detachment and re-initiation by the RNA-dependent RNA-polymerase<sup>[23]</sup>.

**1.1.4 Soil-borne wheat mosaic virus and Chinese wheat mosaic virus** SBWMV was first recognized in Illinois and Indiana in 1919 and identified as the cause of a major disease of winter wheat in 1923. In 1993, we determined the complete RNA2 sequence of an Oklahoma field isolate of SBWMV, and then constructed the full length cDNA clone of it<sup>[24]</sup>.

In China, a virus with similar particle morphology and serologically related to SBWMV has been known for the last twenty years from Shandong Province where it is always associated with WYMV<sup>[23]</sup>. We have now determined the complete nucleotide sequence of an isolate of this virus from Yantai, Shandong Province, and analysis shows that it only shares 76.1% homology in coat protein amino acid sequence and 71.1% full nucleotide sequence with SBWMV, therefore it is different from SBWMV, and is identified as a new member of the genus *Furovirus*, named Chinese wheat mosaic virus (CWMV)<sup>[14, 15]</sup>. Later, we have also determined the nucleotide sequences of an isolate of CWMV from Rongcheng, Shandong Province, where the infected wheat crop developed particularly severe symptom, but the two RNAs shared 95.5% identity to those of the Yantai CWMV isolate<sup>[26]</sup>.

**1.1.5 Oat golden stripe virus and soil-borne cereal mosaic virus** OGSV was first reported in the UK in 1977. Since it is serologically related but differs in host range from SBWMV, it has ever been classified as a strain of SBWMV<sup>[27]</sup>. OGSV causes oat disease in France, Britain and the USA. In Europe, there is

also a *P. graminis* transmitted rod-shaped virus from wheat and rye and it had been thought for many years to be SBWMV based on similar features of fungal transmission, symptom, serology and particle morphology.

Recently, we have determined the full sequences of OGSV and the wheat infecting furovirus from France and Italy. Both viruses have a similar genomic organization to SBWMV and CWMV, but have less than 70% nucleotide identical to them, and RNA2 has greater differences than RNA1. Obviously, these two viruses are different from SBWMV, and are separate new members of genus *Furovirus*<sup>[13]</sup>. The French and Italian isolates have been named European wheat mosaic virus (EWMV)<sup>[13]</sup>. Phylogenetic analyses supported the recognition of these isolates as distinct viruses in the genus *Furovirus*<sup>[13]</sup>. Meanwhile, Dr. Koenig and her co-workers have also determined the sequences of three isolates of fungus transmitted rod-shaped virus from South Germany, of which two isolates were from rye and the other one from wheat, and named them soil-borne rye mosaic virus (SBRMV)<sup>[28]</sup>. Sequence comparisons demonstrate that there are substantial similarities between EWMV from wheat in France and Italy and the SBRMV from rye or wheat in Germany. These isolates are best regarded as the same virus and clearly distinct from SBWMV, OGSV and CWMV. Although EWMV isolates were obtained from wheat, and SBRMV-G and SBRMV-O were from rye, there were no obvious molecular differences correlated with the host. It seems that two different names have been given independently to the same virus. Following discussions with Dr. Koenig, we consider that a different name should be chosen that does not imply restriction to a particular geographical region (Europe) or specificity to a particular, and possibly minor, host (rye). The name soil-borne cereal mosaic virus seems to meet these criteria<sup>[29, 30]</sup>.

