PROCEEDINGS OF THE SEVENTH SYMPOSIUM OF THE INTERNATIONAL WORKING GROUP ON PLANT VIRUSES WITH FUNGAL VECTORS

Editor: C.M. Rush

Julius Kühn-Institute
Federal Research Centre for Cultivated Plants
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PREFACE

The International Working Group on Plant Viruses with Fungal Vectors (IWGPVFV) was formed in 1988 at Kyoto, Japan, with Dr. Chuji Hiruki as chairperson, Drs. Renate Koenig, John Sherwood, Gail Wilser, and most recently Ueli Merz also have served as Chairperson. Currently, there are approximately 65 members, representing 16 countries. The goal of the working group is to provide a forum to facilitate international collaboration and multidisciplinary research on all aspects of plant viruses with fungal vectors. Members of the group meet every three years to present research on a wide variety of topics including virus taxonomy and characterization, vector biology and ecology, virus-vector interactions, and disease epidemiology and management. The high quality of information presented in an informal setting to an international group with common interests always makes for an enjoyable, professionally rewarding meeting.

Symposia of the working group have been held at the Biologische Bundesanstalt (BBA), Braunschweig, Germany (1990), McGill University, Montréal, Canada (1993), West Park Conference Centre, University of Dundee, Dundee, Scotland (1996), Asilomar Conference Center, Monterey, California (1999), Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland (2002), Alma Mater Studiorum, Universita Di Bologna, Bologna, Italy (2005), and Julius Kühn Institute Federal Research Centre for Cultivated Plants, Quedlinburg, Germany (2008). This volume serves as a record of material presented at this most recent meeting. It is hoped that the information in this Proceedings will be useful to members of the IWGPVFV and all those interested in viruses with fungal vectors.

The IWGPVFV is a totally volunteer group and success of its meetings is in large part a result of the hard work and contributions of the local organizing committee and sponsors. The 2008 meeting in Quedlinburg was no exception, and the hospitality and good times will be remembered fondly. Those most responsible for the success of the recent meeting are

listed on the next page. The names and e-mail addresses of the current program committee are also listed. Please contact a member of the program committee if you wish to be included in any future mailings of the IWGPVFV. The next symposium is scheduled to be held in Louvain-la-Neuve, Belgium during 2011 (local organizer Dr. Claude Bragard). Further details will be posted at the IWGPVFV website http://www.rothamsted.bbsrc.ac.uk/ppi/Iwgpvfv as they become available.

Charlie Rush
Proceedings Editor

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DISTRIBUTION AND CHARACTERIZATION OF IRANIAN *BEET BLACK SCORCH VIRUS*

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Summary

The Iranian provinces where sugar beet is cultivated have been surveyed for the presence of *Beet black scorch virus*. Sixty two samples (31%) out of two hundred and three samples showed positive response by RT-PCR in ten out of thirteen Iranian provinces (Khorasan Shomali, Khorasan Razavi, Ghazvine, Zanjan, Ardabil, Azarbijaan Sharghi, Azarbijaan Gharbi, Kermanshah, Hamadan and Kerman). The BBSV satellite was found in nineteen samples (9.3 %) out of 203 in all provinces except Azarbaeijan Shargi. The presence of BBSV was systematically associated by the presence of BNYVV and/or other soil-borne pomoviruses. The complete genome sequences of three Iranian BBSV isolates have been determined. The three isolates, one with satellite designated BBSV-Ha1 and two without satellite designated BBSV-AzGh and BBSV-Ha2 showed 97 % nucleotide identity between the first two and 88 % between the two first and third one. The identity between two Iranian isolates of BBSV-AzGh, BBSV-Ha1 and one isolate of BBSV-Ha2 by both genomes of the 'Ningxia' isolate of BBSV (BBSV-N) from China and 'BBSV-Co' from US were 89 % and 88 % respectively in nt level of whole genomes. In all of the cases Iranian BBSV was accompanied by BNYVV causal agent of rhizomania.

Introduction

Beet black scorch virus (BBSV) was first identified in Inner Mongolia, China, in the late 1980s (Cui *et al*., 1988; Liu and Xian, 1995; Zhang *et al.*, 1996). It was described as a new *Necrovirus* species (Cao *et al*., 2002; Lommel *et al*., 2005). Recently it was reported from the US (Weiland *et al*., 2007) and from Iran (Koenig and Valizadeh, 2008).

The virus elicits severe systemic disease symptoms described as black scorched leaves of sugar beet (*Beta vulgaris* L.) in China. The BBSV isolates reported from the US did not show such a black scorching in leaves, but severe symptoms of Rhizomania-like rootlet proliferation (Weiland *et al*., 2007). Symptomless infections have been observed on sugar beet. The virus can be detected in each of these hosts by RT-PCR and by retro-inoculation to *Chenopodium quinoa*.

The virus belongs to the *Tombusviridae* family and is transmitted efficiently through the soil in a non-persistent manner by zoospores of *Olpidium brassicae* (Jiang *et al*., 1999). The virus comprises an icosahedral particle of 28 nm that encapsidates a positive-sense, single-stranded (ss) genomic (g) RNA of circa 3644 nt. BBSV shares the highest sequence identity (61%) with *Tobacco necrosis virus* D (TNV-D) (Coutts *et al*., 1991; Bo *et al.*, 1996).
Normally, only a single RNA is associated with infections, but a small single-stranded RNA (ssRNA) that is similar to the satellite RNAs (sat-RNA) of other necroviruses has been found in isolates from the Xinjiang province of China (Guo *et al*., 2005).
To date, the extent of BBSV distribution has not been reported. The aim of the present study is to appraise the BBSV and its satellite distribution in Iran, with a special attention to other *Polymyxa*-transmitted soil-borne viruses of sugar beet.

Materials and Methods

Plant material and virus isolates

Two hundred and three sugar beet root samples were collected either directly from the field or from bioassays (Meunier *et al*., 2003). Samples were localized by GPS (Global Positioning System) and a picture from the field sampled as well as from the gathered samples was taken. Rootlets from infected sugar beet and single chlorotic lesion of *C. quinoa* leaves were used for RNA extraction using a SV Total RNA Extraction Kit (Promega). Approximately 171 mg of rootlets or leaves were ground in 1000 µL of lysis buffer in a sterile pestle and mortar (According to Kit Instruction adjustments) and RNA extracted as described in the manufacturer's manual; RNAs were used directly or stored at minus 70 °C.

RT-PCR and Sequencing

For the detection of BBSV, primers by Weiland *et al.* (2007) were used, which amplify a fragment of 315 bp. within the 3' UTR of BBSV RNA. Two hundred nanograms of purified PCR product (DNA), 1 µL of primer (4 µM) and 4 µL of Biomol, mixed with water (Amersham Pharmacia Biotech) were mixed and sequenced. Sequences were analyzed by the Clustal W program of the Genetic Computer Group.

Viral genome characterization and sequence comparison

Plasmid pBBSV-Ha1, pBBSV-Ha2, pBBSV-Msh, pBBSV-AzGh, pBBSV-Ksh were subjected to double-stranded sequencing using primers positioned at an average of 400-bp intervals and the sequence assembled using vector NTI Advance 10 program (Invitrogen) and ORF finder program (NCBI), Major ORFs present within the sequence of BBSV-Ha1, BBSV-Ha2, pBBSV-Msh, BBSV-AzGh, pBBSV-Ksh were detected using NTI Advance 10 program (Invitrogen) and ORF finder program (NCBI). Sequence alignments were performed using Clustal W.

Results

In September 2006, a survey was carried out in all sugar beet growing areas in Iran, to assess the current of rhizomania. When the 203 gathered samples were tested for the presence of BBSV, the virus was found in 62 (31 %) of the tested Iranian sugar beet field. It was distributed in Khorasan Shomali, Khorasan Razavi, Zanjan, Ghazvine, Ardabil, Azarbaeijan Sharghi, Azarbaeijan Gharbi, Kermanshah, Hamadan and Kerman (Fig. 1). In terms of symptoms mostly it was mixed by BNYVV symptoms which are root proliferation but in greenhouse some extend of leaves black scorch on sugar beet leaves just in BBSV-AzGh isolate appeared.

Also the satellite of BBSV was found in the nine provinces of Ardabil, Azarbaeijan Gharbi, Ghazvine, Zanjan, Hamadan, Kerman, Kermanshah, Khorasan Razavi and Khorasan shomali. In some cases we have seen some huge damage to the sugar beet production in the field because of a strong presence of BNYVV, BSBV, BVQ and BBSV which may explain a synergistic affect on sugar beet yet to be established. BBSV satellites in different Iranian provinces, proportionally to the extent of the sugar beet cultivated area.

Fig. 1. Distribution and number of infected and uninfected sugar beet samples by BBSV.

The highest incidence rate was found in the Khorasan Razavi (35%) and Kermanshah (16 %) provinces followed by Khorasan Shomali (11 %), Azarbaeijan Gharbi (9.6 %), Hamadan (8 %) and Ghazvine (8 %). Ardabil and Kerman (3.2 %) and Zanjan and Azarbaeijan Sharghi (1.6 %) were less affected by the virus (Fig. 1).

Mechanical inoculation of *C. quinoa* resulted in the production of the typical necrotic local lesions with chlorotic halos at three to five days post inoculations, but occasionally extended in necrotic areas on most of the inoculated leaves, also in BBSV-AzGh isolate symptoms were more systemic and extended along with leaves main veins. Transfer of the infection to leave of sugar beet resulted in no symptoms. The complete nucleotide sequences and genome organization of BBSV-AzGh, BBSV-Ha2 and BBSV-Ha1 as well as it's satellite were determined and compared with that of BBSV-CO, BBSV-N, -X, and other viruses. Linear alignments of the 3,644-nt genomes of BBSV-AzGh, BBSV-Ha1 with BBSV-CO, BBSV-N and BBSV-X using clustal W indicated a 89 % identity between the genome of these virus isolates, For BBSV-Ha2 it was 88%.

We detected also the satellite of BBSV in Iranian BBSV-Ha1. This BBSV satellite has already reported from China but not from US isolate. The satellite was detected in 30% of the samples positive for the presence of BBSV. Iranian BBSV was mostly accompanied by BNYVV, the casual agent of sugar beet Rhizomania.

Discussion

Up to now, BBSV has only been found in China, Iran and U.S.A. (Weiland *et al.*, 2007; Koenig and Valizadeh, 2008). To date, there is no data publicly available on the distribution of the virus and its economical importance, though, by comparison with BNYVV, another soil-borne virus of sugar beet, it might be inferred as of strategic stake for the future, especially in warmer areas. The recent discovery of the BBSV raised also the question of its origin. In this study, the widespread occurrence of BBSV, alone or with its satellite, in most sugar beet growing areas of Iran was assessed by bioassays and RT-PCR. The virus is present in 10 provinces and was recorded in 31 % of the samples analyzed while the satellite was detected in only 8.8%.

The analysis of the BBSV diversity in Iran reveals a wider diversity than the one reported from China or the U.S.A.

Approximately ¼ of the BBSV-containing samples found in Iran are associated with the virus satellite. Such satellite has been found to enhance the symptom expression, though the symptoms observed were mostly similar to rhizomania symptoms, instead of the beet black scorch symptoms initially described by Cao *et al*. (2002). Weiland *et al*. (2007) did observe similar results in the States, though the sugar beet in their trials were not contaminated by BNYVV. This raised the question of the presence of other soil-borne viruses in co-infection with the BBSV and its satellite.

In Iran, BBSV was always found together with other *Polymyxa*-transmitted soil-borne viruses. Mostly, BBSV is associated with *Beet necrotic yellow vein virus* alone or in combination with sugar beet infecting pomoviruses, *Beet soil-borne virus* and *Beet virus Q*. Such association is possibly an explanation for the observation of the root beardedness also noted by Koenig and Valizadeh (2008) and Weiland *et al*. (2007). The co-occurrence of up to four different soil-borne viruses is also raising questions about potential synergism or competition.

In terms of distribution, the prevalence of BNYVV is much stronger than the one of BBSV. A possible explanation for this is the difference of vector between both viruses. Though the lack of information on the host range and potential biotypes of *Olpidium* found in association with sugar beet, several reports mention the association. Interestingly, several reports have also documented TNV occurrence on sugar beet in the past, and it might be worth exploring if these were not related with BBSV, since a weak cross reaction between both viruses was found by ISEM (Cao *et al*., 2002).

The quasi absence of BBSV infection in the warmer provinces of Fars is possibly due to the fact that *O. brassicae* zoospore are less tolerant to warmer temperature than other *Olpidium* (Gharbi and Verhoyen, 1993, Campbell and Lin, 1976).

References

Bo, Y.-X., Cai, Z.-N., Ding, Q., Yu, J.-L. and Liu, Y. (1996). *Characterization of beet black scorch virus* RNA, partial cDNA cloning and sequencing of the small component. *J. Agric. Biotechnol.* **4**, 269-276.

Campbell, R.N. and Lin, M.T. (1976). Morphology and thermal death point of *Olpidium brassicae*. *Amer. J. Botany* **63**, 826-832.

Cao, Y.-H., Cai, Z.-N., Ding, Q., Li, D.-W., Han, C.-G., Yu, J.-L. and Liu, Y. (2002). The complete nucleotide sequence of *Beet black scorch virus* (BBSV), a new member of the genus Necrovirus. *Arch. Virol*. **147**, 2431-2435.

Coutts, R.H., Rigden, J.E., Slabas, A.R., Lomonossoff, G.P. and Wise, P.J. (1991). The complete nucleotide sequence of *Tobacco necrosis virus* strain D. *J. Gen. Virol*. **72**, 1521-1529.

Cui, X.-M., Gong, Z.-X. and Yu, N.-J. (1988). Studies on two virus isolates from sugar beet in Xinjiang. *J. Shihezi Agric. Coll*. **10**, 73-78.

Gharbi, S. and Verhoyen, M. (1993). Sterilization by UV irradiation of nutrient solutions with a view to avoiding viral infections transmitted by *Olpidium brassicae* in hydroponic culture of lettuce. Mededelingen Faculteit Landbouwwetenschappen. *Universiteit Gent* **58**, 1113-1124.

Guo, L.-H., Cao, Y.-H., Li, D.-W., Niu, S.-N., Cai, Z.-N., Han, C.-G., Zhai, Y.-F. and Yu, J.-L. (2005). Analysis of nucleotide sequences and multimeric forms of a novel satellite RNA associated with *Beet black scorch virus*. *J. Virol*. **79**, 3664-3674.

Jiang, J.-X., Zhang, J.-F., Che, S.-C., Yang, D.-J., Yu, J.-L., Cai, Z.-N. and Liu, Y. (1999). Transmission of *Beet black scorch virus* by Olpidium brassicae. *J. Jiangxi Agric. Univ*. **21**, 525–528.

Koenig, R. and Valizadeh, J. (2008). Molecular and serological characterization of an Iranian isolate of *Beet black scorch virus. Arch. Virol*. **153**, 1397-1400.

Liu, J.-X. and Xian, H.-Q. (1995). Preliminary report on *Beet black scorch virus*. *China Sugarbeet* **3**, 30–31.

Lommel, S.A., Martelli, G.P., Rubino, L. and Russo, M. (2005). Tombusviridae. In: *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 906–936. Edited by C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball. London: Elsevier/Academic Press.

Meunier, A., Schmit, J. F., Stas, A., Kutluk, N. and Bragard, C. (2003). Multiplex RT-PCR for the simultaneous detection of *Beet necrotic yellow vein virus*, *Beet soil-borne virus*, *Beet virus Q* and their vector *Polymyxa betae* KESKIN on sugar beet. *Appl. Environ. Microbiol*. **69**, 2356-2360.

Weiland, J., Winkle, D., Edwards, M. C., Larson, R. L., Shelver, W. L., Freeman, T. P. and Liu, H. (2007). Characterization of a U.S. Isolate of *Beet black scorch virus*. *Phytopathology* **97**, 1245-1254.

Zhang, K.-W., Wang, S.-L., Cai, Z.-N. and Zhang, H.-S. (1996). Preliminary study on beet black scorch disease in Ningxia Province. *China Beet Sugar* **2**, 3–8.

THE COAT PROTEIN-READTHROUGH OF *BEET VIRUS Q* IS LONGER

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Summary

Compared to other closely related furoviruses *sensu lato*, the *Beet virus Q* (BVQ) coat protein (CP) readthrough (RT) domain is reported to be much shorter, allowing place for two small ORFs coding for proteins of 9 and 18 kDa. However, the partial sequencing of 10 BVQ sources from six countries evidenced a longer RT domain for the CP, with three distinct nucleotide additions of 5, 285 and 1 nt being responsible for this rearrangement. It is hypothesized that this special pattern of deletions in the RNA-2 of BVQ previously described resulted from multiple inoculations cycles on *Chenopodium quinoa*. This triple deletion in the coat protein RT domain is, however, specific to BVQ, compared with *Beet necrotic yellow vein virus* (BNYVV), *Potato mop top virus* (PMTV) and *Soil-borne wheat mosaic virus* (SBWMV) where only single-deletion events have been reported.
The 10 strains were reported to share a very high similarity of sequence, at the nucleotide as well as the amino acid level.

Introduction

Two soil-borne *Pomovirus*, the *Beet soil-borne virus* (BSBV) and the *Beet virus Q* (BVQ), are often associated with the rhizomania syndrome and its causal agent, the *Beet necrotic yellow vein virus* (BNYVV). Their pathogenic role and their frequent occurrence on sugar beet, even in symptomless associations, is still a matter of debate (Prillwitz and Schlösser, 1992; Kaufmann *et al*., 1993; Lindsten, 1993; Rush and Heidel, 1995).
The coat protein (CP) readthrough (RT) domain is thought to play a major role in the transmission by *Polymyxa betae*, the vector (Tamada and Kusume, 1991; Diao *et al*., 1999; Adams *et al*., 2001). Therefore, the genomic organization described for the BVQ RNA-2, different from the one of BSBV, with a truncated readthrough domain and two small ORFs encoding for 9 and 18 kDa proteins (p9, p18), is raising questions (Koenig *et al*., 1998). Here is reported the common organization of BVQ CP-RT domain for 10 viral strains from six countries.

Materials and Methods

Viral RNAs of BVQ were purified as described by Meunier *et al*. (2003), using soils with rhizomania symptoms from Belgium (Beclers, Mazy, Seneffe, Silly, Walviert), France (Pithiviers), Germany (Langendorf), Hungary (Tompaladony), Spain (Ciudad Real) and The Netherlands (Kamperland). The region between the STOP codon of the coat protein and the 3' end of the RNA-2 was amplified for each BVQ strain by RT-PCR using Expand Reverse Transcriptase (Roche, Basel, Switzerland) and Expand Long Template PCR System (Roche). The 10 amplicons were purified from gel using QIAquick Gel Extraction Kit (Qiagen, Venlo, The Netherlands) and subsequently sequenced with four pairs of primers, forward and reverse, in an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA). Consensus sequences were obtained with ContigExpress and Vector NTI version 7.1 software (Invitrogen, Carlsbad, USA).

Results

The sequencing results revealed a different arrangement for the coat protein RT domain of *Beet virus Q* from the one described to date (Fig. 1) (Koenig *et al*., 1998). The sequenced RT was longer, with 291 nucleotides (nt) within three distinct nucleotide additions I1, I2 and I3 being responsible for this rearrangement (Fig. 1). Five additional nucleotides were located at positions 864-868, almost 100 base pair before the putative 35 kDa coat protein RT domain STOP codon (975-7) (Fig. 1, addition I1). A supplement of 285 nucleotides was present at position 1223-1507, that is, in the STOP codon of the putative p9 coding sequence (Fig. 1, addition I2). Finally, a single-nucleotide addition was noted at position 2043 (Fig. 1, addition I3).

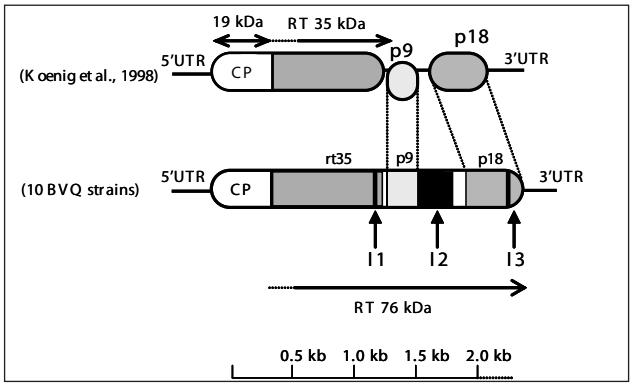

Fig. 1. Schematic representation of the RNA-2 genomic organization for BVQ previously described (Koenig *et al*., 1998) compared with the 10 from this study that all contain three nucleotide (nt) addition of 5 (I1), 285 (I2) and 1 (I3) nt responsible for the longer putative RT domain of 76 kDa instead of 35 kDa. Nucleotide additions are represented with black boxes. The rt35, p9 and p18 nucleotide sequences conserved in the RT 76 kDa coding sequence are shown, as well as the untranslated regions (UTRs), the coding sequences of the coat protein (CP) and the putative readthrough domains (RT).

These three genetic modifications in the nucleotide sequence of *Beet virus Q* RNA-2 induced major changes in the coding sequence length and amino acid composition of the coat protein RT domain (Fig. 1). Indeed, nucleotides additions I1 and I2 suppressed putative 35 kDa RT and p9 STOP codons, respectively, whereas the I3 moves the final STOP codon from position 2142-2144 to 2098-2100. Reading frames between the I1 site and the beginning of the putative p9 sequence, and the I3 site and the final STOP codon are moved by +1 and +2 nt respectively. The expected molecular weight of the putative coat protein RT domain common for the 10 sequenced BVQ strains is therefore 76 kDa instead of 35 kDa. This longer RT integrates the full amino acid sequence of the putative p9 but, because of the I1 and I3 nucleotide additions, the C-terminal extremities of amino acid sequences suggested for putative 35 kDa RT and p18 are modified. With a total number of 32 and 13 varying positions out of 2097 nucleotides and 699 amino acid respectively, the variability of the RT domain between the 10 BVQ strains was comprised between 98.95 and 100 % at the nucleotide level, and 98.71 and 100 % at the amino acid level. This low diversity had already been reported with BVQ coat protein sequences (Stas *et al*., 2001; Lennefors *et al*., 2005).

Discussion

The sequencing of 10 *Beet virus Q* strains from Belgium, France, Germany, Hungary, Spain and The Netherlands seems to lift the veil on the genomic organization of RNA-2. Indeed, the shortness of a putative coat protein 35 kDa RT domain that allowed space for two small putative ORFs p9 and p18 was quite a striking arrangement for the RNA-2 of BVQ described to date, compared with other closely related viruses such as BSBV, PMTV and BNYVV. When first described, it was already proposed that such a genomic arrangement might have resulted from deletion events in a longer sequence that originally resembled that of BSBV (Koenig *et al.*, 1998). This hypothesis is now confirmed with the identification of the common longer putative 76 kDa RT domain. Spontaneous deletions in the coat protein RT domain have been reported for other furoviruses *sensu lato* (Tamada and Kusume, 1991; Chen *et al*., 1994; Chen *et al*., 1995; Tamada *et al*., 1996; Reavy *et al*., 1998) that, when mechanically inoculated in a repeated way, show some deletion events in the C-terminal part of the RT domain. Therefore, as the *Beet virus Q* strain described in 1998 had been submitted to multiple mechanic inoculation cycles on *Chenopodium quinoa*, it can be assumed that three deletion events of 5, 285 and 1 nt occurred in genomic RNA-2. This triple deletion in the coat protein RT domain is, however, specific to BVQ, compared with BNYVV, PMTV and *Soil-borne wheat mosaic virus* (SBWMV) where only single-deletion events have been reported (Tamada and Kusume, 1991; Chen *et al*., 1994; Chen *et al*., 1995; Tamada *et al*., 1996; Reavy *et al*., 1998) (Table 1).

Virus	Deletions positions and lengths
SBWMV	△ 1418-1936 / 1419-1937 / 1420-1938 → 519 nt (1.6 kb clone; Chen *et al*., 1995) △ 1421-2179 → 759 nt (1.4 kb clone; Chen *et al*., 1995) △ 1466-2429 / 1467-2430 / 1468-2431 → 964 nt (1.2 kb clone; Chen *et al*., 1995) △ 1459-2488 → 1030 nt (1.0 kb clone; Chen *et al*., 1995)
BNYVV	△ 1438-1917 → 480 nt (Isolate 2a; Tamada and Kusume, 1991) △ 1407-1985 → 579 nt (Isolate 2b ; Tamada and Kusume, 1991)
PMTV	△ 1732-2274 → 543 nt (PMTV-T; Reavy *et al*., 1998)
BVQ	△ 864-868 → 5 nt △ 1223-1507 → 285 nt (Koenig *et al*., 1998; Crutzen *et al*., △ 2043 → 1nt submitted for publication)

Table 1. Position and length of spontaneous deletions reported in the readthrough domain of the coat protein for four furoviruses *sensu lato*.

Acknowledgements

François Crutzen is a fellow of the Belgian National Fund for Scientific Research (FNRS).

References

Adams, M. J., Antoniw, J. F. and Mullins, J. G. L. (2001). Plant virus transmission by plasmodiophorid fungi is associated with distinctive transmembrane regions of virus-encoded proteins. *Arch. Virol.* **146**, 1139-1153.

Chen, J., MacFarlane, S. A. and Wilson, T. M. A. (1994). Detection and sequence analysis of a spontaneous deletion mutant of *Soil-borne wheat mosaic virus* RNA2 associated with increased symptom severity. *Virology* **202**, 921-929.

Chen, J., MacFarlane, S. A. and Wilson, T. M. A. (1995). An analysis of spontaneous deletion sites in *Soil-borne wheat mosaic virus* RNA-2. *Virology* **209**, 213-217.

Diao, A., Chen, J., Gitton, F., Antoniw, J. F., Mullins, J., Hall, A. M. and Adams, M. J. (1999). Sequence of *European Wheat mosaic virus* and *Oat golden stripe virus* and Genome analysis of the genus *Furovirus*. *Virology* **261**, 331-339.

Kaufmann, A., Koenig, R. and Rohloff, H. (1993). Influence of *Beet soil-borne virus* on mechanically inoculated sugar beet. *Plant. Pathol.* **42**, 413-417.

Koenig, R., Pleij, C. W. A., Beier, C. and Commandeur, U. (1998). Genome properties of *Beet virus* Q, a new furo-like virus from sugarbeet, determined from unpurified virus. *J. Gen. Virol.* **79**, 2027-2036.

Lennefors, B.-L., Savenkov, E. I., Mukasa, S. B. and Valkonen, J. P. T. (2005). Sequence divergence of four soil-borne sugarbeet-infecting viruses. *Virus Genes* **31**, 57-64.

Lindsten, K. (1993). Rhizomania – are both *Beet necrotic yellow vein virus* (BNYVV) and *Beet soil-borne virus* (BSBV) involved? In *Proceedings of the 2nd Symposium of the International Working Group on Plant Viruses with Fungal Vectors*, Montreal, 67-70.

Meunier, A., Schmit, J.-F., Stas, A., Kutluk, N. and Bragard, C. (2003). Multiplex reverse transcription-PCR for simultaneous detection of *Beet necrotic yellow vein virus, Beet soil-borne virus* and *Beet virus* Q and their vector *Polymyxa betae* KESKIN on sugar beet. *Appl. Environ. Microbiol.* **69**, 2356-2360.

Prillwitz, H. and Schlösser, E. (1992). *Beet soil-borne virus*: occurrence, symptoms and effect on development. *Med. Fac. Landbouww. Univ. Gent* **57**, 295-302.

Reavy, B., Arif, M., Cowan, G. H. and Torrance, L. (1998). Association of sequences in the coat protein/readthrough domain of *Potato mop top virus* with transmission by *Spongospora subterranea*. *J. Gen. Virol*. **79**, 2343-2347.

Rush, C. M. and Heidel, G. B. (1995). Furovirus diseases of sugar-beets in the United States. *Plant. Dis.* **79**, 868-875.

Stas, A., Meunier, A., Schmit, J.-F., Marlier, A., Steyer, S. and Bragard, C. (2001). The beet soil-borne pomovirus in Belgium and relationship with rhizomania. *Med. Fac. Landbouww. Univ. Gent* **66**, 39-45.

Tamada, T. and Kusume, T. (1991). Evidence that the 75K readthrough protein of *Beet necrotic yellow vein virus* RNA-2 is essential for transmission by the fungus *Polymyxa betae*. *J. Gen. Virol.* **72**, 1497-1504.

Tamada, T., Schmitt, C., Saito, M., Guilley, H., Richards, K. and Jonard, G. (1996). High resolution analysis of the readthrough domain of *Beet necrotic yellow vein virus* readthrough protein: a KTER motif is important for efficient transmission of the virus by *Polymyxa betae*. *J. Gen. Virol.* **77**, 1359-1367.