## 1.2 Disease and host range

These viruses all cause serious diseases of cereal crops and their biological features extremely depend upon their property of fungal transmission. After sowing of cereal seeds in autumn, the resting spores of *P. graminis*, under suitable temperature and moisture in soil, germinate primary zoospores which infect root hairs and epidermal cells of the cereal crops and then transmit the viruses into the host cells. It is still not clear whether the viruses as intact

particles or RNA form to invade the host cells. However, the viral RNAs and coat proteins can be detected prior to the symptoms development on the leaves. The symptoms on infected leaves of cereals usually appear from late December to early March (Fig. 2(a) and 2(b)). The first symptoms on new leaves are chlorotic spots, which then develop gradually to become yellow and mosaic stripes. In addition, necrosis symptom also develops in March to April in some barley cultivars. With temperature rising in May, new growing leaves are symptomless and the symptoms on infected old leaves gradually disappear, however, the infected plants are stunt with fewer tillers and smaller grain heads. Loss of grain yield, usually 10%–90%, depends on the degree of virus infection, as well as cultivar, climate condition and type of soil. Temperature controls growth of plants and movement of the viruses from roots up to leaves, therefore is the main factor for disease development and symptom appearance. Symptom appearance also differs on different cultivars of cereal crops, usually, the susceptible cultivars show severe symptoms whereas the resistant ones show mild even no symptoms. Successive growing of susceptible cultivars on a large scale is the main reason for outbreak of the diseases.

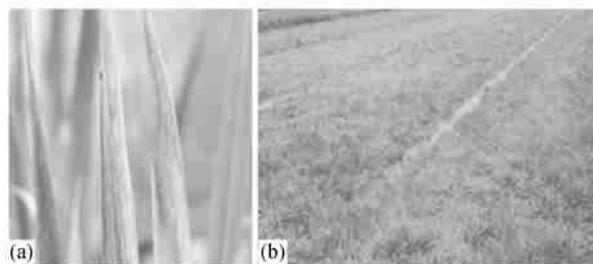


Fig. 2. Symptoms of wheat yellow mosaic virus infected wheat leaves (a) and wheat field (b).

Most of the cereal viruses with the fungal vector have a narrow host range. Under natural conditions, BaYMV and BaMMV infect barley only, WSSMV, WYMV and CWMV wheat only, but CWMV also infects tobacco by mechanical inoculation. OMV and OGSV only infect oats. SBWMV has a wider host range and infects wheat and barley. Japanese SBWMV infects wheat and barley as well as tobacco and maize. SBCMV naturally infects rye and wheat. Differences in the genomic sequences of the viruses are not related to their origins of the natural hosts.

These viruses can survive in the air dried resting spores of *P. graminis* for many years. The period of survival tested for the following viruses is 9 years for

OGSV, 10 years for SBWMV, 10 years for BaYMV, 5 years for OMV and 5 years for WSSMV.

### 1.3 Particle morphology

Particles of genus *Bymovirus* are filamentous with typical lengths of 275–300 nm and 600–625 nm and 12–13 nm in diameter (Fig. 3(a)). The lengths of a few particles of purified viruses often exceed 2000 nm because of end-to-end aggregations of the particles<sup>[3,5]</sup>. Particles of genus *Furovirus* are rod-shaped and their lengths range mainly in 281–300 nm and 138–160 nm with the diameter of 20 nm (Fig. 3(b))<sup>[14,25]</sup>.

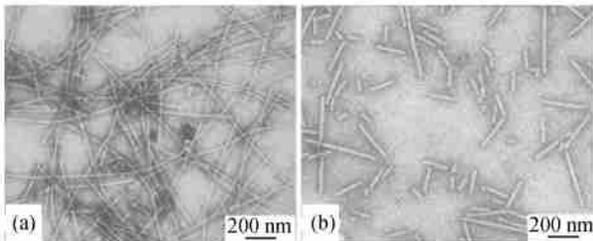


Fig. 3. Purified particles of wheat yellow mosaic virus (a) and Chinese wheat mosaic virus (b).