GENETIC ANALYSIS OF *BEET NECROTIC YELLOW VEIN VIRUS* POPULATIONS FROM RESISTANT AND SUSCEPTIBLE SUGAR BEET GENOTYPES

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Summary

According to virus evolutionary theory, a population of mutants can be rapidly generated in an individual host plant by mutagenesis of RNA virus progeny during replication of the parental molecule. Such a population may be referred to as a quasispecies, based on the idea that it might be the functional unit subjected to natural selection. One of our working hypotheses is that the genetic structure of viral populations follows predictable behavior that defines its adaptive capabilities. Recently, we have explored variability of *Beet necrotic yellow vein virus* (BNYVV) at the p25 region (RNA 3), during its interaction with susceptible and resistant sugar beet (*Beta vulgaris*) genotypes. Individual plants were naturally inoculated with a well characterized wild-type strain of BNYVV in a serial host-passage experiment. By sequencing numerous viral RNAs isolated from these plants, we found that their viral populations mostly consisted of one or two dominant haplotypes surrounded by distinct but related low-frequency haplotypes, in agreement with the quasispecies model. The same dominant haplotype was consistently found in 12 out of 13 susceptible plants. However, substitution of the dominant haplotype from susceptible plants, with new haplotypes, occurred in 20% of the plants carrying the *Rz1* resistance gene and 45% of the plants carrying the unrelated *Rz2* resistance gene. No selection of specific mutations was observed at this stage of the virus adaptive process. However, nucleotide diversity was significantly higher in resistant than susceptible plants. These data suggest that nucleotide diversity is directly proportional to plant resistance strength, since the virus titer was lower in *Rz2*-than in *Rz1*-plants. Also, they corroborate the higher interisolate variability observed during the breakdown of *Rz1*-mediated resistance in the field, and the high genetic stability of the wild type virus in susceptible cultivars (Acosta-Leal *et al*., 2008).

Introduction

The benyvirus BNYVV is transmitted by zoospores of the plasmodiophorid *Polymyxa betae*, and its genome is divided into 4 to 5 single-stranded plus-sense RNA species. Despite its multiparticulate genome and, consequently, the potential for mixed infections with different strains, BNYVV exhibits high genetic stability (Koenig *et al.*, 1995). This observations suggests the existence of strong selective

constraints on virus diversification and effective isolation mechanisms operating among sympatric populations. Recently, we found that the complexity of field populations of BNYVV is conditioned by its host compatibility. Thus, in susceptible sugarbeet cultivars, BNYVV populations were relatively homogeneous and composed of the same predominant haplotype, whereas in cultivars encoding the *Rz1* resistant gene, the populations were 2 to 4 times more heterogeneous and most of them were typified by a different predominant haplotype (2). Based on these findings, we decided to test the hypothesis that the genetic structure of single-plant BNYVV populations is predictably affected by host resistance.

Materials and Methods

BNYVV-resistant sugar beet cultivars carrying the dominant *Rz1* or *Rz2* alleles, and a susceptible (*rz1rz2*) control were grown in individual pots. Twelve plants of each cultivar were seeded into a commercial potting soil mix containing approximately 2 g of field soil infested with a wild type BNYVV (Accesion No. EU480492). Plant roots were harvested 12-14 weeks after planting. Samples were processed according to the RNAqueous®-Mini kit (Ambion Inc. Austin, TX) protocol with modifications described previously (2). RT-PCR (High fidelity) products were cloned and sequenced as previously described (Acosta-Leal *et al.*, 2008). Populational genetic analyses of the sequences were performed with MEGA 3.1 (Kumar *et al*., 2004), DnaSP v4.10.7 Rozas *et al.*, 2003), and/or Arlequin v.2.000 (Schneider *et al.*, 2000) depending of the tasks.

Results and Discussion

A preliminary exploration of the variation of BNYVV after passage through susceptible and resistant sugarbeets was performed by direct DNA sequencing of the high fidelity amplicons obtained from 39 single-plant insolates selected at random. Higher number of nucleotide substitutions were incorporated in the consensus sequence of BNYVV RNA 4 in resistant than in susceptible plants. (Table 1). For instance, only one out of 13 sequenced isolates from susceptible plants exhibited a mutation, which was a $U_{1219}C$ transition in the 3'-terminal noncoding region. By contrast, 3 and 5 consensus mutations were detected in Rz1- and Rz2-plants, respectively. Only two of these mutations were nonsynonymous, and one of them occurred in the hypervariable amino acid residue 68. Interestingly, a high proportion of transversions and double-mutations were observed only in the most resistant *Rz2*-plants.

Table 1. Effect of sugarbeet resistance in the frequency of mutations incorporated into the consensus sequence of single-plant BNYVV populations.

R-gene	Host Passage	Mutations in the consensus sequence		
		Frequency [1]	Mutations	Type
Rz1	1	2/9 (22)	$A_{633}G$	Synonymous
			$U_{646}C$	$C_{68}R$
	2	1/6 (18)	$U_{1212}G$	Noncoding
Rz2	1	2/6 (33)	$U_{396}G$	Noncoding
			$C_{1083}A$	$D_{213}E$
			$U_{1451}C$	Noncoding
	2	3/5 (60)	$A_{355}U$	Noncoding
			$U_{1183}C$	Noncoding
			$U_{672}C$	Synonymous
rz1rz2	1	1/9 (11)	$U_{1219}C$	Noncoding
	2	0/4 (0)	(-)	(-)

[1] Number of single-plant populations with one or more nucleotide substitution in the consensus sequence of the p25 region over total number of sequenced populations.

In total, 385 cDNA fragments, representing 26 single-plant populations, were sequenced in both directions. The number of clones, mutation frequency, and nucleotide diversity per isolate are presented in Table 2. The overall nucleotide diversity (π) in the first host passage was greater ($P = 0.07$) in resistant (0.00149 and 0.00146 for Rz1-, and Rz2-plants, respectively) than susceptible plants (0.00069). This difference was more accentuated during the second host passage. Pairwise comparison between populations of the second host passage revealed that all populations from susceptible plants were undifferentiated and, therefore, represented the same population (Table 3). IN the case of 2Rz1 – only the 2Rz1 (17) population significantly differentiated from those isolated from the same plant genotype. Almost all populations from Rz2-plants differentiated each other. Thus, the type and/or strength of plant resistance markedly affected the genetic diversification of BNYVV from plant to plant.

Table 2. Effect of sugarbeet resistance in the genetic diversity of BNYVV single-plant populations.

Host Passage [a]	Population	Clones No.	Mutation Freq. ($\times 10^{-4}$) [b]	N. Diversity ($\pi \pm$ SE ($\times 10^{-3}$) [c]
1	Rz1(18)	8	6.42 (5/7,792)	1.29 ± 0.6
	Rz1(19)	9	2.28 (2/8,766)	0.46 ± 0.2
	Rz1(21)	8	8.98 (7/7,792)	2.16 ± 0.9
	Rz1(22)	7	0 (0/6,818)	0
	Rz2(05)	9	4.56 (4/8,766)	0.91 ± 0.4
	Rz2(16)	11	1.87 (2/10,714)	0.37 ± 0.3
	Rz2(17)	11	2.80 (3/10,714)	0.56 ± 0.3
	Rz2(24)	9	3.42 (3/8,766)	0.69 ± 0.4
	S(18)	10	1.03 (1/9,740)	0.21 ± 0.2
	S(20)	12	0.86 (1/11,688)	0.31 ± 0.3
	S(21)	10	5.13 (5/9,740)	1.37 ± 0.6
	S(23)	10	2.05 (2/9,740)	0.41 ± 0.4
2	Rz1(15)	17	5.00 (12/24,021)	1.14 ± 0.4
	Rz1(17)	18	2.75 (7/25,434)	0.84 ± 0.4
	Rz1(18)	17	2.91 (7/24,021)	0.92 ± 0.4
	Rz1(19)	19	4.47 (12/26,847)	0.90 ± 0.2
	Rz1(21)	9	2.36 (3/12,717)	0.47 ± 0.3
	Rz2(14)	25	6.23 (22/35,325)	1.25 ± 0.3
	Rz2(16)	22	6.43 (20/31,086)	1.49 ± 0.4
	Rz2(18)	22	2.25 (7/31,086)	0.57 ± 0.2
	Rz2(19)	21	6.07 (18/29,673)	1.56 ± 0.4
	Rz2(23)	24	5.90 (20/33,912)	1.38 ± 0.3
	S(13)	20	1.06 (3/28,260)	0.33 ± 0.2
	S(14)	19	3.35 (9/26,847)	0.67 ± 0.2
	S(15)	18	3.15 (8/25,434)	0.70 ± 0.3
	S(16)	20	2.48 (7/28,260)	0.74 ± 0.3

[a] Sugar beet varieties encoding the dominant *Rz1* or *Rz2* alleles for resistance to BNYVV infection and susceptible (S) control.

[b] Number of mutations over total sequenced nucleotides of the p25 (RNA 3) region. The sequenced fragment was 974 and 1413 nucleotides long for the first and second host passage, respectively.

[c] Nucleotide diversity (π) estimated by the Kimura 2-parameter model as implemented in MEGA 3.1 software (Kumar *et al*., 2004), and standard error (SE) estimated by 500 replicates bootstrapping. Calculations corroborated by using DnaSP v4.10.7 software (Rozas *et al*., 2003).

Table 3. Pairwise genetic differentiation of single-plant populations of BNYVV.

Populations from susceptible plants					
	2S(13)	2S(14)	2S(15)	2S(16)	
2S(13)		0.35	0.32	2.37	
2S(14)	0.007 (0.343)		-0.91	0.35	
2S(15)	0.007 (0.279)	-0.016 (0.659)		0.84	
2S(16)	0.043 (0.108)	0.006 (0.317)	0.014 (0.224)		
Populations from *Rz1*-plants					
	2Rz1(15)	2Rz1(17)	2Rz1(18)	2Rz1(19)	2Rz1(21)
2Rz1(15)		8.66	0.26	-1.33	-1.22
2Rz1(17)	**0.101 (0.013)**		7.52	10.13	12.45
2Rz1(18)	0.003 (0.394)	**0.085 (0.020)**		1.10	2.21
2Rz1(19)	-0.019 (0.999)	**0.121 (< 0.01)**	0.015 (0.213)		-1.68
2Rz1(21)	-0.024 0.687)	**0.140 (0.023)**	0.020 (0.268)	-0.031 (0.999)	
Populations from *Rz2*-plants					
	2Rz2(14)	2Rz2(16)	2Rz2(18)	2Rz2(19)	2Rz2(23)
2Rz2(14)		15.51	30.23	9.41	14.20
2Rz2(16)	**0.155 (< 0.01)**		30.74	8.18	11.30
2Rz2(18)	**0.299 (< 0.01)**	**0.307 (< 0.01)**		24.63	29.43
2Rz2(19)	**0.094 (< 0.01)**	**0.083 (< 0.01)**	**0.248 (< 0.01)**		1.46
2Rz2(23)	**0.142 (< 0.01)**	**0.117 (< 0.01)**	**0.292 (< 0.01)**	0.016 (0.103)	

Values below the diagonal in each matrix are the Wright's FST index of dissimilarity and its statistical significance after 16,000 permutations. Pairs of different populations at $P \leq 0.05$ are in bold.

Values above the diagonal are the percentage of haplotype pairwise comparisons where the haplotype of each comparing population differed in at least one nucleotide. These values were corrected by subtracting the percent difference obtained when the haplotypes were from the same population. All calculations performed by using Arlequin v2.000 (Schneider *et al.*, 2000).

References

Acosta-Leal, R., Fawley, M.W. and Rush, C.M. (2008). Changes in the intraisolate genetic structure of *Beet necrotic yellow vein virus* populations associated with plant resistance breakdown. *Virology* **376**, 60-68.

Koenig, R., Lüddecke, P. and Haeberlé, A.M. (1995). Detection of *Beet necrotic yellow vein virus* strains, variants and mixed infections by examining single-strand conformation polymorphisms of immunocapture RT-PCR products. *J. Gen. Virol*. **76**, 2051-2055.

Kumar, S., Tamura, K., and Nei, M. (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief. Bioinform*. **5**, 150-163.

Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X. and Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**, 2496-2497.

Schneider, S., Roessli, D. and Excoffier, L. (2000). Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

EVIDENCE THAT *BEET NECROTIC YELLOW VEIN VIRUS* HAS AN ENHANCED ACTIVITY OF RNA SILENCING SUPRESSION IN ROOTS

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Summary

Beet necrotic yellow vein virus (BNYVV) RNA3 and RNA4 are not essential for virus multiplication, but are associated with vector-mediated infection and disease development in sugar beet roots. The RNA3-encoded p25 protein is responsible for production of rhizomania symptoms of sugar beet roots. On the other hand, the RNA4 encoded p31 protein was involved in efficient vector transmission and slight enhancement of symptom expression in some *Beta* species. Moreover, p31 was involved in the induction of severe symptoms by BNYVV in *Nicotiana benthamiana* plants without affecting viral RNA accumulation, but p25, previously identified as a major contributor to symptoms in sugar beets, had no such effect on *N. benthamiana*. In two different silencing suppression assays, neither p31 nor p25 was able to suppress RNA silencing in leaves, but the presence of p31 enhanced a silencing suppressor activity in roots without alteration in viral RNA accumulation. Thus, although BNYVV RNA2 encoded p14 protein is known to be a silencing suppressor, p31 also was found to contribute silencing suppression in a root-specific manner.

Introduction

Beet necrotic yellow vein virus (BNYVV) RNA3 encoded p25 is involved in induction of rhizomania symptoms in sugar beet. On the other hand, BNYVV RNA4 is important for efficient transmission of the virus by the vector *Polymyxa betae* and has minor effects on symptom expression (Tamada and Abe, 1989; Tamada *et al*., 1989). It is known that viral proteins associated with symptom severity often function as suppressors of RNA silencing (Silhavy and Burgyán, 2004). In the case of BNYVV, the RNA2-encoded p14 protein (cysteine-rich protein) has a transgene silencing suppressor activity in leaf tissue (Kondo *et al*., 2005). Because of the involvement of BNYVV RNA3 and RNA4 in symptom expression, it is of interest to know whether these RNA-encoding genes p25 and p31 contribute to suppression of RNA silencing in shoots and roots. Previously, we presented evidence that the RNA4-encoded p31 open reading frame (ORF) is required for efficient vector transmission but also is involved in enhanced symptom expression in a host-specific manner (Rahim *et al*., 2007). Here, we

show that neither p25 nor p31 is able to suppress RNA silencing in leaves, but that p31 enhances the ability of BNYVV to suppress silencing in roots.

Materials and Methods

Plant materials, virus isolates and inoculations: *Nicotiana benthamiana* was used for virus propagation or observation of symptom phenotypes (Andika *et al*., 2005). The BNVYY field isolate O11 (RNA1+2+3+4) and the laboratory isolates O11-0 (RNA1+2), O11-3 (RNA1+2+3) and O11-4 (RNA1+2+4) were used. Four RNA4 internal deletion mutants ΔMd1, ΔMd2, ΔMd3 and ΔCter that were derived from the full-length cDNA clone of RNA4 were used (Rahim *et al*., 2007). Foliar rub-inoculation was conducted as described previously (Rahim *et al*., 2007).
Silencing suppression assay: For the patch co-infiltration assay, each of the p31 and p25 ORFs was amplified by PCR on the BNYVV cDNA clones and inserted between the *Xba*I and *Bam*HI restriction sites of the pBin61 binary Ti vector (Voinnet *et al*., 2000) to obtain pBin-p31 and pBin-p25. pBin-HC-Pro, which carries the coding domain of HC-Pro (a known silencing suppressor of *Potato virus Y* (PVY)), was used for a positive control. The *Agrobacterium* (strain C58C1) culture containing pBin-p31, pBin-p25 or pBin-HC-Pro, was mixed with bacteria carrying pBin-GFP and the mixtures were infiltrated in leaves of *N. benthamiana* plant line 16c as described by Voinnet *et al*. (1998). For another silencing suppression assay, leaves of *N. benthamiana* plant line 16c were infiltrated with the bacteria containing pBin-GFP. After 20–25 days, when silencing was achieved against GFP transcripts in the whole plant, systemic leaves were inoculated with BNYVV isolates. At 14 to 17 days post-inoculation (dpi), when systemic symptoms began to appear, roots of the infected plants were partially removed, and the plants were transplanted into new pots to enhance root growth. The GFP fluorescence in leaves and new roots was examined under a UV lamp and with a fluorescence microscope (Zeiss Axioskop), respectively. Northern blot and Western blot analyses were performed as described previously (Andika *et al*., 2005; Rahim *et al*., 2007).

Results and Discussion

BNYVV p31 ORF is required for expression of severe symptoms in N. benthamiana.
N. benthamiana is a systemic host of BNYVV, in which BNYVV induces severe symptoms or mild symptoms. To further confirm the effects of RNA3 and RNA4 on symptoms and viral RNA accumulation, BNYVV O11-3, O11-4 or O11-0 were inoculated to *N. benthamiana* leaves. Infection by O11-4 induced severe symptoms, whereas infection by O11-3 and O11-0 produced mild symptoms in all plants (data not shown). Accumulation levels of viral RNA1 and RNA2 fluctuated slightly, but no correlation was found between amount and symptom severity. Thus, BNYVV RNA 4 is associated with symptom severity on *N. benthamiana,*

whereas RNA3 is not implicated in these severe symptoms.
To determine whether the p31-coding region is responsible for symptom severity, each of the mutated RNA4 transcripts was mixed with O11-0 RNAs and inoculated into *N. benthamiana* seedlings. Plants infected with RNA4 mutants ΔMd1, ΔMd2, ΔMd3 or ΔCter, had mild symptoms, indistinguishable from those induced by O11-0, whereas plants infected with O11-0/wt or O11-4 had severe symptoms (data not shown). Accumulation levels of viral RNAs were not correlated with symptom severity on *N. benthamiana* plants. Thus, BNYVV p31 is required for expression of severe symptoms in *N. benthamiana*.

BNYVV p31 increases a silencing suppressor activity in roots

Many viral proteins involved in enhancement of viral pathogenicity are known to function as silencing suppressors (Silhavy and Burgyán, 2004). First, we employed a patch co-infiltration assay to determine whether the product of BNYVV p31 and p25 could suppress silencing in *N. benthamiana*. Co-infiltration of 16c plant leaves with two *Agrobacterium* cultures carrying pBin-GFP and pBin-HC-Pro resulted in bright green fluorescence at 5 days post-infiltration (Fig. 1a), whereas co-infiltration with two cultures carrying pBin-GFP and the empty vector pBin-61 gave no visible fluorescence under the same conditions due to efficient silencing of the *GFP* gene (Fig. 1a). When a mixture of *Agrobacterium* cultures containing pBin-GFP and pBin-p31 or pBin-p25 was infiltrated, the infiltrated leaf tissues showed no visible fluorescence (Fig. 1a). Northern blot analysis also revealed that levels of GFP mRNA were greatly reduced in poorly fluorescent areas of leaves co-infiltrated with *Agrobacterium* cultures containing pBin-GFP and pBin-p31, pBin-p25 or the empty vector (Fig. 1b), whereas GFP mRNA was abundant in the green fluorescent tissue co-infiltrated with cultures containing the *GFP* and *HC-Pro* genes (Fig. 1b). Thus, expression of both p31 and p25 failed to suppress RNA silencing in the infiltrated leaves of *N. benthamiana*.

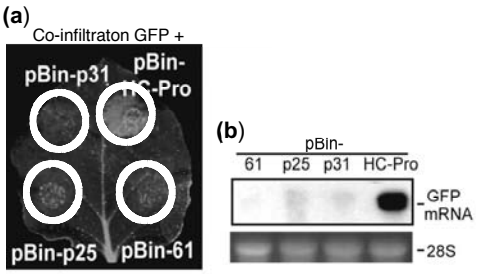

Fig. 1. Assessment of silencing suppression of BNYVV p31 and p25 by the patch co-infiltration assay using GFP-expressing *N. benthamiana* line 16c. (a) An agro-infiltrated leaf was viewed under UV light at 5 days after infiltration. (b) Northern blot analysis of total RNA from agro-infiltrated patches shown in (a).

Secondly, we compared silencing suppressor activity of BNYVV isolates in leaves and roots using GFP-silenced *N. benthamiana* line 16c plants. Plants that had been completely silenced by agro-infiltration of pBin-GFP were inoculated with O11-4 and O11-0. No green fluorescence was observed in leaves of plants infected with both O11-0 and O11-4 (Fig. 2a). However, it is interesting to note that obvious green fluorescence was observed in most of the new emerging roots infected with O11-4 (Fig. 2a), whereas weak green fluorescence was seen

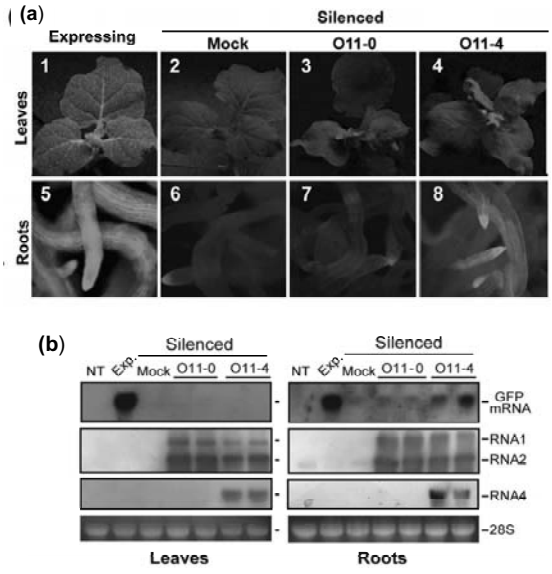

Fig. 2. Assessment of silencing suppression of BNYVV p31 by the reversal assay using GFP-silenced *N. benthamiana* line 16c. (a) Leaves and roots of GFP-expressing and silenced plants viewed under UV light at 21 dpi with BNYVV isolates. (b) Northern blot analysis of total RNA from leaves and roots shown in (a).

in some parts of roots infected by O11-0 (Fig. 2a). As controls, in mock-inoculated plants, faint fluorescence was seen only in root tips (Fig. 2a), while no green fluorescence was observed in shoots (Fig. 2a). In GFP-expressing 16c plants, strong green fluorescence was observed in both shoots and roots (Fig. 2a). In Northern blot analysis, GFP mRNA was abundant in roots infected with O11-4, whereas a lower level of GFP was detected in roots infected with O11-0 (Fig. 2b). In contrast, GFP mRNA was not detected in leaves infected with either O11-4 or O11-0 (Fig. 2b). Thus, GFP mRNA accumulation levels in the leaves and roots were in accordance with the visual observations. In either leaves or roots of the silenced plants, RNA1 and RNA2 were readily detected regardless of the presence or absence of RNA4 (Fig. 2b).

BNYVV RNA2-encoded p14 protein is known to be a silencing suppressor (Kondo *et al*., 2005), and we therefore tested whether p31 affected expression of the *p14* gene. Western blot analysis showed no difference in expression of the p14 protein or the coat protein between roots infected with O11-4 and O11-0 (data not shown).

Taken together, our results indicate that wt RNA4-encoded p31 is able to contribute to silencing suppression of a transgene in the roots, but not in the leaves. This is the first evidence that a viral factor is involved in RNA silencing suppression in a root-specific fashion.

References

Andika, I. B., Kondo, H. and Tamada, T. (2005). Evidence that RNA silencing-mediated resistance to *Beet necrotic yellow vein virus* is less effective in roots than in leaves. *Mol. Plant Microbe Interact*. **18**, 194-204.

Kondo, H., Andika, I.B., Kurokawa, E., Bouzoubaa, S. and Tamada, T. (2005). Comparisons of RNA silencing suppressors encoding by two benyviruses*, Beet necrotic yellow vein viru*s and *Burdock mottle virus.* In *Proceedings of the Sixth Symposium of International Working Group on Plant Viruses with Fungal Vectors*, pp. 13–16. Edited by C.M. Rush, Denver: American Society of Sugar Beet Technologists.

Rahim, M.D., Andika, I.B., Han, C., Kondo, H. and Tamada, T. (2007). RNA4-encoded p31 of *Beet necrotic yellow vein virus* is involved in efficient vector transmission, symptom severity and silencing suppression in roots. *J. Gen. Virol*. **88**, 1611-1619.

Silhavy, D. and Burgyán, J. (2004). Effects and side-effects of viral RNA silencing suppressors on short RNAs. *Trends Plant Sci*. **9**, 76-83.

Tamada, T. and Abe, H. (1989). Evidence that *Beet necrotic yellow vein virus* RNA-4 is essential for efficient transmission by the fungus *Polymyxa betae*. *J. Gen. Virol*. **70**, 3391-3398.

Tamada, T., Shirako, Y., Abe, H., Saito, M., Kiguchi, T. and Harada, T. (1989). Production and pathogenicity of isolates of *Beet necrotic yellow vein virus* with different numbers of RNA components. *J. Gen. Virol*. **70**, 3399-3409.

Tamada, T., Uchino, H., Kusume, T. and Saito, M. (1999). RNA 3 deletion mutants of *Beet necrotic yellow vein virus* do not cause rhizomania disease in sugar beets. *Phytopathology* **89**, 1000-1006.

Voinnet, O., Vain, P., Angell, S. and Baulcombe, D.C. (1998). Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. *Cell* **95**, 177-187.

Voinnet, O., Lederer, C. and Baulcombe, D.C. (2000). A viral movement protein prevents spread of the gene silencing signal in *Nicotiana benthamiana*. *Cell* **103**, 157-167.

THE P25 PROTEIN OF *BEET NECROTIC YELLOW VEIN VIRUS* HAS A DUAL ROLE AS A VIRULENCE AND AVIRULENCE DETERMINANT IN LEAVES OF BETA VULGARIS PLANTS

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Summary

The RNA3-encoded p25 nuclear shuttle protein of *Beet necrotic yellow vein virus* (BNYVV) is responsible for production of rhizomania symptoms of sugar beet roots. Moreover, the p25 protein also is associated with resistance response in rub-inoculated leaves of sugar beet or wild beet plants. The resistance phenotype had symptoms ranging from no visible lesions to necrotic lesions at the inoculation sites, whereas the susceptible phenotype developed bright yellow local lesions. There was a virus strain-cultivar-specific resistance interaction. It was found that three amino acid residues at positions 68, 70 and 179 are important in determining the resistance phenotype, and that the host-genotype specificity is controlled by single amino acid changes at position 68. Microscopic observations using GFP-tagged BNYVV suggested that, in the incompatible interaction, the presence of p25 is involved in inhibition of cell-to-cell movement, probably due to callose deposition in cell walls. Thus, we show that the p25 protein of BNYVV has a dual function as a virulence and avirulence determinant in leaves of beet plants.

Introduction

Plants have evolved an array of defense mechanisms to prevent or limit infection of viruses. These include the hypersensitive response (HR) and extreme resistance (ER); HR is characterized by rapid cell death at the primary infection site, whereas in ER there are no obvious symptoms and virus multiplication is generally limited to initially infected cells (Hull, 2002). Both HR and ER are usually controlled by dominant host genes which confer resistance through matched specificity between a disease resistance gene (R) and a pathogen avirulence (avr) gene.

Beet necrotic yellow vein virus (BNYVV) causes rhizomania disease of sugar beet and is transmitted by soil-borne protozoan *Polymyxa betae*. The RNA3-encoded p25 nuclear shuttle protein of BNYVV is responsible for production of rhizomania symptoms of sugar beet roots (Tamada *et al*., 1999; Vetter *et al*., 2004). Although resistance to BNYVV has been generally estimated by the degree of virus infection in roots of sugar beet plants, we found that resistance response

to BNYVV can also be evaluated, at least in part, on the basis of phenotypes on the inoculated leaves of beet plants infected by foliar rub-inoculation (Tamada, 2007). The resistance phenotype had symptoms ranging from no visible lesions (ER-like) to necrotic lesions (HR-like) at the inoculation sites, whereas the susceptible phenotype developed bright yellow local lesions. Furthermore, three differential sea beet (*Beta vulgaris* subsp. *maritima*) lines MR0, MR1 and MR2 were selected (Tamada, 2007). We also found that host-genotype specificity is controlled by a single amino acid change (Chiba *et al*., 2008). The aim of this study is to understand further mechanism of resistance responses in rub-inoculated leaves.

Materials and Methods

Plant materials, virus isolates and inoculations

Tetragonia expansa plants were used for virus propagation (Tamada *et al*., 1989). Wild beet *B. vulgaris* subsp. *maritima* lines MR0, MR1 and MR2 were used (Tamada, 2007). BNYVV wild-type (wt) isolates O11, GW, S113, FP, IV4, IP7, USTH and T41 were used (Chiba *et al*., 2008). The laboratory isolates O11-4 and GW-4, both of which lack RNA3, were obtained from the original O11 and GW isolates, were used (Chiba *et al*., 2008). Two full-length RNA3 cDNA clones, pT3O (wt O11) and pT3G (wt GW), of the BNYVV-O11 and -GW, respectively, and the mutated cDNA clones pT3O(F68Y), pT3O-H(F68H), pT3O-C(F68C) and pT3O-A(F68A) were used (Chiba *et al*., 2008). Inoculation with transcripts was conducted as described previously (Chiba *et al*., 2008). The progeny mutant viruses from rub-inoculated leaves of beet plants were confirmed by sequence analyses as described previously (Chiba *et al*., 2008).

Northern blot and sequence analyses

Northern blotting and sequence analyses were conducted as described previously (Chiba *et al*., 2008).