### 1.4 Serological affinities

Members of genus *Bymovirus* have no serological relationship with those of genus *Furovirus*. Among the members of genus *Bymovirus*, BaYMV is serologically related to WSSMV and WYMV, WSSMV and WYMV appear to be similar, and OMV and BaMMV have no affinities to any members of the genus<sup>[34]</sup>. Among the members of genus *Furovirus*, they are closely serologically related to each other. We have produced polyclonal antiserum and monoclonal antibodies against some members of this genus for studying serological relationship between them. The results indicated that there are some differences of epitopes distributed on coat proteins of different viruses<sup>[32]</sup>. CWMV and OGSV share the common epitopes on the coat proteins at the 30–40th amino acids while the common epitopes shared by SBWMV, CWMV, SBCMV and OGSV are located at C-terminal half of the coat proteins<sup>[32,33]</sup>. We have also produced 7 monoclonal antibodies against SBWMV. These antibodies have been successfully used to distinguish the different origins of SBWMV isolates<sup>[33]</sup>. The coat proteins of both SBWMV Lab 1 and SBWMV-F differed from SBWMV Okl-WT and Okl-7 by one amino acid near the N-terminus (Gly to Ser at position 6), and the monoclonal antibody SCR

134 reacted only with SBWMV Okl-WT and Okl-7. This can be explained by assuming that SCR 134 reacts with an epitope that contains Gly-6 near the N-terminus of the coat protein and this conclusion is supported by the Pepscan data. While monoclonal antibody SCR 132 reacted with three isolates of SBWMV (SBWMV-Lab 1, Okl-WT and OKL-7) but not SBWMV-F, which differs from the previous three isolates towards the C-terminus (Tyr-159 and Thr-160 were changed to Ile and Ser, respectively). Thus, it is possible that the SCR 132 epitope contains the amino acids Tyr-159 and Thr-160. The monoclonal antibody SCR 133 reacted with a continuous epitope located at one end of the liner coat protein sequence. The epitope was exposed on the surface along the sides of the particles and was readily removed by trypsin-treatment. Since SCR 133 reacted with all four isolates, it seems unlikely that it discriminates the SER-6 for Gly-6 change in SBWMV-F and SBWMV-Lab1. It is, therefore, probable that it reacts with an epitope near the C-terminus, the amino acid residues of which are identical in SBWMV Lab1, SBWMV Okl-WT and SBWMV Okl-7<sup>[33]</sup>.

### 1.5 Deletion mutants

In 1994, we found that repeated passage of SBWMV by manual inoculation resulted in deletion of part of SBWMV RNA2. Deletion was apparent in the population of RNA2 molecules after 11 weeks in primary inoculated wheat plants and after 5 passages no full-length RNA2 remained. In earlier passages (passages 1–4), plants generated several deleted forms of RNA2 whereas after passage 5 only a stable one remained. All naturally deleted forms were cloned and the sequences flanking each deletion site were compared. The results indicated that all deletions occurred within the region encoding the coat protein read-through domain and the 5'-site of each deletion laid in the region between genome coordinates 1417–1465 while the 3'-sites were very different. After 5th mechanical passage the stable 759 nt deletion became dominant<sup>[24]</sup>. The results also showed that, in general, the smaller deletions are not intermediates in the larger deletion process<sup>[35]</sup>. Plants infected by *P. graminis* and maintained at high temperatures (25–30 °C) also showed extensive deletions in the RT domain of CP-RT gene within 4–12 weeks. In contrast, plants kept at 17 °C over the same period contained only full-length RNA2 molecules. Our observations confirm that only full-length SBWMV RNA2 is transmitted to wheat roots by viruliferous

*P. graminis* from field soil and there is no intraplant barrier to the movement of deleted forms of RNA2 between roots and leaves. Deleted forms of SBWMV RNA2 appear to cause more severe symptoms only after mechanical inoculation to young, healthy plants. These studies may also help elucidate the mechanism of spontaneous RNA deletions<sup>[34]</sup>.

For deletion formation in SBWMV we hypothesize that during synthesis of plus-strand RNA the viral replicase pauses and dissociates at minus-strand structures present at the 5' site. Subsequent reassociation of the replicase occurs at various 3' sites which are located randomly (as far as we can tell) in the minus-strand RNA<sup>[35]</sup>. This model is similar to those proposed to explain the formation of deletions within the genomic RNAs of some animal virus.