GFP-tagged viruses and fluorescence observation

GFP-tagged virus (BNYVV-GFP) was produced by a mixture of an RNA1 transcript and an RNA2 transcript that is derived from pB2-RT-GFP3 (Erhardt *et al*., 2001; Tamada *et al*., 2005) was used. BNYVV-GFP was mixed with each of RNA transcripts pT3O and pT3O-C and produced BNYVV-GFP+pT3O and BNYVV-GFP+pT3O-C, respectively. These GFP viruses were inoculated into MR0 and MR1 plants. The fluorescence on the inoculated leaves at 2 to 5 dpi was observed with a fluorescence microscope (Zeiss Axioskop) equipped with the No 10 Blue (BP450-490) and No15 Green (BP546) filters.

Results and Discussion

Response of natural BNYVV isolates in different beet plants

For foliar rub-inoculation, resistance phenotype displays a range of symptoms from no visible lesions to necrotic lesions at the inoculation site, and only very low levels of virus and viral RNA accumulate (Fig. 1). The susceptible phenotype shows large, bright yellow lesions and high levels of virus and viral RNA accumulation (Fig. 1).

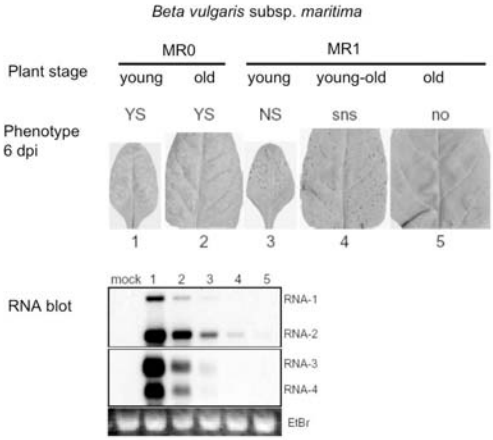

Fig.1. Phenotypes and viral RNA accumulation on the leaves of *B. vulgaris* subsp. *maritima* MR1 and MR0 plants inoculated at young and old stages. Phenotypes are as follow; bright yellow lesions(YS), necrotic lesions (NS), small necrotic lesions (sns) and no lesions (no). Lane numbers in the RNA blot correspond to panel figures in the phenotype.

We tested response of eight BNYVV isolates (O11, S113, FC, IV4, IP7, USTH, T41 and GW) in wild beet *B. vulgaris* subsp. *maritima* lines MR0, MR1 and MR2 (Chiba *et al*., 2008). O11 induced the resistance phenotype in MR1 and MR2 plants, while S113, FC and IV4 showed the resistance phenotype in MR2 plants, but not in MR1 plants. The other four isolates GW, IP7, USTH and T41 showed the susceptible phenotype in MR1 and MR2 plants. All isolates used produced the susceptible phenotypes in MR0 plants. Viral RNAs accumulated to very low levels or were below the detection limits in inoculated leaves with the resistance phenotype, whereas high levels of viral RNA accumulation were observed in the leaves with susceptible phenotype. Thus, there was a virus strain-cultivar-specific resistance interaction (Chiba *et al*., 2008).

Identification of amino acids of the p25 protein required for resistance response

From comparisons of amino acid sequences of p25 proteins from different isolates, we noticed that the amino acid residue at position 68 appears to be one of the major key residues for determining the resistance phenotype. Thus, BNYVV isolates IV4, IP7 and USTH, which showed different phenotypes, differed only at this position in the p25 protein sequence. A His (H) at position 68 was associated with the resistance phenotype in MR2 plants, but a Leu (L) or Cys (C) substitution at this position was not. In addition, O11 induced the resistance phenotype in both MR1 and MR2 plants; therefore, a Phe (F) at position 68 might be associated with the resistance phenotype of both plant lines, although six other amino acid differences were found between the p25 proteins of O11 and IV4. Finally, in the case of Tyr (Y) at position 68, S113 and FP displayed the resistance phenotype

in MR2 plants, whereas T41 and GW showed the susceptible phenotype. There were several other amino acid substitutions in the p25 proteins of these isolates, however, raising the possibility that position(s) other than position 68 may also be involved in inducing the resistance phenotype in MR2 plants.

To identify which amino acid residues at position 68 in the p25 protein are important for the resistance phenotype determinant, we constructed four single mutants in which Phe at position 68 of the p25 protein of RNA3 (pT3O) in BNYVV-O11 was replaced by Tyr (F68Y), His (F68H), Cys (F68C) or Ala (F68A). The F68Y and F68H mutations induced the resistance phenotype in MR2 plants and the susceptible phenotypes in MR1 plants. Inoculation of MR2 plants with F68H resulted in large numbers of necrotic lesions and accumulation levels of viral RNAs were higher than those inoculated with the wt virus (pT3O) or the mutant virus F68Y. The other two single mutants (F68C and F68A) failed to show any resistance phenotype. Levels of viral RNA accumulation in leaves with the resistance phenotype were lower than in the susceptible phenotype, excepted for the F68C mutant. These data indicate that single amino acid residues at position 68 in the p25 protein control the degree of resistance in MR1 and MR2 plants; thus the p25 protein with Phe-68 may act as a stronger elicitor than p25 proteins with Tyr-68 or His-68. Taken together with the results from the natural isolates and other mutated viruses, we concluded that, in addition to amino acid residues (either Phe or Tyr) at position 68, two amino acid residues, Gly and Asn at positions 70 and 179, respectively, are important in determining the resistance phenotype.

Experiments using GFP-tagged BNYVV

In MR1 plants, BNYVV-GFP+pT3O induced no or necrotic lesions (resistance phenotype), whereas BNYVV-GFP+pT3O-C produced bright yellow lesions (susceptible phenotype). Both viruses showed the susceptible phenotype in MR0 plants. In virus-host combinations showing the susceptible phenotype, the GFP fluorescence was observed in a few epidermal cells at 2 dpi, and then GFP-expressing cells were spread in a concentric pattern as time progressed (3 to 5 dpi). By contrast, in virus-host combinations of resistance phenotype, GFP-expressing epidermal cells were limited in a few to several tens at 3 to 4 dpi. In that case, many cells were collapsed and dead, and their cell walls were aberrant sometimes. From these observations, we suggested that in the incompatible interaction, the presence of p25 may be involved in inhibition of cell-to-cell movement, probably due to callose deposition in cell walls. We also speculate that ER-like and HR-like phenotypes display a continuous resistance mechanism that may be activated following the recognition of the R and avr genes.

Although it is not clear how BNYVV p25 is involved in the resistance response, our observations that the p25-lacking or defective mutant virus can replicate and move from cell-to-cell in the leaves of either susceptible or resistant plants and that, in incompatible interactions, tiny lesions are often observed at the inoculation site suggest that p25 is involved in suppression (inhibition) of cell-

to-cell movement rather than virus replication. It is thus likely that the virus can replicate in initially infected cells, but that its movement to adjacent cells is blocked or its accumulation is suppressed. There are many studies showing that viral Avr factors are involved in a suppression of cell-to-cell movement in hosts, but BNYVV is a unique virus in the sense that p25, encoded by one of the extra genomic RNAs other than viral major genes encoding replicase, protease, movement protein and coat protein, has a dual function as a virulence and Avr determinant.

References

Chiba, S., Miyanishi, M., Andika, I. B., Kondo, H. and Tamada, T. (2008). Identification of amino acids of the *Beet necrotic yellow vein virus* p25 protein required for induction of the resistance response in leaves of *Beta vulgaris* plants. *J. Gen. Virol*. **89**, 1314-1323.

Erhardt, M., Dunoyer, P., Guilley, H., Richards, K., Jonard, G. and Bouzoubaa, S. (2001). *Beet necrotic yellow vein virus* particles localize to mitochondoria during infection. *Virology* **286**, 256-262.

Hull, R. (2002). Matthews Plant Virology. 4th ed. Academic Press, San Diego, CA, U.S.A.

Tamada, T. (2007). Susceptibility and resistance of *Beta vulgaris* subsp. *maritima* to foliar rub-inoculation with *Beet necrotic yellow vein virus*. *J. Gen. Plant Pathol.* **73**, 76-80.

Tamada, T., Shirako, Y., Abe, H., Saito, M., Kigushi, T. and Harada, T. (1989). Production and pathogenicity of isolates of *Beet necrotic yellow vein virus* with different numbers of RNA components. *J. Gen. Virol*. **70**, 3399-3409.

Tamada, T., Uchino, H., Kusume, T. and Saito, M. (1999). RNA 3 deletion mutants of *Beet necrotic yellow vein virus* do not cause rhizomania disease in sugar beets. *Phytopathology* **89**, 1000-1006.

Tamada, T., Kondo, H., Andika, I. B., Rahim., M. D. and Bouzoubaa, S. (2005). Analysis of the vascular movement of *Beet necrotic yellow vein virus* in *Nicotiana benthamiana* and *Beta vulgaris* spp. *maritima* M8 using a GFP-expressing virus. In: *Proceedings of the Sixth Symposium of International Working Group on Plant Viruses with Fungal Vectors*, pp. 9-12. Edited by C.M. Rush, Denver: American Society of Sugar Beet Technologists.

Vetter, G., Hily, J-M., Klein, E., Schmidlin, L., Haas, M., Merkle, T. and Gilmer, D. (2004). Nucleo-cytoplasmic shuttling of the *Beet necrotic yellow vein virus* RNA-3-encoded p25 protein. *J. Gen. Virol*. **85**, 2459-2469.

MOLECULAR AND SEROLOGICAL PROPERTIES OF AN IRANIAN ISOLATE OF *BEET BLACK SCORCH VIRUS*

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Summary

In an attempt to obtain an isolate of *Beet necrotic yellow vein virus* (BNYVV) from Iranian sugar beet showing pronounced symptoms of root beardedness we have isolated a virus which produced necrotic local lesions on C*henopodium quinoa* leaves just three days after inoculation. The virus reacted strongly with antisera to Chinese and US isolates of *Beet black scorch virus* (BBSV) and by means of PCR almost the complete genomic RNA of this virus isolate was amplified and sequenced. The genetic organization of the Iranian BBSV closely resembles those of the Chinese and US BBSV isolates, but there are considerable differences in the nucleotide sequences of the three isolates. Sequence comparisons suggested that our Iranian isolate is more distantly related to the Chinese and US BBSV isolates than these two are to each other. Most of the nt sequence differences are, however, silent and the results of agar gel double diffusion tests suggested that the three isolates are serologically closely related, if not identical.

Introduction

Beet black scorch virus (BBSV) belongs to the genus Necrovirus and was first described and characterized in detail by Cai *et al*. (1999), Cao *et al*. (2002), Guo *et al*. (2005) and Yuan *et al*. (2006). The virus is believed to be the causal agent of a severe lethal disease of sugar beet in China typified by black scorched leaves and necrotic fibrous roots (Yuan *et al.*, 2006). Root beardedness is not considered to be typical symptom of this disease (Cai *et al*., 1999). More recently a deviant strain of this virus was isolated in the USA (Colorado) by Weiland *et al*. (2006; 2007) from sugar beet which did show pronounced root beardedness, although many plants on the affected field had tested either negative for *Beet necrotic yellow vein virus* (BNYVV) or contained this virus in very low concentrations only. In an attempt to obtain an isolate of *Beet necrotic yellow vein virus* (BNYVV) from Iranian sugar beet showing pronounced symptoms of root beardedness we have recently obtained an isolate of BBSV which differed considerably from the

Chinese and the US isolates of BBSV in the nt sequence of its genomic RNA. The genome organization of the three viruses, however, was almost identical and most of the nt exchanges were silent which is probably the reason that the three viruses are serologically closely related, if not identical. A detailed description of this virus has been given by Koenig and Valizadeh (2008).

Materials and Methods

For purifying the virus 100 g of infected *C. quinoa* leaves were homogenized in 300 ml 0.1 M sodium acetate pH 6.0 containing 1% mercaptoethanol. The sap was filtered through cheese cloth and stirred for 20 min at 4° C with a 1:1 (v/v) mixture of butanol and chloroform. The supernatant obtained after low speed centrifugation was subjected to high speed centrifugation through a 20% (w/v) sucrose cushion and the sedimented virus was further purified by cesium chloride density gradient centrifugation. An antiserum was produced in a rabbit by two intramuscular injections spaced one week apart of virus emulsified in Freund's complete and incomplete adjuvant, respectively. Primers were designed on the basis of the published sequences of the Chinese and American BBSV isolates in conserved regions to produce PCR products of overlapping sequences from which almost the entire nucleotide sequence of the genomic RNA of the Iranian BBSV isolate, except for the 5' and 3' terminal primer regions, was assembled. Sequences were analysed by means of the Invitrogen Vector NTI Advance 10 software and 'homology trees' showing the percentages of sequence identities were generated by the DNAMAN software (Lynnon Bio/Soft). For routine detection of BBSV by means of RT PCR we used sense and antisense primers derived from nts 3341 – 3360 and 3604 – 3625, respectively, of the closely related Chinese BBSV sequences (accession Nos. AF452884 and AY626780). These sequences are conserved also in the RNA of the US isolate of BBSV (accession No. EF153268).

Results and Discussion

The genome organization of the Iranian BBSV (Fig. 1) closely resembles that of the Chinese and US strains of BBSV (Cao *et al*., 2002; Weiland *et al*., 2007). The genomes of all these BBSV isolates differ from those of other necroviruses by having three, rather than two, movement protein genes (Yuan *et al.,* 2006) and by the smaller size of the coat protein genes which code for 24 kD rather than c. 30 kD proteins. We failed to detect a satellite RNA in our Iranian BBSV isolate using primers derived from the sequence of the satellite RNA detected in one of the BBSV Chinese isolates (Guo *et al.,* 2005).

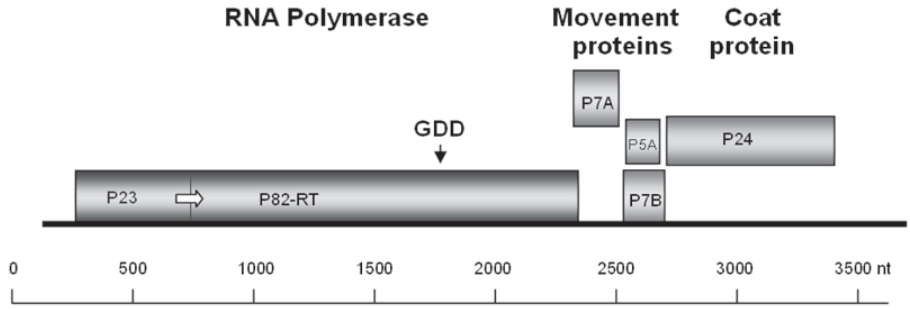

Fig. 1. Genome organization of BBSV.

The RNAs of the Iranian and the US BBSV strains show considerable nt sequence differences to those of two closely related Chinese isolates which originate from rather distant geographic areas, i.e. the Ningxia and Xianjiang provinces, respectively (Yuan *et al.*, 2006). These nt differences are distributed along their entire lengths and are especially pronounced in the coat protein gene region (Fig. 2). Most of the nt exchanges, however, are silent. The percentages of amino acid sequence identities are, therefore, much higher than the percentages of nt sequence identities (Fig. 2). This may indicate that the BBSV isolates from the three countries have separated during evolution a long time ago. There must, however, been a strong selection pressure to retain the original protein structures. In the serological agar gel double diffusion test we were unable to distinguish the Chinese and Iranian BBSV isolates; the US isolate could not be included in these tests for quarantine reasons, but an antiserum to this isolate kindly provided by Drs. J. Suttle and J. Weiland readily detected the two other isolates suggesting that all these isolates are serologically closely related, if not identical. By means of ELISA we were not able to detect the virus in other beet samples from the Iran and in 124 samples from 14 European countries. We cannot exclude the possibility, however, that some of samples might have contained the virus, but due to the necrotic reactions, it has been reported to cause, it may not have been detectable any more in the deteriorating tissues. The beet plants from which the Iranian BSBV was obtained, like those from which the US isolate (Weiland *et al.*, 2008) was obtained, did not show black scorch symptoms. They did, however, exhibit pronounced root beardedness like the beets infected by the US isolate did. However, whereas most of the US plants did not contain detectable amounts of BNYVV, this virus was readily detected in the Iranian beets.

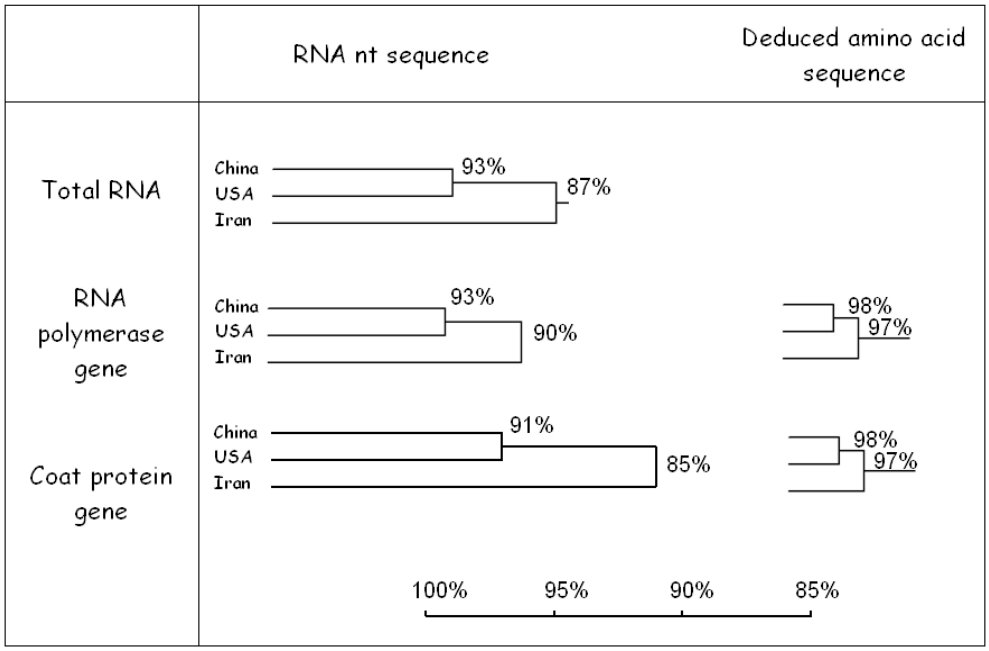

Fig. 2. 'Homology' trees showing the percentages of nt sequence identity for the total RNAs, the RNA polymerase genes and the coat protein genes and of the deduced amino acid sequences of the translation products of these genes of the Chinese, the US and the Iranian isolates of BBSV. The GenBank accession numbers are EU545828, AF452884 and EF153268 for the Iranian, Chinese (Ningxia) and US (Colorado) isolates, respectively.

The Iranian BBSV may or may not have contributed to the severity of the rhizomania symptoms. Further research will be necessary to study possibly existing differences in the pathogenicity of various BBSV isolates. In a preliminary experiment we have been unable to transmit the Iranian and the Chinese BBSV isolates to seedlings of two sugar beet cultivars by means of the 'vortexing' method which is highly efficient for transmitting BNYVV to beet seedlings in the absence of its vector (Koenig *et a*l., 2009a and b),

Acknowlegdements

The excellent technical assistance of Petra Lüddecke and Steffi Loss is gratefully acknowledged. We also thank Dr. K. Richert-Pöggeler, S. Schuhmann and C. Maß for immunoelectron-microscopical testing, Prof. Dr. J. Yu (China Agricultural University, Beijing) and Drs. J. Weiland and J. Suttle (USDA-ARS, Fargo, ND, USA) for gifts of viruses and antisera. The financial support given by the German Federal Ministry of Economics and Technology (AiF Grant 14163 N72, GFP BR42/04) is gratefully acknowledged.

References

Cai, Z.-N., Ding, Q., Cao, Y.-H., Bo, Y.-X., Lesemann, D.-E., Jiang, J.-X., Koenig, R., Yu, J. and Liu, Y. (1999). Characterization of a sugar beet (*Beta vulgaris* L.) virus causing black scorch symptom in China, a possible new member of the Genus *Necrovirus. Proceedings of the Fourth Symposium of the International Working Group on Plant Viruses with Fungal Vectors, Asilomar, October 5-8,1999 J. L. Sherwood and C. M. Rush, eds*, 9-12.

Cao, Y., Cai, Z., Ding, Q., Li, D., Han, C., Yu, J. and Liu, Y. (2002). The complete nucleotide sequence of *Beet black scorch virus* (BBSV), a new member of the genus Necrovirus. *Arch. Virol.* **147**, 2431-2435.

Guo, L. H., Cao, Y. H., Li, D. W., Niu, S. N., Cai, Z. N., Han, C. G., Zhai, Y. F. and Yu, J. L. (2005). Analysis of nucleotide sequences and multimeric forms of a novel satellite RNA associated with *Beet black scorch virus*. *J. Virol.* **79**, 3664-3674.

Koenig, R. and Valizadeh, J. (2008). Molecular and serological characterization of an Iranian isolate of *Beet black scorch virus*. *Arch. Virol.* **153**, 1397-1400.

Koenig, R., Loss, S., Specht, J., Varrelmann, M., Lüddecke, P. and Deml, G. (2009a). A single C/U nucleotide substituion in position 200 of the P25-coding sequence in *Beet necrotic yellow vein virus* RNA 3 greatly enhances the accumulation rate of the virus in rootlets of mechanically inoculated, partially resistant sugarbeet seedlings, *J. Gen. Virol.* (in press).

Koenig, R., Loss, S., Specht, J., Varrelmann, M., Lüddecke P. and Deml, G. (2009b). The accumulation of *beet necrotic yellow vein virus* in roots of mechanically inoculated, partially resistant sugar beet seedlings is greatly increased by a single C/U nucleotide substitution changing alanine to valine in the viral p25 protein. Proceedings of the Seventh Symposium of the International Working Group on Plant Viruses with Fungal Vectors, Quedlinburg, Germany, September 1-4, 2008 (this volume)

Weiland, J. J., van Winkle, D., Edwards, M. C., Larson, R. L., Shelver, W. L., Freeman, T. P. and Liu, H.Y. (2007). Characterization of a U.S.isolate of *Beet black scorch virus*. *Phytopathology* **97**, 1245-1254.

Weiland, J. J., Larson, R. L., Freeman, T. P., and Edwards, M. C. (2006). First report of *Beet black scorch virus* in the United States. *Plant Dis.* **90**, 828.

Yuan, X. F., Cao, Y. H., Xi, D. H., Guo, L. H., Han, C. G., Li, D. W., Zhai, Y. F. and Yu, J. L. (2006). Analysis of the subgenomic RNAs and the small open reading frames of Beet of *Beet black scorch virus*. *J. Gen. Virol.* **87**, 3077-3086.

ARE *POLYMYXA* SPP. SPECIFIC CARRIERS OF PLANT VIRUSES?

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Summary

Little is currently known about the interactions between plant viruses and their plasmodiophorid vectors *Polymyxa betae* and *P. graminis*. More than 15 viruses belonging to the *Beny-, Bymo-, Furo-, Peclu-* and *Pomovirus* genera are transmitted and associated with severe diseases, such as sugar beet rhizomania, mosaics on cereals and peanut clump. The pathosystems associated with these syndromes are complex. Distinct species of viruses may be associated within a region, a field or even a plant (*Beet necrotic yellow vein virus*, *Beet soil-borne mosaic virus*, *Beet soil-borne virus* and *Beet virus Q* on sugar beet; *Soil-borne cereal mosaic virus*, *Soil-borne wheat mosaic virus*, *Barley yellow mosaic virus* and *Barley mild mosaic virus* or *Wheat spindle streak mosaic virus* on barley or wheat). Some viruses cause different symptoms on distinct plant species (*Peanut clump virus* on peanut and sugar cane, *Indian peanut clump virus* on peanut and cereals, BNYVV on Chenopodiaceae). Their host ranges are often wider than the ranges of plants showing symptoms. In addition, at least two *P. graminis* form species are involved in pathosystems (cereal mosaics or peanut clump disease). Specific interactions between viruses and plants or between *Polymyxa* spp. and plants have been examined in many host range studies and molecular and genomic characterization studies, but the transmission of these viruses by their vectors needs more investigation. The mode of virus transmission by *Polymyxa* and the interactions between the viruses and their vectors are key factors in the epidemiological development influencing the future evolution of these pathosystems.

Analyses of the spatial distribution of viral and vector populations in both tropical and temperate areas and the occurrence of co-infection in plants suggest possible co-evolutions. However, experiments using virus transmission systems by *P. betae* and *P. graminis* under controlled conditions and the detection of viruliferous zoospores after the co-infection of aviruliferous zoospores and virus indicate a common virus transmission pathway.

Introduction

The plasmodiophorids *Polymyxa graminis* Ledingham and *Polymyxa betae* Keskin are ubiquitous soil-borne root endoparasites. Morphologically similar, they are distinguished by their separate host ranges, *P. graminis* growing mainly on Gramineae and *P. betae* mostly on Chenopodiaceae. Although they are not considered as pathogens, these species are well known to vector economically important phytoviruses on major crops, causing severe diseases such as rhizomania of sugar beet, cereal mosaics, peanut clump and rice stripe necrosis. Characterization studies of *Polymyxa* have shown that isolates from various geographical origins or host plants have particular ecological requirements, reducing the possibility of gene flow between distinct populations. These characteristics were determined on the basis of the separation of several formae speciales among *P. betae* and *P. graminis.* For *P. graminis*, molecular features (particular ribotypes) supported this differentiation (Legrève *et al*., 2002).

The acquisition and transmission of viruses by plasmodiophorid vectors involve *in vivo* relationships: the virus is acquired by the vector that grows in the plant. The virus is present in resting spores and probably in zoospores (Campbell, 1996). The readthrough portion of the coat protein is believed to play an important role in the transmission process: despite the very limited sequence similarity between the RT-domains of any plasmodiophorid-transmitted viruses, Adams *et al.* (2001) identified complementary transmembrane domains potentially involved in facilitating particle movement across the membrane. Currently, little is known about the transmission specificity of the viruses by *Polymyxa* spp. and f. sp., despite the epidemiological importance of the issue. The pathosystems involving *Polymyxa* and viruses are often very complex: several viruses and sometimes several *Polymyxa* f. sp. may be associated in the same syndrome (Table 1) and some viruses cause symptoms on distinct plant species. Studies on the transmission specificity aim at understanding the epidemiology of the diseases and the future evolution of pathosystems.

Table 1. Viruses and plasmodiophorid vectors involved in three pathosystems

Pathosystems/ syndrome	Vectors	Viruses	Plant species affected
Peanut clump Sugar cane red leaf mottle	*P. graminis* f. sp. *tropicalis* *P. graminis* f. sp. *subtropicalis*	*Peanut clump virus* (PCV) *Indian peanut clump virus* (IPCV)	*Arachis hypogaea* *Saccharum officinarum*
Cereal mosaics	*P. graminis* f. sp. *temperata* *P. graminis* f. sp. *tepida*	*Barley yellow mosaic virus* (BaYMV) *Barley mild mosaic virus* (BaMMV) *Wheat spindle streak mosaic virus* (WSSMV) *Wheat yellow mosaic virus* (WYMV) *Soil-borne wheat mosaic virus* (SBWMV) *Soil-borne cereal mosaic virus* (SBCMV)	*Hordeum vulgare* *Triticum aestivum*
Rhizomania	*P. betae*	*Beet necrotic yellow vein virus* (BNYVV) *Beet soil-borne virus* (BSBV) *Beet virus Q* (BVQ) *Beet soil-borne mosaic virus* (BSBMV)	*Beta vulgaris*

Studying transmission specificity requires conducting experiments under controlled conditions to investigate the *in vivo* acquisition of viral particles, physically separated from their vector by an aviruliferous *Polymyxa* isolate on a host plant, and their transmission to another plant (Fig. 1). Such experiments are difficult to conduct because of the difficulties in growing the vector, to separate the virus from its vector or to reproduce the natural transmission and maintain viruliferous inoculum under controlled conditions. Three approaches were adopted in investigating the transmission specificity for the three pathosystems described in Table 1 and are presented here.

Materials and methods

Sources of viruses. Systematically PCV-infected plants were obtained through other natural modes of transmission of this virus by planting PCV-infected seeds of pearl millet (*Pennisetum glaucum* [L.] R. Br.) from a field in Niger and by planting PCV-infected sugar cane stem cuttings originating from Burkina Faso. BNYVV-infected *Beta macrocarpa* plants were obtained after mechanical inoculation of BNYVV Stras 12 isolate with *in vitro* transcripts synthesized from cDNA clones of full length BNYVV-RNA3 and RNA4.

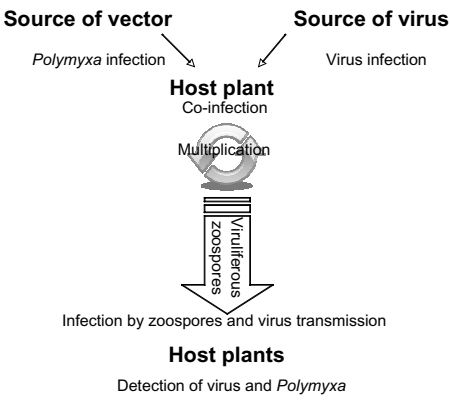

Fig. 1. Virus acquisition and transmission by *Polymyxa* in vivo

Sources of Polymyxa. Aviruliferous cultures of the *P. graminis* f. sp. *tropicalis (P. g. tropicalis*) isolate Ni-I from Niger, *P. graminis* f. sp. *subtropicalis* I9 from India and a BSBV-associated *P. betae* isolate from Belgium were revived and maintained on pearl millet and sugar beet, respectively, using automatic immersion systems (AIS) (Legrève *et al*., 1998). Large amounts of zoospores were released from infected plants.