In addition, we have also identified similar deletion mutants from the mechanically inoculated OGSV and CWMV in which the deletions occur with RT domain of RNA2, but that of the mechanically inoculated OMV occurs within P2 gene as described above. It seems that the deleted forms of all viruses are not transmissible by fungus<sup>[12,22]</sup>.

## 1.6 Pathogenicity

Disease control relies almost exclusively on the deployment of resistant crop cultivars. For many of the fungus-transmitted cereal viruses, little is known about the interactions between virus strains and host genotypes or the viral determinants of virulence. The only significant exception is for the bymoviruses of barley, BaMMV and BaYMV. Seven different strains of BaYMV (in four groups) have been identified in Japan based on the response of different cultivars. In Europe, two strains are recognized on the basis of the response of cultivars carrying the *rym4* resistance gene and the resistance-breaking strain, usually named BaYMV-2, is becoming increasingly important. In the 1990s, we have examined the responses of some selected barley cultivars to BaYMV at 10 sites distributed in different areas of the disease in China and found that at least 6 strains probably present<sup>[36]</sup>. To rapidly detect and diagnose these strains, we have developed some molecular techniques including RT-PCR-single-strand conformation polymorphism (SSCP) and restriction mapping<sup>[9,37,38]</sup>.

For WYMV, we carried out field experiments at 7 sites including Yangzhou, Huangchuan, Yantai,

Rongcheng, Luotian, Hanzhong, and Ya'an in China, and found that the responses of virus isolates at Yangzhou and Ya'an sites to some selected wheat cultivars were very different. For instance, some cultivars (e.g. Brindur, Italo, Vona) were susceptible at both sites, while others (e.g. Hokushin, Newton, Pascal, Tremie and possibly Ernie) appeared to be resistant at both. At Yangzhou site, many plants of Colosseo, Colfiorito, Platani and Akakomugi were infected but these cultivars were not, or only slightly infected at Ya'an. Conversely, Haruyutaka was infected at Ya'an, but not at Yangzhou<sup>[10]</sup>. The field experiments therefore suggest that the two isolates of WYMV differ in their virulence to a range of cultivars. In Japan, Akakomugi is susceptible to WYMV-T but not to WYMV-H, while Haruyutaka is susceptible to both strains, but this pattern is not matched at either of the Chinese sites and their strains are therefore probably different from those reported in Japan<sup>[39]</sup>.

## 2 *Polymyxa graminis*

### 2.1 Life cycle

In 1939, Ledingham first discovered *Polymyxa graminis* on wheat in Canada. Since then, he spent 9 years to observe morphology and life cycle of this fungus<sup>[40]</sup>. In the last decade, we have systematically studied the development of this fungal vector by electron microscopy<sup>[41-44]</sup>. The life cycle of the fungal vector consists of biflagellate zoospores (Fig. 4 (a)), multinucleate plasmodia, zoosporangia and thick-walled resting spores in clusters or cystosori (Fig. 4 (b)). Thick-walled resting spores are formed in plant roots and remain in the soil when the roots decay. The primary zoospores, produced by resting spore germination, swim to host root hairs or epidermal surfaces when they encyst, a process that involves withdrawal of the flagella, adhesion to the host wall and secretion of a thin cyst wall. The zoospore protoplast is injected into the host cell, where it divides into a multinucleate plasmodium (sporangia plasmodium) and develops into zoosporangium. This sac-like structure liberates secondary zoospores (which do not differ morphologically from the primary zoospores) into soil water or perhaps to penetrate deeper into the host root, thus continuing the infection cycle. The factors leading to resting spore formation are not understood; both external factors (e.g. host nutrition, stress) and internal ones (karyogamy) have been invoked. In early stages of development, there is no ap-

parent difference between sporogenic and sporangial plasmodia. The first evidence of sporogenesis is the development of synaptonemal complexes and this is followed by meiotic division and formation of thick-walled resting spores. However, when and where cell fusion and karyogamy occur remains a mystery in the life cycle of the fungus.