In vivo acquisition and transmission of viruses by Polymyxa. Zoospores were released from aviruliferous *Polymyxa*-infected roots of plants grown in the AIS in 1/5 diluted Hoagland solution. Zoospores suspensions were used to inoculate roots of virus-infected plants. After incubation for 3-4 weeks, zoospores released from these plants were inoculated into healthy plantlets. The infection of these plants by the virus and *Polymyxa* were analyzed at least 2 weeks after inoculation.

Virus and vector detection. Viruses and vectors were detected in plants by RT-PCR after RNA extraction using the methods described by Legrève *et al.* (2003) for *P. graminis tropicalis* and *P. graminis* f. sp. *subtropicalis* and *P. betae*, by Vaïanopoulos *et al.* (2007) for *P. graminis* f. sp. *temperata* and *P. graminis* f. sp. *tepida,* by Meunier *et al.* (2003) for BNYVV, by Bragard *et al.* (2006) for PCV. The presence of the vector in the roots and the quantification of zoospores were also assessed by microscopic observation.

Results and discussion

Peanut clump: The seed-transmitted Nigerian PCV isolate on pearl millet was transmitted by zoospores of the Ni-I isolate of *P. g. tropicalis* produced on and released from the PCV-infected plant into healthy plants. More than 20% of the inoculated plants were infected by PCV 3 weeks after infection, but the viruliferous potential subsequently decreased, although the vector persisted in all the inoculated plants. The transmission of the PCV isolate involved in the sugar cane red leaf mottle symptom and maintained by stem cuttings of sugar cane by the Ni-I isolate of *P. g. tropicalis* was investigated by inoculating aviruliferous zoospores into the PCV-infected roots of sugar cane grown in AIS. Three weeks after inoculation, zoospores were released from the PCV-infected plants and inoculated into pearl millet plants. PCV was detected in two-thirds of them. A similar experiment was conducted using the I9 isolate of *P. g. subtropicalis* and revealed that this isolate was also able to transmit the PCV isolate from sugar cane to other cereals. These results showed that the isolates of *P.g. tropicalis* and *P. g. subtropicalis*, both originating from peanut clump infested areas, might act as a vector of a PCV isolate originally associated with red leaf mottle symptoms. Experiments to investigate the transmission of PCV by *P. g. temperata* using sugar cane are now under progress.

Cereal mosaics: The approach for determining the transmission specificity of cereal mosaic viruses by *P. g. temperata* and *P. g. tepida* differs because *in vivo* acquisition and transmission under controlled conditions are complicated in this

pathosystem by the slow development of *P. g. temperata*, the absence of viral sources separated from the vector and the need for a culture of *P. g. tepida*. The detection of BaYMV, SBCMV, *P. g. temperata* and *P. g. tepida* in plants collected in fields or grown under controlled conditions on soils from Belgium and France (Table 2) revealed numerous co-infections. The associations of BaYMV with *P. g. temperata* on barley and of SBCMV with *P. g. tepida* on wheat were the most frequent associations, indicating a preferential pathway of transmission for a given virus by a *P. graminis* form species. However, the occurrence of SBWMV in plants with *P. g. temperata* or of BaYMV with *P. g. tepida* challenged the specificity of the transmission. Despite the formal evidence showing the *in vivo* acquisition and transmission following a scheme presented in Fig. 1, the occurrence of multiple associations suggests that the epidemiology of cereal mosaics viral diseases might be influenced by other agents involved in the same pathosystem (Table 1).

Rhizomania: Co-infections of BNYVV-BSBV and BNYVV-BSBV-BVQ are frequent in the nature (Meunier *et al.,* 2003). The isolation of cultures of *P. betae* associated only with BNYVV, BSBV or BVQ from soils was hampered by the difficulty in separating BNYVV from the other viruses. The transmission of BNYVV viral particles from infected clones with a *P. betae* isolate associated with BSBV demonstrated the ability of this isolate to transmit two viruses from the same pathosystems. The combination of monosporosorus aviruliferous isolates and viral infection clones will help to assess the transmission specificity.

Table 2. Frequency of co-infections of BaYMV, SBCMV, *P. g. temperata* and/or *P. g. tepida* in barley and wheat plants (Vaïanopoulos, 2008)

	Detection in plants by RT-PCR				Frequency of association (number of tested plants)	
Plant Species	BaYMV	SBCMV	*P. g. temperata*	*P. g. tepida*	on plants grown under controlled conditions	on plants collected in fields
Barley	+	+	+	-	46% (26)	0%
	-	+	+	+	4% (26)	0%
	+	-	+	-	27% (26)	70% (20)
	-	+	+	-	23% (26)	0%
	+	-	+	+	0%	30% (20)
Wheat	+	+	+	-	8%(26)	0%
	-	+	-	+	57% (26)	64% (25)
	-	+	+	+	8% (26)	16% (25)
	+	+	-	+	19% (26)	0%
	+	+	+	+	8% (26)	0%
	+	-	+	+	0%	8% (25)
	+	-	-	+	0%	12% (25)

References

Adams, M.J., Antoniw, J.F., and Mullins, J. (2001). Plant virus transmission by plasmodiophorid fungi is associated with distinctive transmembrane regions of virus-encoded proteins. *Arch. Virol.* **146**, 1139-1153.

Bragard, C., Doucet, D., Dieryck, B., and Delfosse, P. (2006). Pecluviruses. In '*Characterization, Diagnosis & management of Plant viruses. Vol. 3 : Vegetable and Pulse Crops*' (Rao G., Lava-Kumar, P. and R.J. Holguin-Pena, Eds), pp. 125-140. SCI Tech publishing, Studium press, LLC, P.O. Box 722200 Houston, Texas.

Campbell, R.N. (1996). Fungal transmission of plant viruses. *Annual Review of Phytopathology*, Vol. **34**: 87-108.

Legrève, A., Delfosse, P., and Maraite, H. (2002). Phylogenetic analysis of *Polymyxa* species based on nuclear 5.8S and internal transcribed spacers ribosomal DNA sequences. *Mycological Research* **106**,138-147.

Legrève, A., Delfosse, P., Van Hese, V., Bragard, C., and Maraite, H. (2003). Broad-spectrum detection of *Polymyxa* species and form species by polymerase chain reaction. In: *Proceedings of the fifth Symposium of the International Working Group on Plant Viruses with Fungal Vectors* (Rush C.M. and U. Merz, Eds)*,* pp.40-43. American Society of Sugar Beet Technologists, Denver, USA.

Legrève, A., Delfosse, P., Vanpee, B., Goffin, A., and Maraite, H. (1998). Differences in temperature requirements between *Polymyxa* sp. of Indian origin and *Polymyxa graminis* and *Polymyxa betae* from temperate areas. *European Journal of Plant Pathology* **104**, 195-205.

Meunier, A., Schmit, J.F., Stas, A., Kutluk, N., and Bragard, C. (2003). Multiplex Reverse Transcription-PCR for Simultaneous Detection of *Beet necrotic yellow vein virus*, *Beet Soil-borne virus*, and *Beet virus Q* and Their Vector *Polymyxa betae* KESKIN on Sugar Beet. Appl. *Environ. Microbiol.* **69**: 2356-2360.

Vaïanopoulos, C., Bragard, C., Moreau, V., Maraite, H., and Legrève A., 2007. Identification and quantification of *Polymyxa graminis* f.sp. *temperata* and *P. graminis* f.sp. *tepida* on barley and wheat. *Plant Disease,* **91** : 857-864.

MOLECULAR PHYLOGENETIC ANALYSIS OF JAPANESE STRAINS OF *BARLEY YELLOW MOSAIC VIRUS*

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Summary

Complete nucleotide sequences of three strains (I, III and IV) of *Barley yellow mosaic virus* (BaYMV) isolated in Japan were determined. The length of the genome was the same among the three strains; RNA1 was 7,642 nt and RNA2 was 3,585 nt. The molecular phylogenetic analysis showed that strain I was most closely related to the Chinese isolate, and these two strains formed one cluster with European isolates. Strains II, III, IV, and the Korean isolate formed another cluster. Amino acid sequences of each viral gene product were compared among strains. The sequences of the VPg protein showed relatively less identity among almost strains (less than 92%) than for the sequences of other proteins (more than 93%). VPg is thought to be involved in interactions with host factors, especially initiation factor 4E (eIF4E) or eIF(iso)4E, and infection. Therefore, the relationship between amino acid substitutions and infection of host plants is analyzed.

Introduction

Barley yellow mosaic virus (BaYMV) is the type member of the genus *Bymovirus*, family *Potyviridae*, and is transmitted in soil by the plasmodiophorid *Polymyxa graminis*. It has slightly flexuous rod-shaped particles with two modal lengths of 550 and 275 nm and has a single stranded, positive-sense RNA genome with two components of approximately 7.6 (RNA 1) and 3.5 (RNA 2) kb. The complete nucleotide sequences of BaYMV isolates from Japan (BaYMV-J), China (BaYMV-C), Korea (BaYMV-K), Germany (BaYMV-G) and the UK have been determined, and strains in Japan are classified into four types, I to IV. VPg is covalently linked to the 5´ end of viral RNA and is required for potyvirus infectivity (Robaglia and Caranta, 2006). Bymovirus-VPg is also known to interact with the eukaryotic translation initiation factor 4E for infection (Robaglia and Caranta, 2006.

Materials and Methods

Infected barley (*Hordeum vulgare* L.) leaves with typical symptoms were collected from three sites in Tochigi prefecture, Japan. The plants used in this study were cultivated in test fields, where only one virus strain existed. Virus strain I was isolated from cv. Nasu Nijo, strain III from cv. Mikamo Golden, which carries the resistant gene *rym5* and is resistant to strain IV, and strain IV from cv. Sachiho Golden, which carries *rym3* and resistant to strain III (Table 1). Approximately 1.5 µg of total RNA was used for cDNA synthesis in a 20 µl reaction mixture using oligo (dT)18 primer and Expand Reverse Transcriptase (Roche Diagnostics GmbH, Germany) following the manufacturer's instructions. The reaction was carried out for 60 min at 37 °C followed by 60 min at 42 °C.

Table 1. Reactions between test cultivars and BaYMV strain types.

	Virus strains				Resistance gene
	I	II	III	IV	
Amagi Nijo	S	R	S	S	*rym6*
Nasu Nijo	S	R	R	S	?
Mikamo Golden	R	R	S	R	*rym5*
Sachiho Golden	R	R	R	S	*rym3*
Sukai Golden	R	R	R	R	*rym3* and *rym5*

S: Infection and symptom development in susceptible cultivar
R: No infection and no symptom development in resistant cultivar
?: Existence of resistance gene unknown

Using complete sequences of BaYMV isolates in GenBank, some primer pairs spanning virtually all of RNA1 and RNA2 excluding 5´ regions were designed (Nishigawa *et al*., 2008) and PCR reactions were performed using KOD-Plus (TOYOBO, Japan) following the manufacturer's instructions. To determine the 5´-end of the genome we performed 5´ RACE by using the 5´ RACE System for Rapid Amplification of cDNA Ends, Version 2.0 (Invitrogen, CA, USA). PCR fragments were cloned into a pTA2 vector (TOYOBO, Japan) following the manufacturer's instructions. These clones were sequenced by an automated DNA sequencer (ABI PRISM 3100-Avant, Applied Biosystems, CA).
Secondary structure of the VPg protein was predicted using deduced amino acids from strain I of BaYMV. Prediction was conducted using the PSIPRED protein structure prediction method (http://bioinf.cs.ucl.ac.uk/psipred/).

Results and Discussion

In this study, we sequenced genomic RNAs of strains I, III and IV of BaYMV. Complete sequences of all Japanese isolates found to date, representing all four strains, have now been determined. Excluding strain II, the complete sequences of each RNA1 or RNA2 were the same length among strains; RNA1 was 7,642 nt and RNA2 was 3,585 nt. These sequences were similar to those previously reported isolates. All strains sequenced in this study encoded a single polyprotein in RNA1 and RNA2, and all genes were conserved among isolates.

The phylogenetic tree constructed using deduced amino acids of the CP revealed that strain I was closely related to the Chinese isolate, and these two strains formed one cluster with European isolates (Fig. 1). Comparing genes among strains, VPg had the lowest identity (less than 92%) except for Chinese strain (95%), while others had more than 93% from the data example between Japanese strain I and other strains (Table 2), and had relatively more substitutions (Fig. 2).

Secondary structure prediction and multiple alignment of BaYMV-VPg (Fig. 2) showed that positions from 119 to 124 were predicted to form]-sheet. In this sequence, residue120 was acidic Glu (E) in strain I and IV whereas basic Lys (K) in strain II and uncharged Thr (T) in strain III. There are other amino acid substitutions in VPg among strains. Those indicated that these amino acid substitutions may change the three-dimensional structure or property of VPg, resulting in changes in the interaction between VPg and eIF4E. It was reported that the ability to overcome the *rym4*-mediated resistance in European isolates is due to substitution of the basic amino acid Lys-132 for the uncharged Asn-132, which is likely to alter VPg's biochemical properties (Kühne *et al.*, 2003). Among the Japanese isolates, residue 132 was the basic amino acid Lys (K) in strain I, His (H) in strains II and III, and the uncharged amino acid Tyr (Y) in strain IV. The L(MVI)xL(MI)xxH motif that is associated with pathogenicity is conserved among all viruses within the family *Potyviridae* (Kanyuka *et al.*, 2004). This motif is conserved among strains I (MEISPH), II, and III (MDISQH), but in strain IV Ile-135 was substituted to Val-135 (MDVSQH). Low identities of VPg (Table 2) means that the evolutional speed of VPg is faster than other genes. Therefore, another resistance-breaking virus with amino acid change in the VPg may break out in the near future.

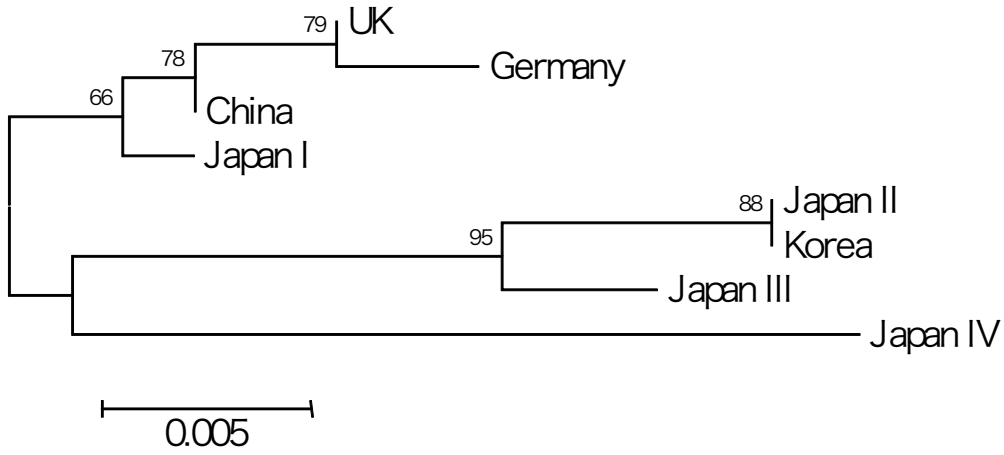

Fig. 1. Phylogenetic tree using deduced amino acid sequences of CP of *Barley yellow mosaic virus* isolates.

A neighbor-joining phylogenetic tree was produced by the MEGA program on the basis of a ClustalW alignment of the amino acid sequences. The robustness of internal branches was tested using bootstrap analyses (1000 replicates).

Table 2. Sequence identity among regions of Japanese strain I and other BaYMV isolates.

	RNA1											RNA2				
	5´ UTR	P3	7K	Hel	14K	VPg	Pro	Rep	CP	3´ UTR	total	5´ UTR	P1	P2	3´ UTR	total
Japan II	88	97 96	96 100	97 98	96 96	94 92	97 93	97 98	98 98	98	98	90	95 95	95 95	98	95
Japan III	96	97 96	95 100	97 99	95 97	94 91	98 99	97 99	98 98	99	97	92	96 99	96 96	98	96
Japan IV	93	97 97	97 98	97 99	96 100	95 91	97 99	98 99	97 97	99	97	99	99 99	99 99	99	99
China	93	97 96	99 100	98 99	96 99	97 95	97 99	98 98	99 99	99	98	95	95 98	95 96	97	99
Korea	88	97 96	96 100	97 98	96 96	94 91	97 94	97 98	98 97	99	97	93	96 97	95 95	96	96
Germany	89	93 93	95 100	94 98	91 95	90 91	93 97	94 98	96 97	99	94	89	90 95	90 94	92	90
UK	89	93 93	93 100	94 98	92 95	89 90	92 96	94 98	96 99	90	93	94	90 94	90 94	92	90

Numbers indicate percentage sequence identity; upper number represents sequence identity of nucleic acids, lower number represents amino acid identity. Amino acids with lower than 92% identity were highlighted.

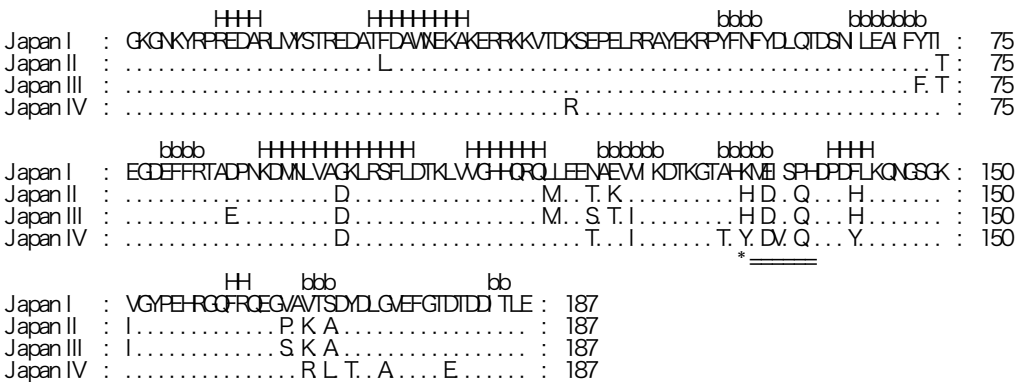

Fig. 2. Alignment of deduced amino acid sequences of VPg among Japanese BaYMV strains I-IV.

Amino acids that are identical to those of Japanese strain I are indicated by dots. Predicted secondary structures are indicated above the amino acid alignment. H: helix: b:]-sheet. Asterisk and double line under the alignment indicate the position related to overcoming *rym4* resistance (Kühne *et al.*, 2003) and the L(MVI)xL(MI) xxH motif (Kanyuka *et al.*, 2004), respectively.

Acknowledgements

This work was supported in part by KAKENHI (19780033 and 17208004) from the Japan Society for the Promotion of Science (JSPS), and Young Scientist's Research at Utsunomiya University, Japan.

References

Kanyuka, K., McGrann, G., Alhudaib, K., Hariri, D. and Adams, M.J. (2004). Biological and sequence analysis of a novel European isolate of *Barley mild mosaic virus* that overcomes the barley *rym5* resistance gene. *Arch. Virol.* **149**, 1469-1480.

Kühne, T., Shi, N., Proeseler, G., Adams, M.J. and Kanyuka, K. (2003). The ability of a bymovirus to overcome the *rym4*-mediated resistance in barley correlates with a codon change in the VPg coding region on RNA1. *J. Gen. Virol.* **84**, 2853-2859.

Nishigawa, H., Hagiwara, T., Yumoto, M., Sotome, T., Kato, T. and Natsuaki T (2008). Molecular Phylogenetic Analysis of *Barley yellow mosaic virus*. *Arch. Virol.* **153**, 1783-1786.

Robaglia, C. and Caranta, C. (2006). Translation initiation factors: a weak link in plant RNA virus infection. *Trends Plant Sci.* **11**, 40-45.

DIAGNOSTIC MULTIPLEX RT-PCR ANALYSIS FOR THE DETECTION OF SOIL-BORNE MOSAIC VIRUSES AND THEIR NATURAL VECTOR *POLYMYXA GRAMINIS*

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Summary

To have the opportunity for rapid, reliable and specific detection and discrimination of *Soil-borne mosaic viruses* affecting cereals, two multiplex reverse transcription-PCR (mRT PCR) protocols both for furoviruses and bymoviruses were developed. The protocols utilises specific primer sets for each virus. Because of the great agronomic importance of *Polymyxa graminis* Ledingham (*P. graminis*) as a viral vector, the diagnostic assays were fulfilled with *P. graminis*-specific primers. It makes possible the simultaneous detection of the viral agents together with their fungal vector in the same reaction. The amplification specificity of all primers designed was tested on artificial mixtures of root plant materials and field samples.

Introduction

Soil-borne mosaic viruses belonging to the genera *Furovirus*, *Bymovirus* and *Pecluvirus* causing severe and economically important mosaic disease in winter cultures of wheat, barley, rye and triticale are transmitted by an obligate root-infecting plasmodiophorid fungus *P. graminis* (Brakke and Langenberg,1988; Adams, 1991), which cannot be controlled by chemical measures. The need of clear and rapid detection of these viruses together with their vector, especially on the early stages of infection, is explained by significant level of disease and yield losses worldwide. Moreover, reliable and rapid diagnostic systems are necessary to investigate epidemiological aspects of appearance and distribution of these viruses, to understand their evolution, to characterize the association between viruses and their natural vector and to find new sources of resistance, which represent the only mean of control. In this study a simple, costs reducing technique for simultaneous detection of soil-borne cereal viruses and *P. graminis* with high degree of specificity and sensitivity is proposed, based on the combination of multiple primer sets to different pathogens into single “multiplexed” amplification reaction, that share single standard amplification conditions. Recently multiplex PCR, where two and more target sequences are simultaneously synthesized in

single reaction mixture, is widely applied for the identification of viruses (Casas *et al*., 1999; Meunier *et al*., 2003; Schubert *et al*., 2007; Deb and Anderson, 2008), and bacteria (Nayak *et al*., 2005). mRT-PCR methods are shown to be significantly more sensitive than an enzyme-linked immunosorbent assays (ELISA).

Materials and Methods

Two separate reaction mixtures are proposed for the differentiation of both the bymoviruses *Barley mild mosaic virus* (BaMMV), *Barley yellow mosaic virus* (BaYMV) and *Wheat spindle streak mosaic virus* (WSSMV) and the furoviruses *Soil-borne cereal mosaic virus* (SBCMV) and *Soil-borne wheat mosaic virus* (SBWMV) together with *P. graminis*. The third mixture was developed for the discrimination in single reaction furovirus SBCMV, bymovirus WSSMV and their fungal vector.

Primers for multiplex RT (mRT)

Starting from the total RNA preparation from approximately 100 mg of root plant material, frozen in liquid nitrogen and ground in an RNase- DNase-free mortar and pestle (for RNA isolation Plant RNA Reagent from Invitrogen was used), RT-PCR was performed to generate the fragments, which showed a good resolution in agarose gel electrophoresis and were easily distinguished from each other by size. Primer sequences that proved to be useful for this aim and PCR-fragment's sizes are given in Tables 1 and 2.

A single universal poly dT-primer was sufficient for the cDNAs syntheses in the case of bymoviruses. For cDNAs generation from genomes of furoviruses virus specific primers were applied (for SBCMV- SBCMVrev_RNA2, for SBWMV - PGRV4b, Table1).

Each mRT reaction mixture was supplemented with reverse primer, proposed by Ward and Adams (1998) and specific to internal transcribed spacer (ITS2 region) of *Polymyxa* species (Pxrev7, Table 1*)*. For bymoviruses two reverse primers were combined in the mRT, for furoviruses – three antisense primers were needed.

In the case of mRT for mixture of SBCMV, WSSMV and *P. graminis* genome-specific antisense primers SBCMVrev_RNA2, 7b and Pxrev7 (Table 1) respectively were added.

Primers for multiplex PCR (mPCR)

Virus specific primers for mPCR were developed based on the sequence differences between relative viruses inside the same genus after visual scoring of the alignment report. For bymoviruses primer pairs specific to RNA 1s were chosen (Table 1). In the case of furoviruses the primer pairs amplifying RNA 2s were applied (Table 1).

For the detection of *P. graminis* primers specific to all *Polymyxa* species described by Ward and Adams (1998), were used (Table 1).

mPCR conditions for amplification of furovirus fragments were 3 min at 96°C followed by 35 cycles (96°C - 30 s / 56°C - 75 s / 2°C - 60 s) and 10 min at 72°C as final extension stage.
For the generation of bymovirus specific PCR products the annealing temperature was slightly increased to 58°C.

Table1. Primers used for multiplex analysis.

		Primer name	Primer sequence (5'-3')
Bymoviruses			
BaMMV	Sense:	MS1- F1	TGCTCGTCACTCTCCTGGT
	Antisense:	P17a	GTGCTTTTGCGGTCTTGATGA
BaYMV	Sense:	MS1- R1	ATCAGCGGAAGCTACTAGAAG
	Antisense:	P18	TGGTTCCTCAATAGCAAAAG
WSSMV	Sense:	7a	CAACCGTTTTCTCAGCACTT
	Antisense:	7b	GCTTTCTCATTCCAACTATCG
Furoviruses			
SBCMV	Sense:	SBCMVfor_RNA2	ACTTACCCATTTAGGTGTAA
	Antisense:	SBCMVrev_RNA2	TTATAATCACGCAAGTACCT
SBWMV	Sense:	PGRV4a	CTGCGACTCACGCTTACATA
	Antisense:	PGRV4b	TAACCGCTTTGGGATGATAG
Polymyxa graminis	Sense:	Pxfwd1	CTGCGGAAGGATCATTAGCGTT
	Antisense:	Pxrev7	GAGGCATGCTTCCGAGGGCTCT

The PCR Extender System (5 Prime) was appropriate for reliable and reproducible results in both cases. Each mPCR reaction included 2 µl of cDNA, 0.5 µl of dNTP mixture with each dNTP at 5 mM, 2.5 µl 10x Polymerase Buffer, 0.3 µl of 5 U/µl Taq Polymerase and 1 µl of each primer set (a mixture of 10µM forward and 10 µM reverse primers). Final reaction volume was 25 µl. Amplified products were electrophoresed on 1.4% agarose gels. The obtained PCR fragment lengths corresponded to expected molecular weights of amplicons. Their specificity was confirmed by sequencing and compared with known deposited in GenBank (NCBI) viral sequences.

Table 2. Expected size of PCR fragments.

Virus / Fungus	**Fragment size (bp)**
BaMMV	700
BaYMV	552
WSSMV	817
SBWMV	681
SBCMV	978
P. graminis type 1	280
P. graminis type 2	320

Results and Discussion

Multiplex assays were validated on the artificial mixtures of root plant materials, harbouring both the viruses and vector (Fig. 1 and 2).

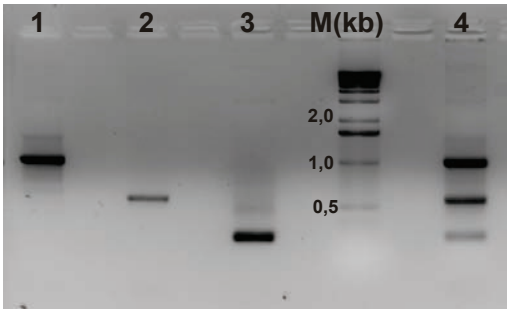

Fig. 1. Triplex of furoviruses together with *P. graminis* in one reaction mixture with primers for SBCMV, SBWMV and *P. graminis* – line 4.
Controls: Line 1 – cDNA for SBCMV, line 2 – cDNA for SBWMV, line 3 – cDNA for *P. graminins* . Each amplified in one reaction mixture with sets of three pairs specific primers.

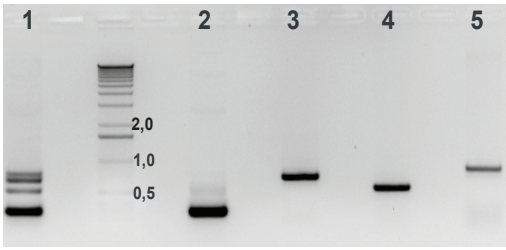

Fig. 2. Quadruplex of bymoviruses together with *P. graminis* in one reaction mixture with primers for BaMMV, BaYMV, WSSMV and *P. graminis* - line 1. Controls: Line 2 – cDNA for *P. graminis*, line 3 – cDNA for BaMMV, line 4 – cDNA for BaYMV, line 5 – cDNA for WSSMV. Each amplified in one reaction mixture with sets of four pairs specific primers.

Two viral species SBCMV and WSSMV, belonging to different families and widely distributed across Europe are devastating for winter wheat (Clover *et al*., 1999; Gitton *et al*., 1999; Rubies Autonell and Vallega, 1987; Koenig and Huth, 2000; Burge *et al*., 2008). They are often found in wheat in mixed infection. Because the special interest to differentiate these two viruses in one single reaction exists, we have pooled them together with *P.graminis* and after optimization of mRT-PCR conditions the specific amplification was performed (Fig. 3).
All mRT-PCR assays were highly specific, convenient and can be used for epidemiological studies as well as for quarantine and breeding programs.

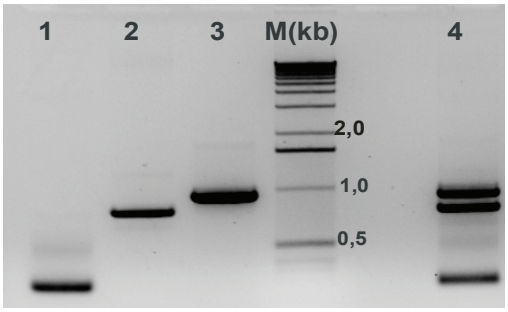

Fig. 3. Triplex of bymovirus WSSMV, furovirus SBCMV together with *P. graminis* in one reaction mixture with primers for WSSMV, SBCMV and *P. graminis* - line 4.
4. Controls: Line 1 - cDNA for *P. graminis*, line 2 - cDNA for WSSMV, line 3 - cDNA for SBCMV. Each amplified in one reaction mixture with sets of three pairs of specific primers.

Up to date we were not successful to perform a one-tube mRT-PCR for all soil-borne viruses and *P. graminis,* probably because of the narrow size range of amplified PCR-fragments and overloading of reaction mixture with big number of different primers. Multiplex assays were applied to study the infection processes in field plants in comparison to ELISA and TBIA. The method described is efficient and more sensitive than immunological procedures and does allow the clear early-season/early-stage detection of single or mixed virus infections simultaneously with the fungal vector (not shown).
Further improvement of diagnostic assay combining the detection of all mentioned above soil-borne viruses and *P. graminis* in one reaction mixture is under way.

References

Adams, M.J. (1991). Transmission of plant viruses by fungi. *Ann. Appl. Biol.* **118**, 479-492.

Brakke, M.K. and Langenberg, W.G. (1988). Experiences with *Soil-borne wheat mosaic virus* in North America and elsewhere. In: *Developments in Applied Biology: Viruses with Fungal Vectors, Vol. 2.* J.I. Cooper and M.J.C. Asher, eds. Association of Applied Biologists, Wellesbourne, England, 183-202.

Burge, G.E., Ratti, C., Rubies-Autonell, C., Lockey, D., Bonnefoy, M., Vallega, V., Pietravalle, S. and Henry, C.M. (2008). Response of UK winter wheat cultivars to Soil-borne cereal mosaic and *Wheat spindle streak mosaic viruses* across Europe. *Eur. J. Plant Pathol*., **120**, 259-272.

Casas, I., Pozo, F., Trallero, G., Echevrria, J.M. and Tenorio A. (1999). Viral diagnosis of neurological infection by RT multiplex PCR: a search for entero- and herpesviruses in a prospective study. *J. Med. Virol*. **57**, 145-151.

Clover, G.R.G., Wright, D. and Henry, C.M. (1999). Occurrence of *Soil-borne wheat mosaic virus* in UK. In: *Proceedings of Fourth Symposium of the International Working Group on Plant Viruses with Fungal Vectors*, Monterey, USA, 105-108.

Deb, M. and Anderson J.M. (2008). Development of a multiplexedx PCR detection method for *Barley and Cereal yellow dwarf viruses*, *Wheat spindle streak virus*, *Wheat streak mosaic virus* and *Soil-borne wheat mosaic virus*. *J. Virol. Methods* **148**, 17-24.

Gitton, F., Diao, A., Durcot, O., Antoniw, J.F., Adams, M.J. and Maraite, H. (1999). A two-step multiplex RT-PCR method for the simultaneous detection of *Soil-borne wheat mosaic virus* and *Wheat spindle streak mosaic virus* from France. *Plant Pathol*. **48**, 635-541.

Koenig, R. and Huth, W. (2000). *Soil-borne rye mosaic* and *European wheat mosaic virus*: Two names for a Furovirus with variable genome properties which is widely distributes in several cereal crops in Europe. *Arch. Virol*. **145**, 689-697.

Meunier, A., Schmit, J.-F., Stas, A., Kutluk, N. and Bragard, C. (2003). Multiplex reverse transcription-PCR for simultaneous detection of *Beet necrotic yellow vein virus*, *Beet soil-borne virus* and *Beet virus Q* and their vector *Polymyxa betae* KESKIN on sugar beet. *Appl. Environ. Microbiol*. **69**, 2356-2360.

Nayak, R., Stewart, T.M. and Nawaz, M.S. (2005). PCR identification of *Campylobacter coli* and *Campylobacter jejuni* by partial sequencing of virulence genes. *Mol. Cell. Probes* **19**, 187-193.

Rubies-Autonell, C. and Vallega, V. (1987). Observation on a mixed *Soil-borne wheat mosaic virus* and *Spindle streak mosaic virus* infection in durum wheat. *J. Phytopathol*. **119**, 111-121.

Schubert, J., Fomitcheva, V., Sztragret-Wiesniewska, J. (2007). Differentiation of *Potato virus* Y strains using improved sets of diagnostic PCR-primers. *J. Virol. Methods* **140**, 66-74.

Ward, E. and Adams, M.J. (1998). Analysis of ribosomal DNA sequences of *Polymyxa* species and related fungi and the development of genus- and species-specific PCR primers. *Mycol. Res*. **102**, 965-974.

NEW *BEET SOIL-BORNE MOSAIC VIRUS* RNA-4 FORM ISOLATION FROM INFECTED SUGAR BEET ROOTS

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Summary

The Benyvirus genus ascribes both *Beet necrotic yellow vein virus* (BNYVV) and *Beet soil-borne mosaic virus* (BSBMV), vectored by the plasmodiophorid *Polymyxa betae.* BSBMV is widely distributed only in the United States and, up to date, it has not been reported in others countries. The described BSBMV genome consists of four RNAs capped at the 5'end and 3' polyadenylated. A 1,203 nt long BSBMV RNA4 form has been so far described with a single putative ORF. We report the detection of unexpected 1,733 nucleotides long form of BSBMV RNA4 from sugar beet roots grown on BSBMV infected soil. The new BSBMV RNA4 form has been molecularly and functionally characterized.

Introduction

Beet soil-borne mosaic virus (BSBMV) is a member of *Benyvirus* genus together with *Beet necrotic yellow vein virus* (BNYVV), both vectored by *Polymyxa betae* (Koenig and Lesemann, 2005). BSBMV is widely distributed only in the United States and, up to date, it has not been reported in others countries. It was first identified in Texas as a sugar beet virus morphologically similar but serologically distinct to BNYVV (Heidel *et al.*, 1997). Subsequent sequence analysis of BSBMV RNAs showed similar genomic organization to that of BNYVV but sufficient molecular differences to distinct BSMBV and BNYVV in two different species (Lee *et al.*, 2001).

The described BSBMV genome consists of four RNAs capped at the 5' end and 3' polyadenylated. RNA1, and RNA2 are, respectively, 6,683 and 4,615 nucleotides (nt) in length and contain putative ORFs similar to which identified on BNYVV RNA1 and RNA2. The 1,720 nt BSBMV RNA3 has a 29 kDa ORF that share 23% amino acid sequence identity with the 25 kDa ORF of BNYVV RNA3. A 1,203 nt long BSBMV RNA4 form (GenBank accession number: NC_003508) has been so far described with a single putative ORF with a predicted mass (13 kDa) considerably smaller than the BNYVV 31 kDa RNA4 product (Lee *et al.*, 2001).

Materials and Methods

Sugar beet plants were grown on BSBMV infected soil kindly supplied by Marc Richard-Molard (ITB, Paris). Total RNA was extracted from infected sugar beet root using Trizol reagent (Invitrogen, Carlsbad, CA).
RNA4 cDNA was synthesized by ImProm-II Reverse Transcriptase system (Promega, Madison, CA) using oligo(dT) primers and then amplified by using a sense primer (nt 1–40), containing a T7 RNA polymerase promoter and an *Not*I restriction site, coupled with the oligo(dT) primer containing *Bgl*II site. PCR products were digested by *Not*I and *Bgl*II restriction enzymes and cloned in *Not*I-*Bgl*II digested pUC19 (Fermentas) to obtain pUC47. Full-length *Bgl*II digested pUC47 cDNA sequence (GenBank acc. no. FJ424610) was used for the synthesis BSBMV RNA-4 infectious run-off transcripts, named tUC47.
Putative ORFs encoding 32 kDa and 13 kDa proteins were amplified by PCR using a sense primer containing *Nco*I restriction site and FLAG epitope sequence (p32NcoIF and p13NcoIF) and an antisense primer carrying *Bam*HI site. PCR products were digested by *Nco*I and *Bam*HI restriction enzymes and cloned in *Nco*I-*Bam*HI digested Rep3 vector (Schmidlin *et al.*, 2005) to obtain Rep3-P32 and Rep3-P13 clones respectively.
A series of mutants was generated by PCR mutagenesis. A translation stop codon was introduced into the 3' primers containing also *Bam*HI restriction site, and p32NcoIF primer was used as the 5' primer. The PCR products were, digested with *Nco*I and *Bam*HI, then ligated to replace the *Nco*I-*Bam*HI fragment in the Rep3-P32 plasmid to obtain Rep3-P32Δ1, Rep3-P32Δ2, and Rep3-P32Δ3 clones. The lengths of the three deletion mutants were 192 bp (7 kDa), 381 bp (14 kDa) and 588 bp (22 kDa), encode, respectively, 64, 127 and 198 amino acids from the N terminus of the p32 protein.

Results and Discussion

An unexpected 1,733 nucleotides long BSBMV RNA4 has been detected from sugar beet roots grown on BSBMV infested soil, during our research program, pointed to the studies of the molecular interactions between sugar beets and Benyviruses.

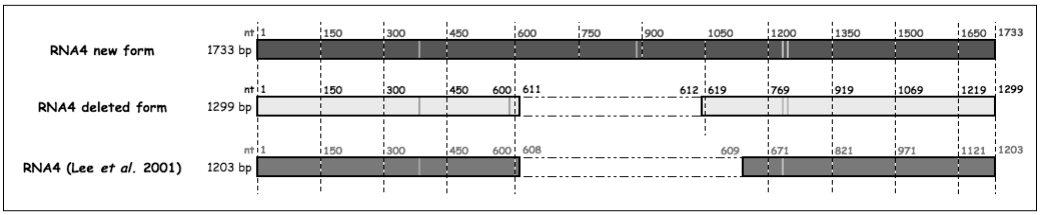

Fig. 1. Schematic comparison of the new BSBMV RNA4 form with RNA sequence described by Lee *et al.* (2001) and RNA4 deleted form obtained after 14 serial mechanical inoculations experiments.

Sequence analysis of the new BSBMV RNA4 form revealed high identity (~100%) with NC_003508 sequence between nucleotides 1-608 and 1,138-1,733, however the new form shows 529 additionally nucleotides between positions 608-1,138 (Fig. 1). Two putative ORFs has been identified, the first one (nucleotides 383 to 1,318), encode a protein with predicted mass of 32 kDa (p32) and the second one (nucleotides 885 to 1,259) express an expected product of 13 kDa (Fig. 2).
Serial mechanical inoculations of wild-type BSBMV on *Chenopodium quinoa* plants were performed every 7 days. Deleted form of BSBMV RNA4 (1299 bp)

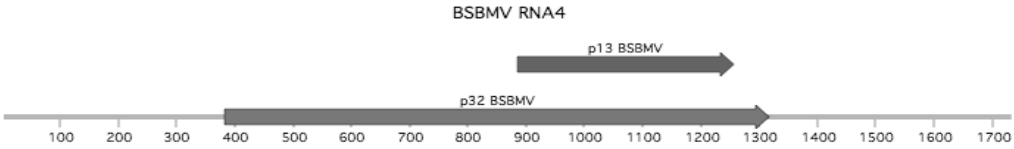

Fig. 2. Genetic map of the new BSBMV RNA4 forms. Two putative ORFs have been identified encoding for proteins with predicted mass of about 13 and 32 kDa.

appeared after 14 passages and its sequence analysis show deletion of 433 nucleotides between positions 611 and 1044 of RNA4 new form. We successfully obtained full-length infectious BSBMV RNA4 cDNA clone. *In vitro* transcripts produced from plasmid pUC47 were rub-inoculated together with BNYVV RNA1 and 2 (namely Stras12) helper strain transcripts (Quillet *et al.*, 1989) onto *C. quinoa* plant leaves. Typical chlorotic spots appeared 7 days post inoculation (dpi) for the Stras12 helper strain but when tUC47 transcripts were added, local necrotic spots appeared (not shown). Northern blot analysis was performed, as previous described (Link *et al.*, 2005; Schmidlin *et al.*, 2005), on total and encapsidated RNAs obtained using Trizol reagent and Protocol TM (Jupin *et al.*, 1990) respectively. Our results demonstrated BNYVV viral machinery ability to replicate and to encapsidate BSBMV RNA4 *in planta* (not shown).
Interestingly, using protocol previous described (Koenig and Burgermeister, 1989) we successfully verified the capability of the new BSBMV RNA4 form to promote BNYVV RNA1, 2 and 3 transmission through the vector *P. betae* in *Beta vulgaris* plants.
The FLAG epitope sequence was fused in frame at the N terminus of p32 and p13 proteins and cloned into Rep-3 to create Rep3-P32 and Rep3-P13. When inoculated onto *C. quinoa* leaves, together with Stras12 helper strain, the Rep3-P32 transcripts induced necrotic local lesions characteristic of tUC47. Otherwise the Rep3-P13 clone, containing p13 sequence, induced yellow local lesion (Fig 3a).
FLAG-tagged above-described p32 deletion mutants were analyzed for their ability to induce necrotic local lesions *in planta*. When inoculated onto *C. quinoa* leaves, together with Stras12, local lesions appeared 5–7 d.p.i. Stras12-like chlorotic spots were obtained with Rep3-P32Δ1 and Rep3-P32Δ2 clones but necrotic spots were found with Rep3-P32Δ3 mutant suggesting that the 64 N terminal amino acids of p32 probably play an important role in the symptoms induction (Fig 3a and b). Lesions obtained in the presence of Rep3-P32, Rep3-P13 and deletion

mutants were analyzed by western blot as previously described (Link *et al.*, 2005).When FLAG-specific antibodies conjugated with peroxidase or alkaline phosphatase were used, no specific signal was detected between 7 and 35 kDa. However, a specific FLAG tagged protein that specifically disappeared in the presence of FLAG-competitor appeared in the high molecular weight portion (> 170 kDa; not shown). These results suggest a possible strong interaction of p32 protein with cellular protein(s) that need to be further investigated.

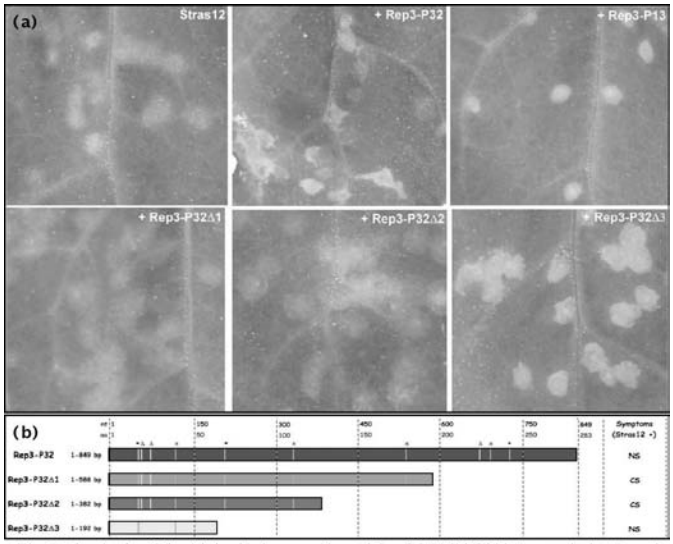

Fig. 3. Analysis of the biological properties of the BSBMV RNA4 encoded p32 and p13 proteins. (a) Local lesions on *C. quinoa* leaves after mechanical inoculation (7 dpi) with Stras12 isolate alone or supplied with infectious run-off transcripts synthesized from Rep3-P32, Rep3-P13, Rep3-P32Δ1, Rep3-P32Δ2, and Rep3-P32Δ3 cDNA clones. (b) Representation and symptoms induced by clones carrying p32 and its deleted forms. NS, necrotic spots; CS, chlorotic spots.* = N-glycosylation site, Δ = Protein kinase C or Casein kinase II phosphorylation site, π = N-myristoylation site.

Internal deletions or loss of smaller RNA species have been described for BNYVV isolates maintained in the greenhouse by repeated mechanical inoculation to local lesion hosts (Hehn *et al.*, 1994; Richards and Tamada, 1992). Moreover prolonged cultivation of field-infected plants or growth at high temperatures results in the spontaneous deletion of part of *Soil-borne mosaic virus* RNA2 (Chen *et al.*, 1995). Our study demonstrated that BSBMV RNA4 is longer than the form previous isolated from leaves of field-infected sugar beet plants maintained in the greenhouse for indefinite period (Lee *et al.*, 2001). Sequence analysis let us to formulate the hypothesis that Lee *et al.* (2001) described a spontaneous deletion mutant of the full-length form identified in our experiments. Our BSBMV RNA4 clone share 46% nucleotide sequence identity with BNYVV RNA4 as compared to the 35% previously described by Lee *et al.* (2001). Furthermore, such hypothesis is confirmed by the occurrence of a shorter RNA4 species, which appeared after several serial mechanical inoculations. Such deleted form was highly similar to the sequence described in 2001 (Fig. 1).

Finally, our results allowed us to associate the p32 expression to the local necrotic lesions phenotype on mechanically inoculated *C. quinoa* plants as well as its role in the transmission of BNYVV RNA1, 2 by the vector *P. betae*.

More experiments will be then needed to better investigate the role of the new BSBMV RNA4 form in the symptoms expression and in the *P. betae* mediated transmission exploiting similarities and divergences between BSBMV and BNYVV.

References

Chen, J., Macfarlane, S.A. and Wilson, T.M. (1995). An analysis of spontaneous deletion sites in *Soil-borne wheat mosaic virus* RNA2. *Virology* **209,** 213-217.

Hehn, A., Bouzoubaa, S., Jonard, G., Guilley, H., and Richards, K.E. (1994). Artificial defective interfering RNAs derived from RNA 2 of *Beet necrotic yellow vein virus*. *Arch Virol* **135,** 143-151.

Heidel, G.B., Rush, C.M., Kendall, T., Lommel, S.A. and French, R.C. (1997). Characteristics of *Beet soil-borne mosaic virus*, a Furo-like Virus Infecting Sugar Beet. *Plant Dis.* **81**, 1070-1076.

Jupin, I., Richards, K., Jonard, G., Guilley, H. and Pleij, C.W. (1990). Mapping sequences required for productive replication of *Beet necrotic yellow vein virus* RNA 3. *Virology* **178,** 273-280.

Koenig, R. and Burgermeister, W. (1989). Mechanical inoculation of sugar beet roots with isolates of *Beet necrotic yellow vein virus* having different RNA compositions. *J. Phytopathology* **124,** 249-255.

Koenig, R. and Lesemann, D.E. (2005). Genus Benyvirus. In *Virus Taxonomy, Eighth report of the International Committee on Taxonomy of Viruses***,** pp. 1043-1048. Edited by C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball. London: Elsevier Academic Press.

Lee, L., Telford, E.B., Batten, J.S., Scholthof, K.B. and Rush, C.M. (2001). Complete nucleotide sequence and genome organization of *Beet soil-borne mosaic virus*, a proposed member of the genus Benyvirus. *Arch Virol.* **146,** 2443-2453.

Link, D., Schmidlin, L., Schirmer, A., Klein, E., Erhardt, M., Geldreich, A., Lemaire, O. and Gilmer, D. (2005). Functional characterization of the *Beet necrotic yellow vein virus* RNA-5-encoded p26 protein: evidence for structural pathogenicity determinants. *J. Gen. Virol.* **86,** 2115-2125.

Quillet, L., Guilley, H., Jonard, G. and Richards, K. (1989). In vitro synthesis of biologically active *Beet necrotic yellow vein virus* RNA. *Virology* **172,** 293-301.

Richards, K.E. and Tamada, T. (1992). Mapping functions on the multipartite genome of *Beet necrotic yellow vein virus. Ann. Rev. Phytopathol.* **30,** 291-313.

Schmidlin, L., Link, D., Mutterer, J., Guilley, H. and Gilmer, D. (2005). Use of a *Beet necrotic yellow vein virus* RNA-5-derived replicon as a new tool for gene expression. *J. Gen. Virol*. **86,** 463-467.

HOST RANGE AND HOST (UN)SPECIFICITY OF DIFFERENT *POLYMYXA BETAE* ISOLATES

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Summary

Five soil samples were taken from sugar beet growing areas in France (3), England (1) and Czech Republic (1). Sugar beet, *Chenopodium album*, *C. murale*, *C. ficifolium*, *Portulaca oleracea*, and *Amaranthus retroflexus* as possible hosts of specific formae speciales were sown in these samples. After six weeks roots of baiting plants were checked for the presence of *P. betae* cystosori. If they were not found every two weeks other plants were checked. Roots containing cystosori were harvested and homogenized in water. The homogenates were used to inoculate the same host plants in sand cultures. Using this method we obtained *P. betae* isolates from sugar beet and *C. murale* from all soil samples and two isolates from *C. album*. Roots of plants were used for the enrichment of *P. betae* population in sand. Then all host species were sown in these sands and roots of plants were checked as described above. Sugar beet and *C. murale* were infected in all soils. *C. ficifolium* and *C. album* were infected in three soils, *A. retroflexus* in one soil and *P. oleracea* has never been infected. On the contrary, after the enrichment of population of given isolate in sand all other hosts were always infected with two exceptional cases of *A. retroflexus* only. It means that there is almost no host specificity of different isolates. In different trials seeds of potential host species were sown into soil containing *P. betae*. The procedure was similar as described above. Some new host of *P. betae* were found.

Introduction

The soil protist *Polymyxa betae* described as a sugar beet parasite by Keskin (1964), is important as a vector of some viruses infecting sugar beet occurring almost worldwide where it is grown, namely *Beet yellow vein virus* (BNYVV), *Beet soil-borne virus* (BSBV), and *Beet virus Q* (BVQ). BNYVV especially causes very serious losses of sugar if resistant varieties are not used on contaminated fields (e.g. Putz, 1982). Other viruses could enhance the losses caused by BNYVV even when this was proved in pot trials only (Prillwitz and Schlösser, 1992). *P. betae* has a rather wide host range. It includes, according to Keskin (1964), *Beta vulgaris*, *B. vulgaris cicla*, *Chenopodium bonus henricus*, *C.*

capitatum, *C. foliosum*, *C. album*, *C. sandwicheanum*, *Spinacia oleracea*, *Salsola kali*, and *Atriplex hortensis*. Later on, Ivanovic *et al*. (1983) added these plants: *Amaranthus retroflexus*, *C. quinoa*, *C. hybridum*, *Montia perfoliata* and *Stellaria media*. Barr and Asher (1992) and Hugo *et al*. (1996) found some new hosts from *Papaveraceae* and *Silenaceae* families. Subikova and Bojnansky (1998) described some objects, resembling cystosori, also in roots of some asteraceous species. Recently the host range of *P. betae* was extended by Legreve *et al*. (2005). Certain differences in ability to infect other plant species were found in some *P. betae* isolates. For instance, according to Barr (1979) isolate from *C. album* does not infect sugar beet, *A. retroflexus*, and *Kochia scoparia*. Goffart *et al*. (1989) tested 7 European *P. betae* isolates. All of them infected sugar beet, fodder beet and spinach, four of them infected *C. murale*, two infected *C. album* and none was able to infect *Portulaca oleracea*. This species is described as a host from Japan (Abe and Ui, 1986) and isolate from it is rather specific for this host. On the other hand, Gerik and Duffus (1987), did not find high specificity of *P. betae* isolates. As great differences among these reports exist we wanted to make the situation clearer.

Materials and Methods

Five soil samples were taken from sugar beet growing areas in France (3 –Northern France, Oise, Bourgogne), South-East England and Czech Republic. Sugar beet, *Chenopodium album*, *C. murale*, *C. ficifolium*, *Portulaca oleracea*, and *Amaranthus retroflexus* as possible hosts of specific formae speciales were sown in these samples. After six weeks, roots of baiting plants were checked for the presence of *P. betae* cystosori by light microscope. If they were not found every two weeks further plants were checked. Roots containing high numbers of cystosori were harvested and homogenized with pestle and mortar in distilled water. The homogenates were used to inoculate the same host plants in sand cultures. Therefore, each isolate obtained was multiplied in this way. Using this method we obtained *P. betae* isolates from sugar beet and *C. murale* from all five soil samples and two isolates from *C. album*. In other plant species/soil combinations the number of cystosori was not high enough to enable further study. Roots of plants were used for enrichment of *P. betae* population in the sand. Then, all host species were sown in these sands and roots of baiting plants were checked as described above.

In separate trials host range of *P. betae* was further studied. Two soil samples were taken from a sugar beet growing area in Central Bohemia. Tested plant species from families *Chenopodiaceae*, *Amaranthaceae*, and *Caryophyllaceae* as the most probable hosts were sown into these soils. Roots of baiting plants were checked as described above with the same repeating and/or resowing of seeds if cystosori were not found. Altogether more than 30 species were tested.

Results and Discussion

Sugar beet and *C. murale* were heavily infected in all 5 soils. *C. ficifolium* and *C. album* were infected in three soils, *A. retroflexus* in one soil and *P. oleracea* has never contained cystosori. On the contrary, after the enrichment of population of given isolate in sand all other hosts were always infected with two exceptional cases of *A. retroflexus* only. It means that there is almost no host specificity of different isolates of *P. betae* (Table 1).

Table 1. Host (un)specifity of P. betae isolates obtained from various soils and hosts. 1-5 number of soil sample; Bv *Beta vulgaris* (species from which the isolate was obtained), Cm *Chenopodium murale*, Ca *C. album;* + cystosori found, - cystosori not found, 0 not tested.

Isolate / Baiting species	Bv5	Bv4	Bv3	Bv2	Bv1	Cm5	Cm4	Cm3	Cm2	Cm1	Ca5	Ca4
Beta vulgaris	0	0	0	0	0	+	+	+	+	+	+	+
C. murale	+	+	+	+	+	0	0	0	0	0	+	+
C. ficifolium	+	+	+	+	+	+	+	+	+	+	+	+
C. album	+	+	+	+	+	+	+	+	+	+	0	0
A. retroflexus	-	+	+	+	+	+	-	+	+	+	+	+

Thus the differences in host range of different isolates described by other authors (Goffart *et al*., 1989) could be simply caused by attractiveness of various plant species. In the case of less attractive hosts the number of cystosori is low and so they are hard to locate within the microscope field of view. The infection pressure could also play significant role. Without enrichment we were not able to find infection of some species in some soils (*A. retroflexus*) but after the enrichment it was infected by at least one isolate from each soil (either from sugar beet or from *C. murale*). Unfortunately we were not able to obtain a population from *A. retroflexus* strong enough to be used for infection of other species and verify whether it is really specific for this host. *P. betae* isolates capable of infecting *P. oleracea* probably occur in Japan and maybe in the USA only, as no infection of this plant species has yet been found in Europe (this study, Goffart *et al*., 1989).

Among all tested plant species for host range of *P. betae,* sugar beet and spinach served as control species. In all cases they were very readily infected. Almost the same results were obtained with three subspecies of *B. vulgaris*: *maritima*, *macrocarpa*, and *orientalis*. Further good hosts are *C. murale*, *C. quinoa*, and *C. amaranticolor*. They were infected in most cases and usually contained high numbers of cystosori. *C. vulvaria*, *C. album*, *C. gigantheum*, *C. polyspermum*, *C. glaucum* and *C. urbicum* were infected in about 50 % of cases and usually contained smaller numbers of cystosori. Only exceptionally and in small amounts cystosori were found in *A. lividus*, *A. blitum*, *A. hybridus*, *C. botrys*, *Atriplex rosea*, and *Arenaria procera*. *A. blitioides*, *C. ambrosioides*, *C. schraderianum*, *C. ugandae, Axyris amaranthoides*, *Atriplex sagittata*, *Atriplex calotheca*, *Stellaria graminea*, *Minuartia laricifolia*, *Petrorhagia saxifragae*, *Cerastium lanatum*,

Celosia argentea and *Beta patellaris* have never contained the cystosori and can be regarded as non hosts and/or very poor hosts (Table 2). The question is whether they could be infected after the enrichment. In any case, for such host range studies it would be useful to use some other method for the detection of *P. betae* (PCR) to be sure that in the case of low number of cystosori it is really infection by *P. betae*. Moreover, in some plant species plasmodia or zoosporangia could be present but without cystosori. PCR could reveal such "hidden" infections. Such tests were done by Legreve *et al*. (2005) only recently.