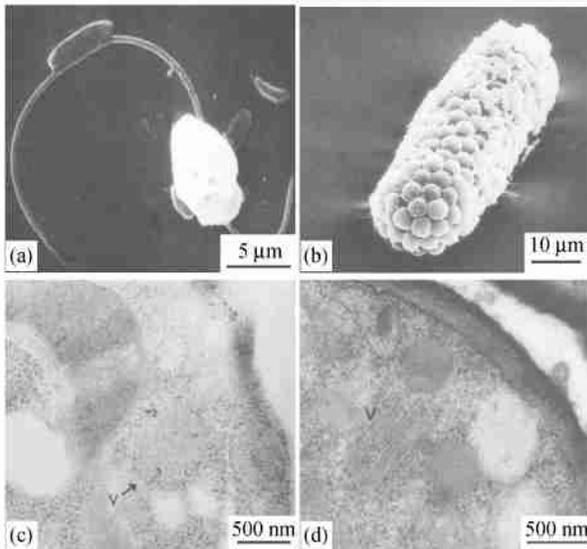


Fig. 4. *Polymyxa graminis*: (a) Zoospore; (b) resting spores; (c) barley mild mosaic virus particles inside zoospore and (d) barley yellow mosaic virus particles inside resting spore. (a) and (b) are provided by M. J. Adams.

## 2.2 Epidemiology

We have studied the effects of environmental factors on the development of the fungal vector, *P. graminis* in the greenhouse using irrigated sand cultures. The isolates collected from the temperate regions were able to grow and infect barley roots over a wide range of temperature, but the fastest development occurred at 17–20 °C and it only took about 2 weeks from inoculation with resting spores to produce secondary zoospores. The isolates from tropical regions, which transmit peanut clump virus, had a higher optimum temperature of 27–30 °C.

*P. graminis* has been detected in roots of cultivated wheat, barley, rice, oats, rye, maize and sorghum. It has also been detected in the roots of Bermuda grass and creeping bent grass as well as various temperate *Agrostis*, *Dactylis*, *Festuca*, *Poa* and *Phleum* species. This is worrying because all these wild grass species might serve as reservoirs for *P. graminis*, the extent to which different isolates can infect all these hosts is largely unknown<sup>[49]</sup>. Most

isolates studied in detail have originated from barley, and they multiplied best on barley and usually less well on wheat; isolates from wheat may have multiplied better in wheat than did the barley isolates and none of these isolates multiplied appreciably in oats<sup>[49]</sup>. Further work is needed to determine the degree of specialization to different hosts and to explore further the susceptibility of weed hosts to the viruses that the fungal vector transmits. In addition, it is possible to determine the absolute numbers of cystosori and the germination ratio of the resting spores of *P. graminis*. Our studies showed that the fungus has a great potential of infection. In baiting test using infested soil, the dilution endpoints for detecting WYMV on wheat roots were 1/625–1/15625, and for resting spores of the fungus vector, *P. graminis*, 1/3125–1/15625<sup>[45]</sup>. Electron microscopic observations showed that resting spores of the fungus collected from roots of wheat after harvest were mostly empty, usually associated with many bacteria, suggesting that germination had occurred *in situ*<sup>[43, 44]</sup>.