Table 2. Hosts and non hosts of *Polymyxa betae.*

Very good hosts	Sugar beet, spinach *B. vulgaris* ssp. *macrocarpa* *maritima* *orientalis*
Good hosts	*C. murale* *C. quinoa* *C. amaranticolor*
Medium hosts	*C. vulvaria* *C. album* *C. gigantheum* *C. polyspermum* *C. glaucum* *C. urbicum*
Bad hosts	*A. lividus* *A. blitum* *A. hybridus* *C. botrys* *Atriplex rosea* *Arenaria procera*
Non hosts and/or very poor hosts	*A. blitioides* *C. ambrosioides* *C. schraderianum* *C. ugandae* *Axyris amaranthoides* *Atriplex sagittata* *Atriplex calotheca* *Stellaria graminea* *Minuartia laricifolia* *Petrorhagia saxifragae* *Cerastium lanatum* *Celosia argentea* *Beta patellaris*

To our knowledge, some of the above mentioned species have never been listed before as hosts of *P. betae*: *A. lividus, A. blitum, Arenaria procera, Atriplex rosea, C. urbicum, C. gigantheum,* and *C. glaucum* and can thus be regarded as new hosts.

Acknowledgements

This work was supported from the grant of the Ministry of Education of Czech Republic No. MSM 604607901.

References

Abe, H. and Ui, T. (1986). Host range of *P. betae* Keskin strains in rhizomania infected soils of sugar beet fields in Japan. *Ann. Phytopath. Soc. Jpn* **52**, 394-403.

Barr, D.J. (1979). Morphology and host range of *Polymyxa graminis, Polymyxa betae* and *Ligniera pilorum* from Ontario and some other areas. *Can. J. Plant Pathol*. **1**, 85-93.

Barr, K.J. and Asher, M.J.C. (1992). The hoste range of *Polymyxa betae* in Britain. *Plant Pathology* **41**, 64-68.

Gerik, J.S. and Duffus, J.E. (1987). Host range of California isolates of *Polymyxa betae*. *Phytopathology* **77**, 1759 (abstract).

Goffart, J.P., Horta, V. and Maraite, H. (1989). Inoculum potential and host range of *Polymyxa betae* and *Beet necrotic yellow vein furovirus*. *EPPO Bull.* **19**, 517-525.

Hugo, S.A., Henry, C.M. and Harju, V. (1996). The role of alternative hosts of *Polymyxa betae* in transmission of *Beet necrotic yellow vein virus* (BNYVV) in England. *Plant Pathology* **45**, 662-666.

Ivanovic, M., Macfarlane, I. and Woods, R.D. (1983). Viruses of sugar beet associated with *Polymyxa betae*. *Rothamsted Exp. Stn. Rep*. *1982*, 189-190.

Keskin, B. (1964). *Polymyxa betae* n.sp., ein Parasit in den Wurzeln von *Beta vulgaris* Tournefort, besonders während der Jugendentwicklung der Zuckerrübe. *Arch. Mikrobiol*. **49**, 348-374.

Legreve, A., Schmit, J.-F., Bragard, C. and Maraite, H. (2005). The role of climate and alternative hosts in the epidemiology of rhizomania. *Proc. 6th Symp. IWGPVFV, Bologna*, 125-128.

Prillwitz, H. and Schlösser, E. (1992). *Beet soil-borne virus*: occurence, symptoms and effect on plant development. *Med. Fac. Landbouww. Univ. Gent* **57/2a**, 295-302.

Putz, C. (1982). La rhizomanie de la betterave. *Phytoma* 1982 (11), 24-25.

Subikova, V. and Bojnansky, V. (1998). Natural reservoirs of *Beet necrotic yellow vein virus. Listy cukrovarnické* **114**, 196-198 (in Slovak).

RHIZOMANIA AS SEEN FROM INSIDE THE BEET CELL: PROTEOME CHANGES IN SUGAR BEET IN RESPONSE TO *BEET NECROTIC YELLOW VEIN VIRUS*

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Summary

Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV), is a devastating viral pathogen of sugar beet. There are limited sources of resistance against the virus and resistance-breaking isolates are becoming increasingly problematic worldwide. Developing more effective disease control strategies starts with gaining a better understanding of the basis for resistance and the mechanism of disease. Multidimensional liquid chromatography was employed to examine proteins differentially expressed in nearly isogenic lines of sugar beet either resistant or susceptible to BNYVV infection. More than 1,000 protein peaks were reproducibly detected in the root extracts from each treatment. Differential protein expression in response to viral inoculation was determined by comparing healthy and BNYVV-challenged chromatogram protein profiles for each sugarbeet genotype. Protein expression was temporally regulated, and in total, 7.4 and 11% of the detected proteome was affected by BNYVV-challenge in the resistant and susceptible genotype, respectively. Sixty-five of the proteins induced or repressed by the virus were identified by tandem MALDI-TOF mass spectrometry and expression of key defense- and disease-related proteins was further verified using qualitative reverse transcriptase polymerase chain reaction. The proteomic data suggests involvement of classic systemic resistance components in *Rz*1-mediated resistance and phytohormones in hairy root symptom development.

Introduction

Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV), is one of the most economically important diseases affecting sugar beet (*Beta vulgaris* L.), and is widely distributed in most sugar beet growing areas of the world. The disease is characterized by excessive growth of lateral roots and constriction of the taproot which reduces sugar yield. BNYVV is transmitted by the plasmodiophorid, *Polymyxa betae* Keskin. Fields remain infested with BNYVV indefinitely in *P. betae* cystosori that remain dormant up to 25 years, therefore rotation to non-host crops

or lengthening rotations is ineffective at reducing disease incidence (Rush *et al*., 2006), and the only viable means of control has been natural resistance.

The first source of natural resistance was discovered in 1983 and is now known as *Rz*1 (Lewellen *et al*., 1987; Scholten *et al*., 1997). This gene, now incorporated into most major sugar beet breeding lines, confers strong resistance to BNYVV pathotypes A and B. However, other isolates, such pathotype P found in France and England and pathotype IV found in the Imperial Valley of California are not controlled by *Rz*1 resistance and are becoming an increasing threat. Additional sources of resistance have been identified, known as *Rz*2, *Rz*3 and *Rz*4. While these additional sources of resistance hold promise, widespread planting of new resistance sources will likely lead to further selection favoring the emergence of additional resistance-breaking BNYVV pathotypes. Furthermore, several minor genes in addition to *Rz*1 may contribute to more enhanced resistance (Gidner *et al*., 2005), the identity of which remain largely unknown. Until the epidemiology behind the spread of resistance-breaking isolates is understood, alternative disease control methods and additional sources of resistance will be required to control this pathogen. The first step toward more rapid, accurate selection of resistance and creation of novel disease control methods is to gain a better understanding of the underlying mechanisms of resistance and disease. This is especially critical with *Rz*-mediated resistance, since different genetic sources map to different chromosomal positions and appear to have different underlying mechanisms (Scholten *et al.,* 1997).

Proteomics is a powerful method for investigating plant response to stimuli, detecting protein differences as a result of *de novo* production and post-translational modification. Multidimensional liquid chromatography (MDLC) is an extremely sensitive, highly reproducible method of separating proteins. Differentially expressed proteins can be rapidly identified by comparing chromatograms derived for treated and control samples. In the current study, MDLC was used to compare proteins from healthy and BNYVV-challenged resistant (*Rz*1) and susceptible (*rz*1) sugar beet. Differentially expressed proteins were identified using tandem MALDI-TOF mass spectrometry and expression of key defense- and disease-related proteins was verified by semi-quantitative reverse transcriptase polymerase chain reaction.

Materials and Methods

Detailed information on materials and methods employed in the studies described herein are described in Larson et al. (2008).

Results and Discussion

Rhizomania is a destructive viral pathogen of sugar beet. There are limited sources of resistance for use against BNYVV and resistance-breaking isolates are becoming an increasing threat. Furthermore, evidence suggests the alternate

Rz sources of resistance have variable mechanisms (Scholten *et al.,* 1997) and there are minor gene contributors to resistance that remain unknown (Gidner *et al.,* 2005). A greater understanding of the mechanisms underlying resistance and disease will help to identify markers for use in more rapid, accurate molecular selection, aid in the development of novel disease control strategies and provide a framework for understanding the breakdown of certain sources of resistance in sugar beet. Our initial efforts in this process, outlined in this manuscript and in Larson et al. (2008), were to characterize sugar beet proteins affected by a non-resistance breaking isolate (A-type) of BNYVV in the most widely deployed source of resistance, *Rz*1. This response was compared to a nearly isogenic line of sugar beet lacking resistance to BNYVV.

Prior to protein analysis, the presence/absence of BNYVV in the two genotypes was verified by ELISA assay (Larson et al., 2008). Subtractive analysis of MDLC data proved to be a highly reproducible method for detecting sugar beet proteins qualitatively and quantitatively affected by BNYVV. Of the 66 total protein fractions containing differentially expressed peaks from the resistant genotype, 35 peaks eluted at concentration levels that make identification with MALDI-TOF/TOF mass spectrometry difficult, therefore only 31 were subjected to further analysis. Similarly, only 50 of the 86 differentially expressed peaks in the susceptible genotype were at levels conducive to analysis by MALDI-TOF/TOF. Sugar beet is not well represented in public databases therefore several approaches were necessary for protein identification. For all proteins, first attempts at protein assignment were completed using Mascot analysis software searching with combined peptide mass fingerprint (PMF) and MS/MS spectra. Additional identification with Mascot was achieved by running MS/MS spectra individually against the non-redundant database. Lastly, identification of the remaining proteins was attempted through homology-based searching with the *de novo* peptide sequence derived from the MS/MS spectra using PEAKS (Detailed in Larson et al., 2008). Using all three approaches, 65 proteins were identified with greater than 90% confidence. All protein identifications were further validated by comparing the degree of homology of the peptide sequence across five diverse plant species (data not shown). Furthermore, proteins identified with single peptide matches were only accepted if the matched sequence was located within a highly conserved functional domain as determined using NCBI's conserved domain database.

In total, 7.4% and 11% of the entire proteome detected in this current study was affected by BNYVV for the resistant and susceptible genotype, respectively. Protein expression was temporally regulated. With the resistant genotype, 22 and 29 proteins were uniquely affected at 3 and 6 weeks, respectively and 15 were coordinately affected at both time points. Likewise, 24 and 50 proteins were uniquely affected at 3 and 6 weeks, respectively in the susceptible genotype and 12 were coordinately affected at both time points. For the resistant genotype, at 3 weeks post germination, 10 of the 22 uniquely affected proteins were induced and 12 were repressed and at 6 weeks post germination, 18 were induced and

11 repressed. For the susceptible genotype, at 3 weeks post germination 11 proteins were induced, 13 repressed and at 6 weeks post germination, 26 were induced and 24 repressed. Lastly, 11 proteins were similarly affected in both genotypes.

Only 42% of the differentially expressed proteins were able to be identified based on correlation of the acquired spectra to proteins in the public database. Although limited due to limited knowledge of the beet genome, this information provided a great deal of information regarding changes that occur in the beet proteome in response to BNYVV infection and resistance. Subtractive proteomics demonstrated the protein response to BNYVV was fairly limited, only 7.4% and 11% of the entire proteome showed reproducible differential expression following BNYVV challenge for the resistant and susceptible genotype, respectively. The protein response was also temporally regulated as illustrated in Figure 3. Not surprisingly, several of the proteins differentially affected by BNYVV in both the resistant and susceptible genotype are related to protein expression and turnover (Larson et al., 2008).

Several proteins affected by BNYVV are classically associated with plant defense, suggesting inducible resistance may contribute to viral disease suppression. These include pathogenesis-related proteins, such as chitinase, protease, glucanase, peroxidase and defensin. Interestingly, induction of these proteins was not always limited to the resistant genotype. Significant overlap was noted by Li and colleagues between resistant and susceptible tomato following challenge with powdery mildew, half of which were more rapidly induced in the resistant line (see reference in Larson et al., 2008). In the current study, similar observations were made. Chitinase, defensin and protease were induced in both genotypes with increased expression occurring more rapidly in the resistant line. This suggests timing of expression is critical to defense. When examined at the transcript level, significant induction of chitinase was limited to the resistant line. Transcript expression was transient, but protein levels were sustained at increased levels through 6 weeks post germination, suggesting low protein turnover (Larson et al., 2008). Some oxidative enzymes, which are also known to contribute to plant defense appear to have similar timing-dependent expression. Polyphenol oxidase (PPO), a protein responsible for physical barrier development, and toxic compound and reactive oxygen production is more highly and rapidly expressed in the resistant genotype when compared with expression patterns from the susceptible genotype. However, another protein peak also identified as PPO was repressed in both treatments following BNYVV challenge. Since protein modifications were not examined and molecular weights are unable to be determined using MDLC, the apparent contradictory expression noted between the three proteins might be explained as the latter PPO being modified in the challenged plants, causing a significant retention time or isoelectric point shift. The importance of PPO in resistance was confirmed by RT-PCR since the gene was induced in the resistant line and repressed in the susceptible (Larson et al., 2008). This is similar to the findings with oxalate oxidase (OXO), a protein

which produces hydrogen peroxide which can act as a messenger for defense gene activation. Two protein peaks identified as OXO were more highly induced in the resistant line than the susceptible. This expression pattern was further confirmed by RT-PCR (Larson et al., 2008).

Rhizomania is associated with development of a “hairy root” phenotype symptomatic of infection. The proliferation of lateral roots creates a major constriction of taproot growth, the main sucrose storage site in beet. Therefore, the symptoms of viral infection greatly reduce sugar accumulation in beet. To date, the specific physiology responsible for hairy root development remains unknown. This proteomic investigation has uncovered several potential clues regarding the cause of BNYVV-induced symptoms. Several phytohormone-related proteins induced in the susceptible line were repressed or had no noticeable change in expression in the resistant genotype. The most prevalent phytohormone correlation was with auxins although there were ethylene responsive transcription factors and abscisic acid (ABA) associated proteins induced as well. In an attempt to further evaluate the role of auxin and ABA in hairy root development, RT-PCR of several contributors to the auxin signal transduction cascade (AGC2-2 and PIP) and auxin-inducible (GH3, SAUR, auxin-inducible transcription factor) genes and ABA signaling (ABA kinase) and inducible (ABA protein) proteins was performed. Results with the signal transduction components were inconclusive in both instances (data not shown). This is not surprising since most are regulated through phosphorylation cascades. The others had slight increases following BNYVV challenge, but the current methods were not sensitive enough to determine statistical differences between treatments. To further evaluate the role of these phytohormones in hairy root development, future investigations will include quantification of phytohormone levels in the beet roots as well as isolation and identification of phosphorylated proteins in the susceptible genotype.

This characterization of the sugar beet proteomic response to BNYVV has uncovered the potential contribution of systemic resistance and phytohormone activity in defense and symptom development, respectively, providing a better understanding of resistance and disease mechanisms in sugar beet. More detailed information on these studies can be found in Larson et al. (2008). Future investigations will include comparing the host protein response in *Rz*1 beet to the responses of other *Rz* resistance sources in beet, as well as the differential host response to resistance-breaking pathotypes of BNYVV. In the quest to develop more effective means of screening for resistance, the transcripts for the proteins with differential expression limited to the resistant line (chitinase, polyphenol oxidase, and oxalate oxidase) will be examined for future exploitation as biomarkers for resistance screening. Furthermore, over expression of these resistance-specific genes will be examined as a potential novel disease control mechanism.

Selected References

Gidner, S., Lennefors, B.L., Nilsson, N.O., Bensefelt, J., Johansson, E., Gyllenspetz, U., and Kraft, T. 2005. QTL mapping of BNYVV resistance from the WB41 source in sugar beet. Genome. 48: 279-285.

Larson, R.L., Wintermantel, W.M., Hill, A., Fortis, L., and Nunez, A. 2008. Proteome changes in sugar beet in response to *Beet necrotic yellow vein virus*. Physiol. Mol. Plant Pathol. 72: 62-72.

Lewellen, R.T., Skoyen, I.O., Erichsen, A.W. 1987. Breeding sugarbeet for resistance to rhizomania: Evaluaton of host-plant reactions and selection for and inheritance of resistance. In Proc. of the IIRB 50th Winter Congress, Brussels, Belgium, 11-12 February 1987. Brussels: Institut International de Recherches Betteravieres. Pp. 139-156.

Rush, C.M., Liu, H.-Y., Lewellen, R.T., and Acosta-Leal, R. 2006. The continuing saga of rhizomania of sugar beets in the United States. Pant Dis. 90: 4-15.

Scholten, O.E., Klein-Lankhorst, R.M., Esselink, D.G., De Bock, T.S.M., and Lange, W. 1997. Identification and mapping of random amplified polymorphic DNA (RAPD) markers linked to resistance against *Beet necrotic yellow vein virus* (BNYVV) in *Beta* accessions. Theor. Appl. Genet. 94: 123-130.

INDEXED DATA FOR COMPARING THE REACTION OF DURUM WHEAT CULTIVARS TO *SOIL-BORNE CEREAL MOSAIC VIRUS* ASSAYED IN DIFFERENT SEASONS

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Summary

A total of 89 cultivars of durum wheat (*Triticum durum* Desf.) were grown in different trials over seven seasons in a field near Bologna with natural inoculum sources of *Soil-borne cereal mosaic virus* (SBCMV), and evaluated for resistance to this pathogen on the basis of symptom severity, DAS-ELISA readings and grain yield. Each trial comprised 30-33 cvs. The data collected for each cv. in single seasons were subsequently indexed to minimize the confounding effects of differences in disease pressure between seasons on cultivar rankings for each of the three parameters examined. Despite highly significant correlations between symptom score and ELISA value indexes, resistance rankings separately based on each of the two resistance parameters tended to match adequately only for cvs. tested over a relatively high number seasons.

Introduction

SBCMV has been reported from various European countries (Jezewska, 1995; Clover *et al*., 1999; Vaianopoulos *et al*., 2005; Budge *et al*., 2008). In Italy it is widespread (Vallega and Rubies-Autonell, 1989; Ratti *et al*., 2005), and often causes yield reductions of about 50-70% on susceptible cvs. of common wheat (*Triticum aestivum* L.) and durum wheat (Rubies-Autonell *et al*., 2003; Vallega *et al*., 1999a), sometimes as a result of mixed infections with *Wheat spindle streak mosaic virus* (Rubies-Autonell and Vallega, 1987; Vallega *et al*., 2003). Most of the durum wheat cultivars marketed in Italy are either susceptible or moderately susceptible to SBCMV, and none was found completely resistant (Rubies-Autonell, *et al*., 2006); the same was observed for a large number of durum wheat cvs. from other countries (Ratti *et al*., 2006). The nearly 200 durum wheat cvs. thus far analysed exhibit a wide and continuous range of reactions to SBWMV, suggesting that their response is controlled by numerous genes (Vallega *et al*., 1999a,b; Ratti *et al*., 2006; Vallega *et al*., 2006).

Materials and Methods

Eighty-nine cvs. of durum wheat were assayed in 1995/96, 1996/97, 2000/01, 2001/02, 2002/03, 2003/04 and 2004/05 in a field near Bologna with natural inoculum sources of SBCMV. The cvs. were evaluated for resistance to SBCMV on the basis of symptom severity, DAS-ELISA readings and grain yield. Each trial comprised 30-33 cvs.; nineteen cvs. were tested 4-7 seasons and 58 cvs. 1-2 seasons only. In each season symptom severity was scored on 2-4 dates using a 0-4 scale (Vallega and Rubies-Autonell, 1985). DAS-ELISA was performed as reported previously (Ratti, et al., 2006) on extracts from a bulk of the youngest or 2nd youngest fully expanded leaf of 10 or 15 plants per plot collected on one (1996 and 2001) or two dates. The cvs. were grown in 10 m^2 solid-seeded plots, arranged in a randomized block design with three replicates. Symptom score and ELISA data collected for each cv. on different dates in each season were averaged and subsequently indexed as percent of the highest mean observed among all the cvs. assayed in that season; grain yield data were indexed in the same manner.

Table 1. Symptom score and ELISA value indexes (%) and rankings for 89 durum wheat cvs. assayed for SBCMV resistance near Bologna from 1995/96 to 2004/05.

Cultivar	No. of years tested	Symptom score		ELISA value		Cultivar	No. of years tested	Symptom score		ELISA value	
		Index	Rank	Index	Rank			Index	Rank	Index	Rank
Louxor	1	6.7	**3**	6.1	**4**	Norba	1	18.8	**21**	50.6	**54**
Neodur	7	12.1	**12**	3.4	**1**	Torrebianca	5	38.7	**52**	34.9	**41**
Ares (Ionio)	4	9.5	**7**	6.9	**6**	Vendetta	1	35.6	**46**	38.3	**43**
Campodoro	1	4.5	**1**	13.0	**13**	Tresor	2	35.4	**45**	38.9	**44**
Meridiano	5	11.4	**11**	6.4	**5**	Virgilio	2	50.8	**58**	26.6	**33**
Dylan	3	9.0	**4**	10.0	**9**	Quadrato	4	37.0	**47**	40.9	**47**
Nefer	1	4.6	**2**	15.2	**17**	Verdi	3	29.7	**36**	49.0	**52**
Giusto	1	17.4	**17**	10.5	**11**	Appio	2	37.9	**50**	42.8	**48**
Colorado	5	18.2	**20**	10.8	**12**	Exeldur	2	33.9	**41**	53.4	**56**
Valerio	1	24.7	**29**	4.7	**3**	Italo	2	37.6	**49**	52.3	**55**
Ceedur	1	10.1	**8**	19.4	**24**	Plinio	1	37.5	**48**	61.2	**61**
Pietrafitta	2	11.2	**10**	19.1	**22**	Colosseo	4	42.3	**56**	60.4	**60**
Provenzal	5	26.2	**31**	4.4	**2**	Sorrento	1	69.4	**66**	38.9	**45**
Solex	6	12.5	**14**	19.3	**23**	Ixos	3	40.2	**54**	68.2	**63**
Baio	1	10.1	**9**	25.2	**31**	Creso	6	55.3	**60**	58.0	**58**
Levante	2	28.6	**35**	7.3	**7**	Portobello	1	58.4	**61**	55.3	**57**
Tiziana	3	23.0	**25**	13.1	**14**	Giove	1	67.2	**64**	64.3	**62**
Lloyd	3	17.4	**18**	20.4	**25**	Ofanto	2	55.1	**59**	79.5	**66**
Parsifal	2	17.9	**19**	23.5	**28**	Claudio	5	78.8	**73**	75.7	**65**
Cosmodur	2	20.8	**23**	21.9	**26**	Portorico	5	67.3	**65**	89.9	**76**
Duilio	7	24.2	**27**	19.1	**21**	Prometeo	2	98.2	**87**	59.4	**59**
Avispa	3	30.5	**37**	13.2	**15**	Anco Marzio	1	86.5	**78**	72.4	**64**
Fiore	2	39.3	**53**	7.5	**8**	Giemme	2	64.3	**63**	95.5	**85**
Flavio	2	23.3	**26**	24.4	**29**	Ciccio	2	61.0	**62**	99.0	**86**
Svevo	1	33.5	**40**	15.6	**18**	Marco	2	78.4	**72**	83.3	**69**
Gianni	5	24.6	**28**	24.7	**30**	Balsamo	2	75.3	**70**	90.4	**77**
San Carlo	5	26.8	**32**	22.7	**27**	Derrick	2	79.7	**74**	87.1	**71**
Rusticano	1	33.3	**39**	17.5	**20**	Platani	2	75.0	**69**	93.2	**79**
Grecale	2	40.6	**55**	10.3	**10**	Vettore	2	86.1	**77**	82.5	**67**
Peleo	1	9.4	**6**	43.0	**49**	Simeto	7	74.4	**68**	94.5	**80**
Vitron	2	12.3	**13**	40.8	**46**	Sorriso	1	76.5	**71**	94.6	**81**
Canyon	1	38.0	**51**	15.2	**16**	Vinci	1	71.2	**67**	100.0	**89**
Brindur	1	9.4	**5**	44.7	**51**	Orobel	5	82.2	**75**	89.4	**74**
Portofino	2	21.9	**24**	32.3	**38**	Vesuvio	3	88.7	**80**	87.2	**72**
Iride	6	26.0	**30**	29.3	**35**	Cannizzo	3	87.8	**79**	88.3	**73**
Valsalso	1	20.5	**22**	34.8	**40**	Grazia	5	93.0	**84**	86.0	**70**
Preco	1	12.5	**15**	43.6	**50**	Bronte	1	83.0	**76**	99.4	**87**
Vitomax	3	27.3	**33**	29.2	**34**	Carioca	1	100.0	**88**	82.5	**68**
Normanno	2	27.4	**34**	29.3	**36**	Cirillo	3	93.9	**85**	89.5	**75**
Giotto	3	42.8	**57**	16.0	**19**	Peres	1	90.6	**82**	93.2	**78**
Duetto	2	33.9	**42**	25.3	**32**	Vetrodur	3	90.4	**81**	95.5	**84**
Zenit	2	34.1	**43**	30.8	**37**	Granizo	1	96.9	**86**	95.0	**82**
Perseo	1	15.6	**16**	49.8	**53**	Concadoro	1	92.9	**83**	100.0	**88**
Gardena	2	31.2	**38**	36.2	**42**	Agridur	1	100.0	**89**	95.4	**83**
Ermecolle	1	34.7	**44**	33.6	**39**						

Results and Discussion

The 89 cultivars assayed demonstrated a wide and continuous range of reactions to SBWMV Table 1), both in terms of symptom severity (index range = 4.5–100.0%), ELISA value (3.4–100.0%), and grain yield (35.5-100.0%). Many cvs. consistently showed mild symptoms and low ELISA values in various seasons, yet none proved completely resistant. Moreover, although symptom score and ELISA value indexes were closely correlated (r = 0.850**), each index yielded different and sometimes contrasting resistance rankings between cvs., as in the case of cvs. Fiore, Grecale, Peleo, Brindur, tested 1 or 2 seasons only.

Table 2. Symptom score, ELISA value and grain yield indexes (%) and rankings for 19 durum wheat cvs. assayed for SBCMV resistance in 4 or more seasons (Bologna, 1995/96-2004/05).

Cultivar	No. of years tested	Symptom score		ELISA value		Grain yield	
		Index	Rank	Index	Rank	Index	Rank
Neodur	7	12.1	**3**	3.4	**1**	85.3	**4**
Ares (Ionio)	4	9.5	**1**	6.9	**4**	86.7	**2**
Meridiano	5	11.4	**2**	6.4	**3**	86.1	**3**
Colorado	5	18.2	**5**	10.8	**5**	83.0	**5**
Provenzal	5	26.2	**9**	4.4	**2**	89.8	**1**
Solex	6	12.5	**4**	19.3	**7**	78.6	**10**
Duilio	7	24.2	**6**	19.1	**6**	80.1	**7**
Gianni	5	24.6	**7**	24.7	**9**	78.8	**9**
San Carlo	5	26.8	**10**	22.7	**8**	80.7	**6**
Iride	6	26.0	**8**	29.3	**10**	79.3	**8**
Torrebianca	5	38.7	**12**	34.9	**11**	76.1	**11**
Quadrato	4	37.0	**11**	40.9	**12**	74.7	**12**
Colosseo	4	42.3	**13**	60.4	**14**	65.5	**15**
Creso	6	55.3	**14**	58.0	**13**	70.7	**13**
Claudio	5	78.8	**17**	75.7	**15**	35.5	**19**
Portorico	5	67.3	**15**	89.9	**18**	55.6	**16**
Simeto	7	74.4	**16**	94.5	**19**	52.8	**17**
Orobel	5	82.2	**18**	89.4	**17**	66.5	**14**
Grazia	5	93.0	**19**	86.0	**16**	48.2	**18**

A wide and continuous range of reactions to SBWMV in terms of symptom severity and ELISA value, as well as a closer correlation between the two indexes (r = 0.952**) and a high similarity between symptom and ELISA index rankings (except in the case of cv. Provenzal) was observed for the 19 cvs. assayed in four or more seasons (Table 2). The grain yield index too became more closely related to the symptom score (r = -0.923** vs. -0.744**) and ELISA indexes (r = -0.875** vs. -0.769**) when analysis' were restricted to the 19 cvs. assayed in 4 or more seasons. These 19 rather closely related cvs., representing only 22% of those

here examined, originate no less than 6 distinct levels of resistance to SBCMV; indeed, recent results indicate that in lines derived from “Neodur x Cirillo” and “Meridiano x Claudio” SBCMV resistance is governed by at least 4 and 5 genes, respectively (Ratti *et al*., 2008; Maccaferri *et al*., 2008; De Vita *et al*., 2008).

References

Budge, G.E., Ratti, C., Rubies-Autonell, C., Lockley, D., Bonnefoy, M., Vallega, V., Pietravalle, S. and Henry, C. M. (2008). Response of UK winter wheat cultivars to Soil-borne cereal mosaic and *Wheat spindle streak mosaic viruses* across Europe. *Eur. J. Plant Pathol.* **120**, 259-272.

Clover, G.R.G., Wright, D.M. and Henry, C.M. (1999). Occurrence of *Soil-borne wheat mosaic virus* in the UK. In *“Proc. of the Fourth Symposium of the International Working Group on Plant Viruses with Fungal Vectors”* (J.L. Sherwood and C.M. Rush, Eds.), 105-108.

De Vita, P., Mastrangelo, A.M., Ratti, C., Rubies-Autonell, C. and Vallega, V. (2008). Resistance to *Soil-borne cereal mosaic virus* in durum wheat lines derived from the cross “Neodur x Cirillo”. In *“Program of the Seventh Symposium of the International Working Group on Plant Viruses with Fungal Vectors”* (C.M. Rush, Ed.), 44.

Jezewska, M. (1995). Detection of Polish isolate of *Wheat soil-borne mosaic virus* in cereal seeds. *Phytopathologia Polonica* **10**, 61-67.

Maccaferri, M., Ratti, C., Rubies-Autonell, C., Tuberosa, R., Demontis, A., Massi, A., De -Ambrogio, E., Stefanelli, S., Vallega, V. and Sanguineti, M.C. (2008). Mapping genetic factors for resistance to SBCMV in durum wheat. In *“Abstracts of the International Durum Wheat Symposium From Seed to Pasta”*, (E. Borasio and E. DeAmbrogio Eds.), pp. 127.

Ratti, C., Pisi, A., Vallega, V. and Rubies-Autonell, C. (2005). Molecular characterization of Italian *Soil-borne cereal mosaic virus* (SBCMV) isolates. *Parasitica* **61**, 11-16.

Ratti, C., Rubies-Autonell, C., Maccaferri, M., Stefanelli, S., Sanguineti, M.C. and Vallega, V. (2006). Reaction of 111 cultivars of *Triticum durum* Desf. from some of the world’s main genetic pools to *Soil-borne cereal mosaic virus*. *J. Plant Dis. Protection* **113**, 145-149.

Ratti, C., Rubies-Autonell, C., Maccaferri, M., Corneti, S., Stefanelli, S., Sanguineti, M.C., Demontis, A., Massi, A. and Vallega, V. (2008). Inheritance of resistance to SBCMV in a durum wheat population of lines derived from the cross Meridiano/Claudio. *AWN* **54**, 76.

Rubies-Autonell, C. and Vallega, V. (l987). Observations on a mixed *Soil-borne wheat mosaic virus* and *Wheat spindle streak mosaic virus* infection in durum wheat. *J. Phytopathology* **119**, 111-121.

Rubies-Autonell, C., Vallega, V. and Ratti, C. (2003). Reactions of cultivars of common wheat (*Triticum aestivum* L.) to *Soil-borne wheat mosaic virus* in northern Italy during 1996-97. *J. Plant Dis. Protection* **110**, 332-336.

Rubies-Autonell, C., Ratti, C., Pisi, A. and Vallega, V. (2006). Behaviour of durum and common wheat cultivars in regard to *Soil-borne cereal mosaic virus* (SBCMV): results of fifteen trials. In *"Proceedings of the 12th Congress of the Mediterranean Phytopathological Union"* (N. Paplomatas Ed.), pp. 100-102.

Vaianopoulos, C., Legreve, A., Moreau, V., Steyer, S., Maraite, H. and Bragard, C. (2005). Occurrence of bymo- and furoviruses on wheat in Belgium. *Parasitica* **61**, 47-54.

Vallega, V. and Rubies-Autonell, C. (1985). Reactions of Italian *Triticum durum* cultivars to *Soil-borne wheat mosaic. Plant Dis.* **69**, 64-66.

Vallega, V., and Rubies-Autonell, C. (1989). Ulteriori indagini sulla diffusione del virus del mosaico comune in Italia. *Informatore Fitopatologico* **39**, 57-58.

Vallega, V., Rubies-Autonell, C., Turina, M., Ratti C. and Contoli, S. (1999a). Reactions of durum wheat cultivars to infections by SBWMV grown in northern Italy during 1995-96. *J.Plant Dis. Protection* **106**, 284-290.

Vallega, V., Rubies-Autonell, C., Ratti, C., Turina, M. and Contoli, S. (1999b). Reactions of durum wheat (*Triticum durum* Desf.) cultivars to *Soil-borne wheat mosaic furovirus* (SBWMV) in northern Italy during 1996-97. In *"Proceedings of the Fourth Symposium of the International Working Group on Plant Viruses with Fungal Vectors"* (J.L. Sherwood and C.M. Rush Eds), pp. 131-134.

Vallega, V., Rubies-Autonell, C. and Ratti, C. (2003). Reaction of durum wheat cultivars to mixed SBWMV and WSSMV infection in central Italy. *Phytopathologia Mediterranea* **42**, 177-182.

Vallega, V., Rubies-Autonell, C., and Ratti, C. (2006). Resistance to accumulation of *Soil-borne cereal mosaic virus* in eight cultivars of *Triticum durum* Desf. *Parasitica* **62**,79-96.

SOIL-BORNE CEREAL MOSAIC VIRUS: AGRONOMIC PERFORMANCE OF DURUM WHEAT CULTIVARS AS PREDICTED BY VIRUS CONCENTRATION AND SYMPTOM SEVERITY EVALUATIONS ON DIFFERENT DATES

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Summary

Different sets of cultivars of durum wheat (*Triticum durum* Desf.) were tested over seven seasons in a field near Bologna with natural inoculum sources of *Soil-borne cereal mosaic virus* (SBCMV). SBCMV-symptoms were scored on a 0-4 scale on 2-4 dates, and DAS-ELISA was performed on samples collected on 1-2 dates. Grain yield, kernel weight, test weight, plant height and days to head were also recorded. The data collected were used to estimate the damage caused by SBCMV and to identify the most informative dates for rating symptom severity and assessing virus concentration. Correlations between the latter two resistance parameters and agronomic traits, separately computed for different dates, were similar within the 30-60 day span investigated. Symptom scores above 3.0 were associated with grain yield and plant height reductions of 48% and 25%, respectively.

Introduction

SBCMV has been reported from various European countries (Jezewska, 1995; Clover *et al*., 1999; Vaianopoulos *et al*., 2005; Budge *et al*., 2008). In Italy SBCMV is widespread (Vallega and Rubies-Autonell, 1989; Ratti *et al*., 2005), and often causes yield reductions of about 50-70% on susceptible cvs. of common wheat (*Triticum aestivum* L.) and durum wheat (Vallega and Rubies-Autonell, 1985; Vallega *et al*., 1999; Rubies-Autonell *et al*., 2003). Most of the durum wheat cultivars marketed in Italy are susceptible to SBCMV, and none was found completely resistant (Rubies-Autonell *et al*., 2006; Vallega *et al*., 2006); the same was observed for a large number of durum wheat cvs. from other countries (Ratti *et al*., 2006).

Materials and Methods

Eighty-nine durum wheat cvs. were assayed over seven different seasons (1995/96, 1996/97, 2000/01, 2001/02, 2002/03, 2003/04 and 2004/05) in a field near Bologna with natural inoculum sources of SBCMV. Each trial comprised 30-33 cvs. Symptom severity and DAS-ELISA absorbance, as well as grain yield, kernel weight, test weight, plant height and heading date were determined. In each season symptom severity was scored on 2-4 dates using a 0-4 scale (Vallega and Rubies-Autonell, 1985). DAS-ELISA was performed as reported previously (Ratti *et al*., 2006) on extracts from a bulk of the youngest or 2nd youngest fully expanded leaf of 10 or 15 plants per plot collected on one (1995/96 and 200/01) or two dates. The cvs. were grown in 10 m^2 solid-seeded plots distributed according to a randomized block design with three replicates. The effects of SBWMV on the agronomic performance of cvs. manifesting diverse symptom severity were estimated by regression analysis.

Results and Discussion

As summarized in Table 1, symptom scores above 3.0 were associated with mean grain yield and mean plant height reductions of 48% and 25%, respectively, as well as with notable decreases in kernel weight and test weight. Even mild symptoms caused appreciable negative effects on grain yield (-9%) and plant height (-5%).

Table 1. Actual performance and estimated effects of SBCMV for durum wheat cvs. with different symptom severity grown in a field with SBCMV near Bologna over 7 seasons.

Symptom score (0-4)	**Grain yield**	Reduction (%)	**Plant height**	Reduction (%)	**Kernel weight**	**Test weight**	**Heading date**
Theoretical	*5.10*	*0.0*	*85.3*	*0.0*	-	-	-
0.0-1.0	4.66	8.7	81.4	4,6	42.4	78.4	40
1.0-2.0	3.97	22.2	78.2	8.4	41.6	77.6	41
2.0-3.0	2.97	41.8	70.4	17.5	38.5	76.7	41
3.0-4.0	2.64	48.2	64.1	24.9	36.1	77.1	43

Mean ELISA absorbance (Table 2) was significantly correlated (P= 0.05) in 7 seasons with mean symptom severity, grain yield and plant height; in 4 seasons with kernel weight and test weight; and in 2 seasons with heading date. Mean symptom severity too was more closely correlated with grain yield and plant height than with the other three agronomic traits (Table 3).

Table 2. Correlations between ELISA absorbance values on different dates and agronomic characters and mean symptom score, for 89 durum wheat cvs. grown in a field with SBCMV near Bologna in trials comprising 30-33 cvs.

Year	No. of cvs.	Date	Grain yield	Plant height	Kernel weight	Test weight	Heading date	Mean Symptom Score
1996	33	Mar.26	**-0.710** **	**-0.721** **	**-0.516** **	**-0.383** *	**0.523** **	**0.855** **
1997	33	Apr.04	-0.736 **	-0.436 **	-0.351 *	-0.409 *	0.170 n.s.	
		May.07	-0.720 **	-0.462 **	-0.428 *	-0.583 **	0.282 n.s.	
Mean ELISA			**-0.752** **	**-0.468** **	**-0.412** *	**-0.533** **	**0.246** n.s.	**0.826** **
2001	30	Mar.21	**-0.570** **	**-0.404** *	**-0.079** n.s.	**-0.371** *	-	**0.808** **
2002	30	Mar.13	-0.709 **	-0.790 **	-0.231 n.s.	-0.472 **	0.099 n.s.	
		Apr.03	-0.467 **	-0.511 **	-0,074 n.s.	-0.152 n.s.	0.173 n.s.	
Mean ELISA			**-0.703** **	**-0.781** **	**-0.206** n.s.	**-0.422** *	**0.130** n.s.	**0.862** **
2003	31	Mar.13	-0.853 **	-0.667 **	-0.547 **	-0.635 **	0.332 n.s.	
		Apr.02	-0.897 **	-0.773 **	-0.401 *	-0.536 **	0.391 *	
Mean ELISA			**-0.920** **	**-0.758** **	**-0.494** **	**-0.612** **	**0.413** *	**0.942** **
2004	31	Mar.30	-0.816 **	-0.746 **	-0.431 *	-0.313 n.s.	0.244 n.s.	
		Apr.22	-0.861 **	-0.784 **	-0.524 **	-0,310 n.s.	0.267 n.s.	
Mean ELISA			**-0.874** **	**-0.797** **	**-0.497** **	**-0.325** n.s.	**0.266** n.s.	**0.928** **
2005	32	Apr.06	-0.501 **	-0.744 **	-0.179 n.s.	0.240 n.s.	0.119 n.s.	
		Apr.20	-0.507 **	-0.669 **	-0.204 n.s.	0.139 n.s.	0.087 n.s.	
Mean ELISA			**-0.519** **	**-0.732** **	**-0.197** n.s.	**0.200** n.s.	**0.108** n.s.	**0.819** **

In four out of five seasons ELISA correlations with grain yield and plant height were practically identical for the two sampling dates considered; only in 2002 was the earlier date (March 13) distinctly more informative. Correlations with kernel weight and test weight too were similar on different dates, even if more erratic. Symptom score correlations with grain yield and plant height were essentially identical for the various sampling dates considered except for the first scoring date in 2005 (March 18), which proved markedly less informative than the three subsequent ones. Correlations with kernel weight and test weight also proved similar on different dates, even if – as in the case of ELISA values – markedly more erratic.

Table 3. Correlations between SBCMV-symptom severity on different dates and agronomic characters and mean ELISA absorbance for 89 durum wheat cvs. grown in a field with SBCMV near Bologna in trials comprising 30-33 cvs.

Year	No. of cvs.	Date	Grain yield	Plant height	Kernel weight	Test weight	Heading date	Mean ELISA
1996	33	Mar.26	-0.760 **	-0.776 **	-0.647 **	-0.458 **	0.609 **	
		May.17	-0.757 **	-0.786 **	-0.601 **	-0.382 *	0.735 **	
Mean symp.			**-0.773** **	**-0.800** **	**-0.638** **	**-0.425** *	**0.693** **	**0.855** **
1997	33	Mar.26	-0.657 **	-0.558 **	-0.364 *	-0.288 n.s.	0.515 **	
		Apr.04	-0.761 **	-0.484 **	-0.180 n.s.	-0.298 n.s.	0.352 *	
		Apr.16	-0.825 **	-0.494 **	-0.439 **	-0.461 **	0.348 *	
		Apr.24	-0.777 **	-0.541 **	-0.365 *	-0.444 **	0.363 *	
Mean symp.			**-0.812** **	**-0.556** **	**-0.368** *	**-0.408** *	**0.418** *	**0.826** **
2001	30	Feb.19	-0.635 **	-0.655 **	-0.224 n.s.	-0.344 n.s.	-	
		Mar.05	-0.604 **	-0.675 **	-0.306 n.s.	-0.342 n.s.	-	
		Mar.21	-0.615 **	-0.595 **	-0.261 n.s.	-0.279 n.s.	-	
Mean symp.			**-0.630** **	**-0.653** **	**-0.273** n.s.	**-0.325** n.s.	-	**0.808** **
2002	30	Feb.28	-0.514 **	-0.711 **	-0.087 n.s.	-0.223 n.s.	0.110 n.s.	
		Mar.13	-0.606 **	-0.771 **	-0.077 n.s.	-0.225 n.s.	0.247 n.s.	
		Apr.03	-0.653 **	-0.784 **	-0.132 n.s.	-0.286 n.s.	0.319 n.s.	
		Apr.22	-0.698 **	-0.789 **	-0.137 n.s.	-0.442 *	0.189 n.s.	
Mean symp.			**-0.651** **	**-0.803** **	**-0.114** n.s.	**-0.307** n.s.	**0.233** n.s.	**0.862** **
2003	31	Mar.13	-0.914 **	-0.835 **	-0.549 **	-0.515 **	0.332 n.s.	
		Apr.02	-0.859 **	-0.767 **	-0.563 **	-0.499 **	0.469 **	
		Apr.16	-0.912 **	-0.850 **	-0.545 **	-0.531 **	0.367 *	
Mean symp.			**-0.913** **	**-0.834** **	**-0.564** **	**-0.526** **	**0.396** *	**0.942** **
2004	31	Mar.18	-0.790 **	-0.730 **	-0.456 **	-0.327 n.s.	0.335 n.s.	
		Mar.30	-0.823 **	-0.754 **	-0.589 **	-0.317 n.s.	0.318 n.s.	
		Apr.15	-0.891 **	-0.766 **	-0.521 **	-0.363 *	0.319 n.s.	
		Apr.22	-0.775 **	-0.665 **	-0.481 **	-0.286 n.s.	0.283 n.s.	
Mean symp.			**-0.859** **	**-0.765** **	**-0.537** **	**-0.340** n.s.	**0.330** n.s.	**0.928** **
2005	32	Mar.18	-0.395 *	-0.569 **	-0.155 n.s.	0.213 n.s.	0.036 n.s.	
		Apr.01	-0.552 **	-0.712 **	-0.417 *	0.246 n.s.	0.105 n.s.	
		Apr.06	-0.635 **	-0.796 **	-0.435 *	0.257 n.s.	0.105 n.s.	
		Apr.18	-0.687 **	-0.777 **	-0.391 *	0.282 n.s.	0.151 n.s.	
Mean symp.			**-0.633** **	**-0.782** **	**-0.400** *	**0.272** n.s.	**0.116** n.s.	**0.819** **

Symptom score and ELISA value rankings among cvs. are known to change sometimes substantially during the season (Vallega *et al*., 2006; Ratti *et. al*, 2008; Rubies-Autonell *et al*., unpublished) and, therefore, averaging data collected on various dates during the course of a season is mandatory for adequately classifying

cultivar responses to SBCMV. On the other hand, the results reported above indicate that multiple symptom and ELISA observations allow more meaningful SBCMV-damage estimates only with respect to the more loosely associated agronomic traits (kernel weight, test weight and heading date), whereas single symptom scoring and ELISA sampling collection dates is enough to produce reliable grain yield and plant height reduction estimates.

References

Budge, G.E., Ratti, C., Rubies-Autonell, C., Lockley, D., Bonnefoy, M., Vallega, V., Pietravalle, S. and Henry, C. M. (2008). Response of UK winter wheat cultivars to *Soil-borne cereal mosaic* and *Wheat spindle streak mosaic viruses* across Europe. *Eur. J. Plant Pathol.* **120**, 259-272.

Clover, G.R.G., Wright, D.M. and Henry, C.M. (1999). Occurrence of *Soil-borne wheat mosaic virus* in the UK. In *"Proceedings of the Fourth Symposium of the International Working Group on Plant Viruses with Fungal Vectors"* (J.L. Sherwood and C.M. Rush, Eds.), pp.105-108.

Jezewska, M. (1995). Detection of Polish isolate of *Wheat soil-borne mosaic virus* in cereal seeds. *Phytopathogia Polonica* **10**, 61-67.

Ratti, C., Pisi, A., Vallega, V. and Rubies-Autonell, C. (2005). Molecular characterization of Italian *Soil-borne cereal mosaic virus* (SBCMV) isolates. *Parasitica* **61**, 11-16.

Ratti, C., Rubies-Autonell, C., Maccaferri, M., Stefanelli, S., Sanguineti, M.C., and Vallega, V. (2006). Reaction of 111 cultivars of *Triticum durum* Desf. from some of the world's main genetic pools to *Soil-borne cereal mosaic virus*. *J. Plant Dis. Protect.***113**,145-149.

Ratti, C., Rubies-Autonell, C., Maccaferri, M., Corneti, S., Stefanelli, S., Sanguineti, M.C., Demontis, A., Massi, A. and Vallega, V. (2008). Inheritance of resistance to SBCMV in a durum wheat population of lines derived from the cross 'Meridiano / Claudio'. *AWN* **54**, 76.

Rubies-Autonell, C., Vallega, V. and Ratti, C. (2003). Reactions of cultivars of common wheat (*Triticum aestivum* L.) to *Soil-borne wheat mosaic virus* in northern Italy during 1996-97. *J. Plant Dis. Protect.* **110**, 332-336.

Rubies-Autonell, C., Ratti, C., Pisi, A., and Vallega, V. (2006). Behaviour of durum and common wheat cultivars in regard to *Soil-borne cereal mosaic virus* (SBCMV): results of fifteen trials. In *"Proceedings of the 12th Congress of the Mediterranean Phytopathological Union"* (N. Paplomatas Ed.), pp. 100-102.

Vaianopoulos, C., Legreve, A., Moreau, V., Steyer, S., Maraite, H. and Bragard, C. (2005). Occurrence of bymo- and furoviruses on wheat in Belgium. *Parasitica* **61**, 47-54.

Vallega, V. and Rubies-Autonell, C. (1985). Reactions of Italian *Triticum durum* cultivars to soil-borne wheat mosaic. *Plant Dis.* **69**, 64-66.

Vallega, V. and Rubies-Autonell, C. (1989). Ulteriori indagini sulla diffusione del virus del mosaico comune in Italia*. Informatore. Fitopatologico* **39**, 57-58.

Vallega, V., Rubies-Autonell, C., Turina, M., Ratti C. and Contoli, S. (1999). Reactions of durum wheat cultivars to infections by SBWMV grown in northern Italy during 1995-96. *J. Plant Dis. Protect.* **106**, 284-290.

Vallega, V., Rubies-Autonell, C., and Ratti, C. (2006). Resistance to accumulation of *Soil-borne cereal mosaic virus* in eight cultivars of *Triticum durum* Desf. *Parasitica* **62**, 79-96.

POLYGENIC RESISTANCE TO *SOIL-BORNE CEREAL MOSAIC VIRUS* IN A DURUM WHEAT POPULATION OF LINES DERIVED FROM THE CROSS "MERIDIANO X CLAUDIO"

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Summary

A population consisting of 184 recombinant inbred lines (RILs) from a cross between durum wheat (*Triticum durum* Desf.) cvs. "Meridiano" (highly resistant) and "Claudio" (moderately susceptible) was grown during the 2006-07 season in a field with natural inoculum sources of SBCMV near Bologna, and evaluated for SBCMV-symptom severity expression and ELISA absorbance in extracts from leaves. Comparative analysis of symptom severity and ELISA absorbance values in time showed the presence of a sizeable temporal change in the relative degree of resistance of the lines in terms of symptom expression and virus concentration in leaves, responsible for marked changes in resistance ranking order among lines. Symptom expression and ELISA value frequency distributions indicated a complex mode of SBWMV-resistance in the "Meridiano x Claudio" population, involving no less than four genes, the presence of favorable alleles contributed by both parents, and a different timeline of expression of resistance.

Introduction

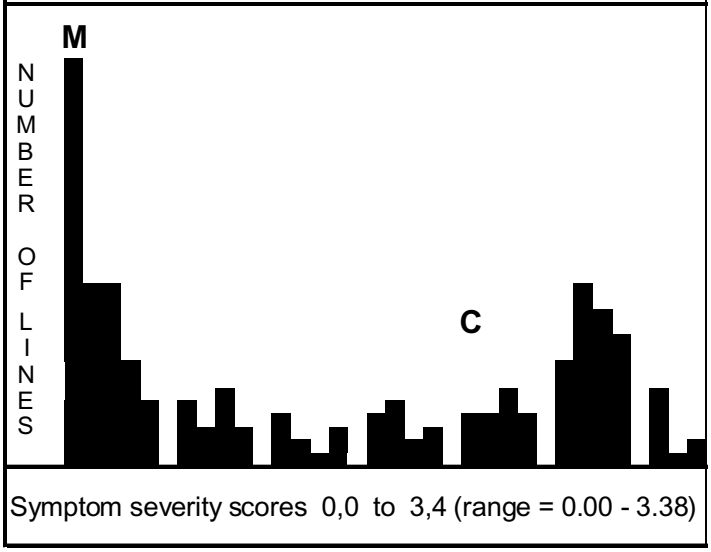
SBCMV has been reported from various European countries (Vaianopoulos *et al*., 2005), and is widespread in Italy (Vallega *et al*., 1999a). Most of the durum wheat cultivars marketed in Italy are susceptible to SBCMV, and none was found completely resistant (Rubies-Autonell, *et al*. 2006; Vallega *et al*., 2006). The same was observed for a large number of durum wheat cvs. from other countries (Ratti *et al*., 2006). Moreover, durum wheat cvs. were found to exhibit a consistent array of intermediate reactions to the virus, suggesting that their response to SBCMV is governed by numerous genes (Vallega *et al*., 1999b; Ratti *et al*., 2006; Vallega *et al*., 2006). To test this hypothesis, 184 RILs from a cross between cvs. Meridiano (highly resistant) and Claudio (moderately susceptible) was grown in a field with natural inoculum sources of SBCMV near Bologna.

Materials and Methods

The lines, specifically designed to identify the quantitative trait loci (QTLs) involved in the control of SBCMV resistance, were developed by Società Produttori Sementi Spa, Italy, via single seed descent. Lines and parental cvs. were grown during the 2006-07 season at Cadriano (Bologna) in 2.4 m^2 solid-seeded plots distributed according to a randomized block design with two replicates, and evaluated for symptom-severity (Feb. 16, Feb. 21, March 12 and April 2) and ELISA absorbance (Feb. 16 and March 12). Symptom severity was scored on a 0 (no symptoms) to 4 (severe stunting and mottling) scale, and DAS-ELISA was determined on extracts from a bulk of the youngest fully expanded leaf of 15 plants per plot (Ratti *et al*., 2006).

Results and Discussion

Fig. 1. Number of lines derived from "Meridiano x Claudio" showing different symptom severity scores (0-4 scale) on February 21, 2007.



Disease pressure at the experimental site was severe, as testified by the comparatively high percentage of lines (6%) with symptom severity scores equal or above 3.5. ANOVA indicated that differences among RILs were significant ($P \leq 0.01$) for ELISA values and symptom scores on all observation dates (data not reported). Symptom scores and ELISA values were closely correlated (r ranging from 0.674 to 0.793, depending on the dates considered; $P \leq 0.001$).
Symptom-severity score frequency distributions showed on all dates 6 to 8 distinct and often bi-modal frequency peaks (Fig. 1), with one or two major peaks

consistently located to the right of the mean value found for cv. Claudio. ELISA frequency distributions showed 6 (March 12, Fig. 2) or 5 (Feb. 21) major frequency peaks and transgressive segregants on Feb. 21 only.

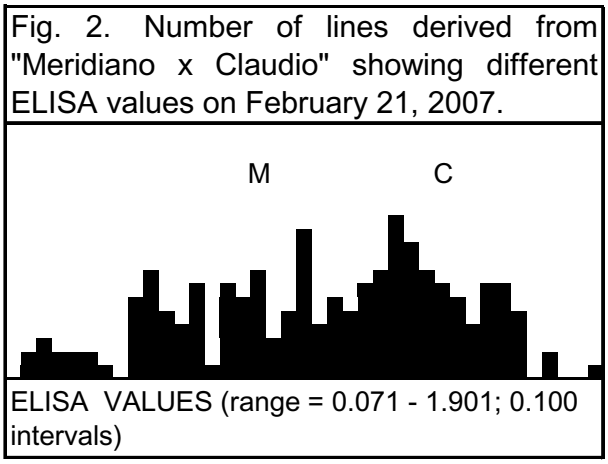


Fig. 2. Number of lines derived from "Meridiano x Claudio" showing different ELISA values on February 21, 2007.

Symptom scores became increasingly polarized during the course of the season, and numerous lines showed a marked decrease in symptom severity on the last observation date. As largely expected, the symptom scores recorded Feb. 16 and March 12 were closely correlated ($P \leq 0.001$), and the same applied between ELISA values recorded on the same dates (Figs. 3 and 4). Interestingly, the correlation between the deviations from each of the two regression equations (Fig. 5) was significant too ($r = 0.334$; $P \leq 0.001$), thus confirming that the relative degree of resistance of the lines - independently measured in terms of symptomatology and ELISA – indeed changed during the time span considered, causing substantial modifications in ranking order among lines. This temporal change may be attributed to genes affecting SBCMV infection directly, or controlling morphological or phenological plant traits affecting the onstart and / or progress of SBCMV infection. Rankings of cultivars based on SBCMV response levels, have been reported to change during the season (Vallega *et al.*, 2006; Rubies-Autonell *et al.*, unpublished); the present experiment offered the opportunity to validate the phenomenon in a common genetic background.

Fig. 3. Correlation between symptom scores on February 16 and April 2 for 184 lines derived from "Meridiano x Claudio".

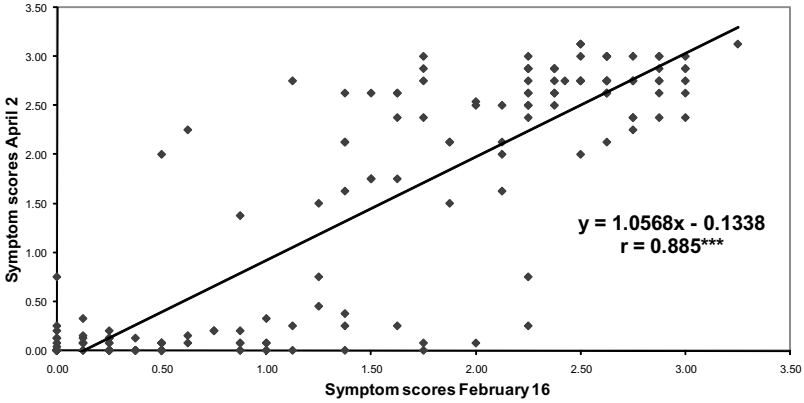


Fig. 4. Correlation between ELISA values on February 16 and April 2 for 184 lines derived from "Meridiano x Claudio".