## 2.3 Relationship between viruses and the fungal vector

Since the 1960s, scientists in the international society of plant pathology have been continuing to explore the relationships between the fungal vectors and plant viruses they transmit although the progress is rather slow. Virus transmission occurs by zoospore, but in one of two different ways. Those viruses transmitted by *Oplidium brassicae* are acquired from soil water on the out surface membrane of zoospores. The virus particles appear to enter the zoospore through the infection canal but they do not enter the resting spore during its formation. By contrast, all viruses transmitted by *Plasmodiophoromycetes* including *P. graminis* are acquired from plant cells and enter the resting spores of vectors and released with zoospores on germination. Members of genera *Bymovirus* and *Furovirus* belong to the second type of transmission, but how and when *P. graminis* acquires viruses and how viruses enter the host plant cell cytoplasm are unknown. However, it is likely that these processes are taking place either when zoospore penetrates the host cells and transfers their contents into the host cell cytoplasm, or at the sporogenic plasmodia stage of *P. graminis* development when there is only a thin membrane boundary separating the plasmodioplodid from the host cell cytoplasm<sup>[49]</sup>. Acquired viruses are thought to be carried inside the fungus spores and cannot be removed from zoospores by

washing or treatment of antiserum, or inactivated by application of NaOH and HCl. Using immunogold labeling electron microscopy technique in 1991, we observed MaMMV particles inside a very small portion (c. 1%) of zoospores and zoosporangial plasmodia of *P. graminis* (Fig. 4(c)) although it is proved that it transmits the virus efficiently<sup>[47, 48]</sup>. Seven years later, we also found BaYMV-like particles inside a few resting spores of the fungus (Fig. 4(d))<sup>[44]</sup>. This is the first evidence in which immunogold labeling has been used to confirm the identity of a virus in its fungal vector.

### 3 Disease resistance

Although *P. graminis* is an obligate parasite of cereal roots, it is not considered a pathogen because it does not cause any disease, and does not seem to reduce crop yield. However, it transmits several plant viruses that do cause serious diseases of cereal crops. Since virus-containing resting spores of *P. graminis* persist in soil and crop debris for decades, cultural practices such as crop rotations or delayed sowing for virus control are of little value, whilst chemical control methods are unacceptable for ecological and economic reasons. Therefore, application of resistant cereal varieties offers the only practical and economically friendly measure of control. In the past decade, we carried out a project of screening resistant genotypes immune to bymoviruses and furoviruses from a large number of gemsplasm of wheat and barley and found 4 Japanese local barley varieties (Chosen, Hagne Mugi, Iwate Mensumy 2 and Mokusekko 3) and a European one (Energy) immune to bymoviruses of barley from China, Japan and Europe, and these resistant resources have been used in barley breeding programmes<sup>[18, 21]</sup>. In addition, we have also identified 47 foreign wheat varieties (Chisholm, Victory etc.) resistant to WYMV and 4 (Karl, Larned, Pandas and Hawk) resistant to CWMV in China<sup>[26]</sup>. Cereal crop cultivars with good resistance to one or more of fungus-transmitted viruses are now commercially available, but gemsplasm of wheat and barley which are immune to *P. graminis* have not been available, or need to be further confirmed. Nevertheless, resistance to the fungal vector could provide the opportunity to control all the viruses which the fungal vector transmits simultaneously and is therefore an attractive idea<sup>[49]</sup>. Some gemplasm of *Hordeum bulbosum* appears to be resistant to *P. graminis* and might provide a source of resistance for barley breeding<sup>[50]</sup>.

### 4 Prospects

Research progress in understanding and controlling the virus diseases has been rather slow because of the difficulties of studying with an obligate, root infecting fungal vector. However, these challenges must be met if many of the intriguing questions about the diseases are addressed. The following areas are of particular interest and relevance: Molecular studies are required to clarify various aspects of the taxonomy and phylogeny of the plasmodiophorids; the biological differences such as host range, transmissibility of different viruses, and difference between the various isolates of *P. graminis* need to be further studied; mechanisms of germination of the resting spores and recognizing hosts of the zoospores, molecular mechanism of requiring and transmission of the viruses by the fungal vector, molecular mechanism of viral pathogenicity, mechanism of resistance of cereal crops to the viruses, and mechanism of resistance breaking of the cereals to the viruses are all important issues to be addressed. Answering these questions has great scientific value to understand the interactions among the fungal vector, viruses and host plants, and to establish new systems of disease control.

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