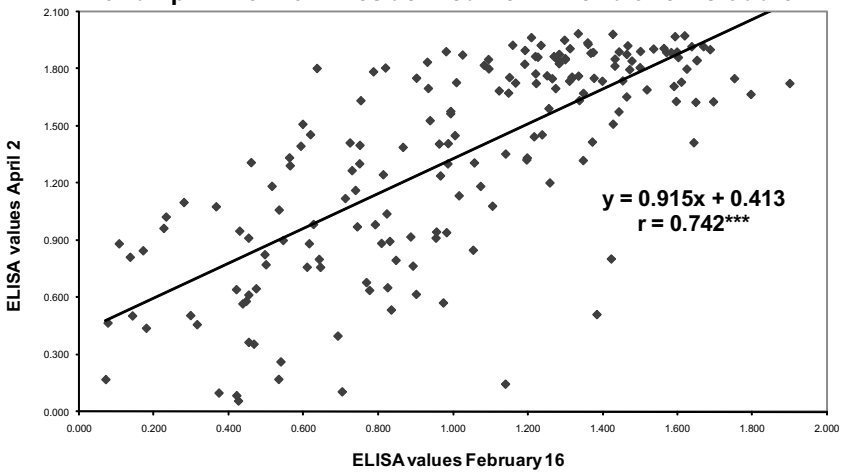
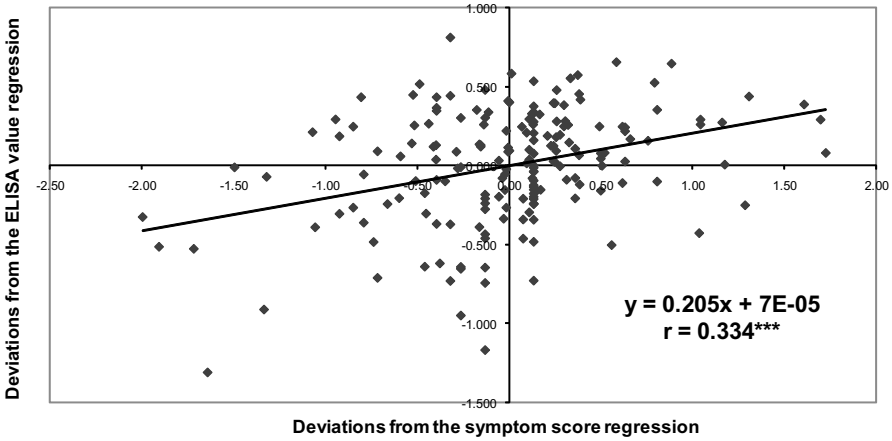


Fig. 5. Correlation between deviations from the symptom score regression (February 16 vs. April 2) and the ELISA score regression (February 16 vs. April 2) for 184 lines derived from "Meridiano x Claudio".



On the whole, results suggest a complex mode of SBCMV-resistance in the "Meridiano x Claudio" cross, involving at least four genes, favourable alleles contributed by both parents and a different timeline of resistance expression. Such conclusions are largely validated by preliminary results obtained with single-marker analysis using 414 simple sequence repeat – SSR - and DArT markers segregating among the RILs, which showed that a major quantitative trait locus responsible for a major SBCMV-response located in the distal end of the 2B chromosome as well as 7 other chromosome regions are significantly associated with SBCMV resistance across symptom scores and ELISA values (Maccaferri *et al*., 2008). The ongoing mapping work with SSR and DArT markers will be most helpful in elucidating the location, effects and nature of the genes controlling SBCMV resistance in the population investigated.

References

Maccaferri, M., Ratti, C., Rubies-Autonell, C., Tuberosa, R., Demontis, A., Massi, A., Stefanelli, S., Vallega, V. and Sanguineti, M.C. (2008). Mapping genetic factors for resistance to *Soil-borne cereal mosaic virus* (SBCMV) in durum wheat. In: *"Proceedings of the 11th International Wheat Genetics Symposium"* (R. Appels, et al., Eds.), pp. 1-3.

Ratti, C., Rubies-Autonell, C., Maccaferri, M., Stefanelli, S., Sanguineti, M.C. and Vallega, V. (2006). Reaction of 111 cultivars of *Triticum durum* Desf. from some of the world's main genetic pools to *Soil-borne cereal mosaic virus*. *J. Plant Dis. Protect.***113**, 145-149.

Rubies-Autonell, C., Ratti, C., Pisi, A. and Vallega, V. (2006). Behaviour of durum and common wheat cultivars in regard to *Soil-borne cereal mosaic virus* (SBCMV): results of fifteen trials. In: *"Proceedings of the 12th Congress of the Mediterranean Phytopathological Union"* (N. Paplomatas Ed.), pp. 100-102.

Vallega, V., Rubies-Autonell, C., Turina, M., Ratti C. and Contoli, S. (1999a). Reactions of durum wheat cultivars to infections by SBWMV grown in northern Italy during 1995-96. *J. Plant Dis. Protect.* **106**, 284-290.

Vallega, V., Rubies-Autonell, C., Ratti, C., Turina, M. and Contoli, S. (1999b). Reactions of durum wheat (Triticum durum Desf.) cultivars to Soil-borne wheat mosaic furovirus (SBWMV) in northern Italy during 1996-97. In*: "Proceedings of the Fourth Symposium of the International Working Group on Plant Viruses with Fungal Vectors"* (J.L. Sherwood and C.M. Rush Ed.), pp. 131-134.

Vallega, V., Rubies-Autonell, C., Ratti, C. and Bianchi, L. (2004). Comportamento di cultivar di frumento duro e tenero rispetto al virus del mosaico comune del frumento (SBWMV): risultati di sei anni di prove. In: *"Proceedings of the Giornate Fitopatologiche"* (A. Brunelli et al., Eds.), Vol. 2, pp. 385-392.

Vallega, V., Rubies-Autonell, C. and Ratti, C. (2006). Resistance to accumulation of *Soil-borne cereal mosaic virus* in eight cultivars of *Triticum durum* Desf. *Parasitica* 62,79-96.

MAPPING AND DIAGNOSTIC MARKER DEVELOPMENT FOR *SOIL-BORNE CEREAL MOSAIC VIRUS* RESISTANCE IN WHEAT

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Summary

Soil-borne cereal mosaic virus (SBCMV) belonging to the genus *Furovirus* is a serious constraint to winter wheat cultivation in Europe. Due to transmission by the plasmodiophorid *Polymyxa graminis,* chemical measures are neither effective nor acceptable for economical and ecological reasons. Therefore, the only possibility of controlling this virus is breeding and growing of resistant cultivars. Using genetic analyses, we found that resistance to SBCMV in cv. ´Tremie´ and ´Claire´ is inherited in a monogenic manner and is located on chromosome 5DL in a region where the *Sbm1* gene for resistance to SBCMV has been recently mapped in wheat cv. 'Cadenza'. 'Cadenza' shares no common ancestry with 'Claire' or 'Tremie'. In all mapping populations, a previously unmapped fragment of SSR *Xgwm469* is the closest linked marker. This marker is of high diagnostic value and therefore well suited to marker based selection procedures. All susceptible genotypes carried a null allele of this SSR, whereas resistant genotypes presumably related to either 'Claire' and 'Tremie' or 'Cadenza' revealed a 152bp or 154bp allele, respectively. In order to identify genes involved

in resistance to SBCMV, expression profiling using RNA of inoculated and healthy plants of resistant and susceptible lines by cDNA-AFLP was carried out. Eight differentially expressed cDNA AFLP fragments were detected. All differentially expressed fragments will be screened for polymorphism and subsequently genetically mapped.

Introduction

Mosaic disease, caused by *Soil-borne cereal mosaic virus* (SBCMV), a member of the genus *Furovirus,* is a serious constraint to winter wheat production in Europe (Budge *et al.*, 2008). SBCMV is transmitted by the plasmodiophorid *Polymyxa graminis*, a eukaryotic soil-borne microorganism that colonizes roots of *Gramineae* plants and has been detected down to a soil depth of 60 cm. Following transmission by *P. graminis*, SBCMV is translocated into the upper parts of susceptible plants causing stunting and mosaic symptoms on leaves that are most prominent in early spring. Winter wheat plants infected in autumn are particularly sensitive to frost damage resulting in increased winter killing or reduced vigour during spring. Chemical measures except soil fumigation, which is unacceptable for economical and ecological reasons, are ineffective against *P*. *graminis*. Furthermore, as virus-containing resting spores of *P. graminis* are distributed by wind, water and machinery and can survive in the soil for decades (Brakke and Langenberg, 1988), crop rotation is not an effective option for disease control either. Therefore, the only possibility of controlling this disease on infested fields is growing of resistant cultivars.

In modern plant breeding programmes, marker-assisted selection (MAS) is an increasingly important tool since it allows breeders to make advanced decisions at several key points during the breeding process. Molecular markers improve breeding efficiency by facilitating reliable selection of resistant individuals on the single plant level independently of symptom expression in the field. Pyramiding of non-linked resistance genes and efficient marker based introgression of resistance genes into high yielding genotype can increase the durability of resistance in the field (Ordon *et al.,* 2004). Efficient breeding for resistance to SBCMV is dependent upon the identification of resistant germplasm followed by the mapping of respective resistance genes and the development of molecular markers that allow monitoring of respective alleles during the breeding process.

cDNA-AFLP is a PCR-based method that allows determination of an expressed part of the genome without any previous sequence knowledge (Vuylsteke *et al.,* 2007). This technique employs the addition of adapters at the ends of restricted cDNA fragments and the subsequent amplification of a subset of these fragments by PCR with primers carrying selective nucleotides at their 3'ends.

In this study we are presenting detailed information on the genetics of SBCMV resistance in elite European wheat cultivars, the development of molecular markers which can be employed for efficient marker assisted selection (MAS) in breeding for SBCMV resistance, and the detection of differentially expressed cDNA AFLP fragments after SBCMV infection.

Material and Methods

Doubled haploids (DH) were derived from crosses between the resistant cv. 'Tremie' and the susceptible cultivars 'Texel', 'Aztek' and 'Soissons' (in total 64 DH lines), and between the resistant cv. 'Claire' and the susceptible cv. 'Savannah' (126 DH lines). To assess resistance of the segregating DH-populations and a collection of 99 wheat genotypes, visual scoring and Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) were carried out. Phenotypic data on the SBCMV resistance gathered in field tests were used for composing resistant and susceptible DNA and cDNA bulks according to Michelmore *et al.* (1991).

Genomic DNA was isolated by the CTAB method according to Graner *et al.* (1991). Resistant and susceptible bulks were screened with 256 *EcoR*I+3/*Mse*I+3 AFLP primer combinations according to the 'AFLP Core Kit manual' (Invitrogen). In addition, a total of 162 genomic and EST-derived microsatellite markers located on 21 wheat chromosomes were amplified as previously described (Röder *et al.,* 1998).
cDNA was synthesised by using the Biorad Smart Kit according to the suppliers recommendations and used for cDNA AFLP analyses. The cDNA was digested with the restriction enzymes *EcoR*I and *MseI* (Fermentas). Parents and bulks were screened with 100 *EcoR*I/*Mse*I + 2 cDNA AFLP combinations and products were resolved on an automated laser fluorescence A.L.F. express sequencer (Amersham Biosciences).

Results and Discussion

In all DH populations, genetic linkage between the resistance locus and marker loci from the long arm of wheat chromosome 5D was detected (Figure 1A). In total, 12 microsatellite loci were integrated into the genetic map of the Savannah x Claire population and 15 microsatellite loci in the consensus map of the Tremie-derived populations. To identify additional closely linked genomic fragments, resistant and susceptible SBCMV bulks were screened with 256 *EcoR*I+3/*Mse*I+3 AFLP primer combinations. Out of 23 AFLP fragments that were polymorphic between DNA bulks, only one was mapped to 5DL. However, in the Savannah x Claire population the AFLP *E37M49* was the closest proximal marker. The closest linked marker in both populations is *Xgwm469* (Figure 1B). This marker co-segregates with the resistance locus in the Tremie-derived population

while in the Claire-derived population it maps 1 cM distal to the resistance locus. To clarify the position of *Xgwm469-5D* in relation to another source of SBCMV resistance this marker was mapped in a population of 204 DH lines derived from the cross Avalon x Cadenza (Bass *et al.,* 2006). In this population *Xgwm469-5D* co-segregated with *Sbm1.* The diagnostic value of *Xgwm469-5D* was assessed using a collection of 99 SBCMV resistant and susceptible wheat cultivars. Importantly, all of the susceptible genotypes carry a null allele of *Xgwm469-5D*, whereas resistant genotypes presumably related to either 'Claire' and 'Tremie' or 'Cadenza' revealed a 152bp or 154bp allele for *Xgwm469-5D*, respectively Perovic *et al.* (2008).

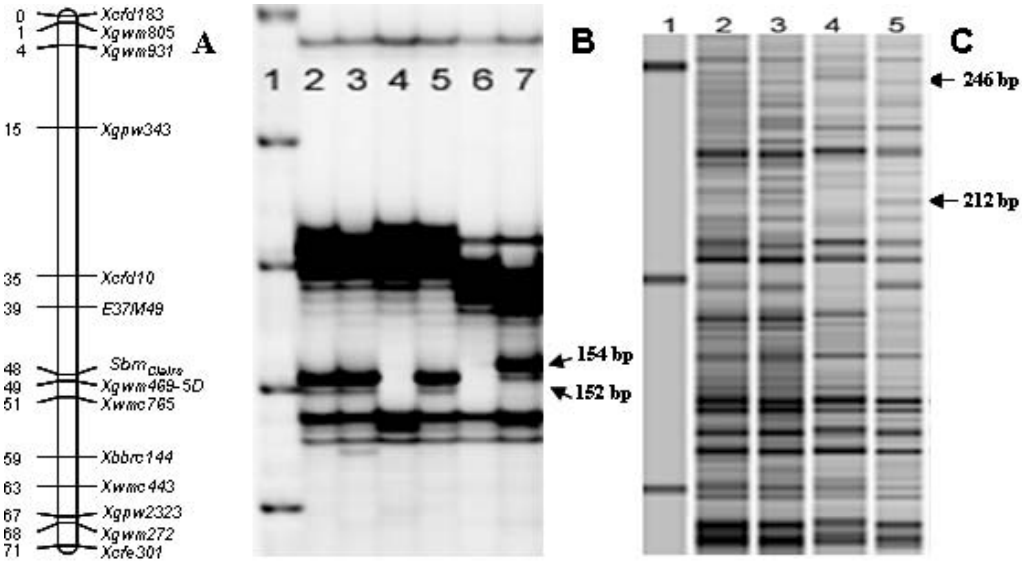

Fig. 1 A. Genetic linkage map of wheat chromosome 5D constructed using mapping population Savannah x Claire. **B.** Li-Cor gel of SSR marker GWM469. *Xgwm469-5DL* alleles linked to SBCMV resistance are indicated by arrows. The order of genotypes is: 1), *micro*STEP-20a (700) DNA ladder (Microzone Ltd). 2), Claire - r; 3), Moulin - r; 4), Wasp – s.; 5), Flame - r; 6), Avalon - r; 7), Cadenza - r. r- resistant genotype, s – susceptible genotype. C. ALF gel of cDNA AFLP combination AA/CA. Differentially expressed cDNA AFLP fragments between reistant and susceptible bulks after SBCMV infection are indicated by arrows. The order of samples is: 1), 50 bp ladder (Fermentas) 2), healthy susceptible bulk 3), healthy resistant bulk 4), infected susceptible bulk 5), infected resistant bulk.

Transcription profiling by cDNA AFLP was carried out to identify differentially expressed genes in susceptible and resistant genotypes. The exact time point for RNA extraction for transcription profiling was determined using RT-PCR for SBCMV detection in different plant organs of susceptible genotype. The first virus detection in roots was 4 weeks after sowing in infested soil under glasshouse conditions, eight weeks in hypocotyls and ten weeks in leaves of infected plants, respectively. Therefore, the plant material for cDNA AFLP was harvested 4, 8

and 10 weeks after infection from roots, leaves and hypocotyls, respectively. Samples were taken in parallel from inoculated and healthy plants of resistant and susceptible lines and bulked for the BSA transcriptome analysis. Five fragments in roots and one in hypocotyls and leaves were identified as differentially regulated in resistant and susceptible bulks. In total, eight differentially expressed cDNA AFLP fragments were isolated (Figure 1C). All differentially expressed fragments will be sequenced and screened for polymorphism in order to map them genetically.

In this study, we identified a previously unmapped microsatellite locus *Xgwm469* as most closely linked to the SBCMV resistance gene on chromosome 5DL and it was shown that GWM469 is well suited for MAS of resistant genotypes at early developmental stages on a single plant level. Besides this, transcript differentiating fragments (TDFs) have been identified by cDNA-AFLP which may facilitate the identification of genes involved in resistance and the development of closely linked markers, as recently shown for the powdery mildew resistance gene Mlg in barley (Korell *et al.,* 2008).

Acknowledgments

We thank B. Knüpfer and M. Weilepp for excellent technical assistance. The work is supported by a grant in the Community's Sixth Framework Programme (EU contract number COOP-CT-2004-512703). Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom. RL was supported by a BBSRC quota studentship awarded to Rothamsted Research.
These results and this publication reflect only the author's view and the *Community* is not liable for any use that may be made of the information contained in this article.

References

Bass, C., Hendley, R., Adams, M,J., Hammond-Kosack, K.E. and Kanyuka, K. (2006). The *Sbm1* locus conferring resistance to *Soil-borne cereal mosaic virus* maps to a gene-rich region on 5DL in wheat. *Genome* **49**, 1140-1148.

Brakke, M.K. and Langenberg, W.G. (1988). Experiences with *Soil-borne wheat mosaic virus* in North America and elsewhere. In: Cooper J.I., Asher M.J.C. (Eds) *Developments in Applied Biology II. Viruses with Fungal Vectors*. Association of Applied Biologists, Wellesbourne, UK, pp. 183-202.

Budge, G.E., Ratti, C., Rubies-Autonell, C., Lockley, D., Bonnefoy, M., Vallega, V., Pietravalle, S. and Henry, C.M. (2008). Response of UK winter wheat cultivars to Soil-borne cereal mosaic and *Wheat spindle streak mosaic viruses* across Europe. *Eur. J. Plant Pathol.* **120**, 259–272.

Graner, A., Jahoor, A., Schondelmaier, J., Siedler, H., Pillen, K., Fischbeck, G., Wenzel, G. and Herrmann, R.G. (1991). Construction of an RFLP map of barley. *Theor. Appl. Genet.*, **83**, 250-256.

Korell, M.T., Eschholz, W., Eckey, C., Biedenkopf, D., Kogel, K.-H., Friedt, W., Ordon, F. (2008). Development of a cDNA-AFLP derived CAPS marker co-segregating with the powdery mildew resistance gene Mlg in barley. *Plant Breeding* **127**, 102-104

Michelmore, R.W., Paran, I. and Kesseli, R.V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* **88**, 9828-9832.

Ordon, F., Friedt, W., Scheurer, K., Pellio, B., Werner, K., Neuhaus, G., Huth, W., Habekuss, A., Graner, A. (2004). Molecular markers in breeding for virus resistance in barley. *J. Appl. Genet.* **45**, 145-159.

Perovic, D., Förster, J., Devaux, P., Hariri, D., Guillerouxm, M., Kanyuka, K., Lyons, R., Weyen, J., Feuerhelm, D., Kastirr, U., Sourdille, P., Röder, M. and Ordon, F. (2008). Mapping and diagnostic marker development for *Soil-borne cereal mosaic virus* resistance in bread wheat (Submitted in Molecular breeding).

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P. and Ganal, M.W. (1998). A microsatellite map of wheat. *Genetics* **149**, 2007-2023.

Vuylsteke, M., Peleman, J.D., van Eijk, M.J.T. (2007). AFLP-based transcript profiling (cDNA-AFLP) for genome-wide expression analysis. *Nature Protocols* 2,1399-1413.

RESISTANCE EVALUATION OF WINTER BARLEY ACCESSIONS AND VARIETIES TO STRAINS OF *BARLEY YELLOW MOSAIC VIRUS* AND *BARLEY MILD MOSAIC VIRUS* UNDER FIELD CONDITIONS

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Summary

In extensive screening programmes genotypic differences in reactions of 82 accessions in the period 2006/2007 and 83 in 2007/2008 and 5 varieties of winter barley to the yellow mosaic virus complex were evaluated at four different locations. The field trials were carried out on two sites where the soil was natural infested with both BaYMV-1 and BaMMV and at two other locations additionally infested with the resistance breaking pathotype BaYMV-2. Resistance to BaMMV and BaYMV-1 caused by *rym4* was found quite frequent within the primary gene pool of *Hordeum vulgaris*. Sixty nine accessions tested in 2006/2007 and 74 tested in 2007/2008 exhibited no visible virus symptoms on the leaves of all inspected plants. A few number of accessions reacted inconsistently due to inhomogeneous splitting of breeding material. Among the 5 tested varieties 3 (Naomie, Jorinde and Fridericus) were resistant to BaMMV and BaYMV-1 in both evaluation periods; no virus could be detected serologically neither in tissue print immunoassay or ELISA nor by RT-PCR. In contrast, only very few accessions were unaffected by the pathotype BaYMV-2. In 2006/2007 seven breeding lines remained virus free and three other lines gave conflicting results due toa single infected plant in the plot. In 2007/2008 three of the accessions previously tested virus free did not became infected by BaYMV-2 and four other winter barley lines reacted inconsistent. The variety Jorinde carrying the resistance gene *rym5* remained virus free in all examinations.

Introduction

The yellow mosaic complex of barley is one of the most severe threats to the production of winter barley in Europe. The disease is caused by *Barley mild mosaic virus* (BaMMV) and *Barley yellow mosaic virus* (BaYMV), both transmitted, beside other cereal viruses, by *Polymyxa graminis* Ledingham (Kanyuka *et al.*,

2003). The two viruses, which belong taxonomically to the genus *Bymovirus* within the family *Potyviridae* (Adams *et al.*, 2005), differ in serological properties, in nucleotide sequences of the two genomic RNAs and their ability to infect different barley genotypes (Huth and Adams, 1990). Based on pathogenicity tests on different barley cultivars, several strains of BaMMV (Lee *et al.*, 1996; Nomura *et al.*, 1996; Hariri *et al.*, 2003; Habekuss *et al.*, 2008) and BaYMV (Huth, 1989; Kashiwazaki *et al.*, 1989; Chen *et al.*, 1996) have been identified worldwide.
The only way to reduce the impact of these viruses BaMMV/ BaYMV on yield performance is to grow resistant varieties. However, mechanical transmissions through finger rubbing or spraying are not very effective and are cumbersome. For these reasons, screening for resistance is commonly done in the field. Therefore, extensive screening programmes were started in cooperation with the Bundessortenamt of the Federal Ministry of Food, Agriculture and Consumer Protection to evaluate genotypic differences in barley accessions and varieties to the yellow mosaic virus complex. The reaction of 82 accessions in the period 2006/2007 and 83 in the season 2007/2008 and 5 standard varieties of winter barley were evaluated at four different locations.

Materials and Methods

The field trials were carried out on two sites near Braunschweig (BBA and Mehrum) where the soil was naturally infested with both BaYMV-1 and BaMMV and at two further locations (Borum and Schladen) additionally infested with the resistance breaking pathotype BaYMV-2. The field trials were inspected for virus symptoms at three different intervals in the early spring. Plants from varieties with without visible symptoms were tested serologically using polyclonal antisera to BaMMV and BaYMV in DAS-ELISA and/or tissues print immunoassay (TPIA) (Johnson *et al.*, 2007). Plants serologically tested negative were further assayed by RT-PCR using virus specific prímer combinations (Steyer *et al.*, 2005). Additionally, plants with suspect symptoms were tested by ELISA or TPIA using specific monoclonal antibodies for the presence of other cereal infecting viruses like *Barley yellow dwarf virus*, *Soil-borne wheat mosaic virus* and *Soil-borne cereal mosaic virus.*

Results and Discussion

Plants with very severe symptoms often had mixed infections of both bymoviruses. However, in plants with vague symptoms none of the other assayed viruses were detected.
The results of field evaluations revealed that resistance to BaMMV and BaYMV-1 caused by the *rym4* gene is quite frequent within the primary gene pool of *Hordeum vulgaris*. Sixty nine accessions tested in 2006/2007 and 74 tested in 2007/2008 exhibited no visible virus symptoms on the leaves of all inspected plants. A few number of accessions reacted inconsistently due to inhomogeneous

splitting of breeding material. Among the 5 tested standard varieties 3 (Naomie, Jorinde and Fridericus) were resistant against BaMMV and BaYMV-1 in both evaluation periods; no virus could be detected serologically neither by tissue print immunoassay or ELISA nor by RT-PCR. The varieties Tiffany and Vanessa were susceptible to both viruses (Table 1).

In contrast, only very few accessions were not infected by the pathotype BaYMV-2. In 2006/2007 seven breeding lines remained virus free and three other lines gave conflicting results due to a single infected plant in the plot.

Table 1. Reaction of five standard barley varieties in field trials of two years

				2006/07		2007/08	
Identification No.	Variety	TKW in g	Germination capacity (%)	BaYMV-1 / BaMMV [1)]	BaYMV-2 [2)]	BaYMV-1 / BaMMV [1)]	BaYMV-2 [2)]
1457	Tiffany	48.00	97	9	9	9	9
1794	Vanessa	53.00	95	9	9	9	9
2092	Naomie	47.00	97	1	9	1	9
2312	Jorinde	54.20	86	1	1	1	1
2345	Fridericus	50.10	96	1	9	1	9

[1)] Field trial at Braunschweig and Mehrum, [2)] field trial at Bornum and Schladen, 1 resistant, 9 susceptible

In 2007/2008 three of the accessions previously tested virus free did not become infected by BaYMV-2 and four other winter barley lines reacted inconsistently. The reason for that could be that the breeding material is heterogenic and still segregating. The variety Jorinde carrying the resistance gene *rym5* remained virus free under field condition in all tests.
Beside the German accessions a few varieties from the UK were included in the screening. The behaviour of 3 varieties in both test seasons is summarized in Table 2. Additionally, 2 further accessions were tested in 2007/2008 showing that none of the varieties was resistant to BaYMV-2 (Table 2).

Table 2. Reaction of five barley varieties from UK in field trials

	2006/07		2007/08	
	BaYMV-1 / BaMMV [1)]	BaYMV-2 [2)]	BaYMV-1 / BaMMV [1)]	BaYMV-2 [2)]
Pelikan	1	9	1	9
Yatzy	1	9	1	9
Maybrit	1	9	1	9
Scarpia	nt	nt	1	9
Nikela	nt	nt	9	9

[1)], [2)] for legends see Table 1, nt - not tested.

Further breeding activities to combine *rym5* with *rym9* which is effective against BaMMV-1 and the other resistance breaking strains known so far, could result in resistance against all currently known yellow mosaic inducing virus pathotypes.

Acknowledgements

We thank Dr. Dirk Rentel (BSA, Hannover), Dr. R. Hempker (Limagrain GmbH Rosenthal), Ms. K. Cais and Mr. M. Dötsch (LfL Freising), Mr. O. Welli-Stephan (DSV Lippstadt), and Vicky Fanstone (Disease Resistance and Diagnostics, NIAB, Cambridge, UK) for fruitful co-operation.

References

Adams, M.J., Antoniw, J.F. and Fauquet, C.M. (2005). Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Arch. Virol.* **150**, 459-479.

Chen, J., Adams, M.J., Zhu, F.T., Wang, Z.Q., Chen,J., Huang, S.Z. and Zhang Z.C. (1996). Responses of foreign barley cultivars to *Barley yellow mosaic viruses* at different sites in China. *Plant Pathol.* **45**, 1117-1125.

Habekuss, A., Kühne, T., Krämer, I., Rabenstein, F., Ehrig, F., Ruge-Wehling, B., Huth, W. and Ordon F. (2008). Identification of *Barley mild mosaic virus* isolates in Germany breaking *rym5* resistance. *J. Phytopathology* **156**, 36-41.

Hariri, D., Meyer, M. and Prud'homme, H. (2003). Characterization of a new *Barley mild mosaic virus* pathotype in France. *Eur. J. Plant Pathol.* **109**, 921-928.

Huth, W. (1989). Ein weiterer Stamm des *Barley yellow mosaic virus* aufgefunden. *Nachrichtenbl. Dt.. Pflanzenschutzd*. **41**, 6-7.

Huth, W. and Adams, M.J. (1990). *Barley yellow mosaic virus* (BaYMV) and BaYMV-M: two different viruses. *Intervirology* **31**, 38-42.

Jonson, G., Park, J.C., Kim, Y.K., Kim, M.J., Lee, M.J., Hyun, J.N. and Kim, J.G. (2007). Direct stem blot immunoassay (DSBIA): A rapid, reliable and economical detection technique suitable for testing large number of barley materials for field monitoring and resistance screening to *Barley mild mosaic virus* and *Barley yellow mosaic virus*. *Plant Pathol. J.* **23**, 260-265.

Kanyuka, K., McGrann, G., Alhudaib, K., Hariri, D. and Adams, M.J. (2004). Biological and sequence analysis of a novel European isolate of *Barley mild mosaic virus* that overcomes the barley *rym5* resistance gene. *Arch. Virol.* **149**, 1469–1480.

Kanyuka, K., Ward, E. and Adams, M. J. (2003). *Polymyxa graminis* and the cereal viruses it transmits: a research challenge. *Mol. Plant Pathol.* **4**, 393-406.

Kashiwazaki, S., Ogawa, K., Usugi, T. and Tsuchizaki, T. (1989). Characterization of several strains of *Barley yellow mosaic virus*. *Ann. Phytopath. Soc. Jpn.* **55**, 16-25.

Lee, K.J., Kashiwazaki, S., Hibi, T., So, I.Y. (1996). Properties and capsid protein gene sequence of a Korean isolate of *Barley mild mosaic virus*. *Ann. Phytopath. Soc. Jpn.* **62**, 397-401.

Nomura, K., Kashiwazaki, S., Hibino, H., Inoue, T., Nakata, E., Tsuzaki, Y. and Okuyama, S. (1996). Biological and serological properties of strains of *Barley mild mosaic virus*. *J Phytopathol*.**144**, 103-107.

Steyer, S., Barbier, A., Denis, F., Collard, M., Vaianopoulos, C. and Bragard, C. (2005). Detection of *Barley yellow mosaic virus* and *Barley mild mosaic virus* by ELISA and RT-PCR: a comparison study. *Parasitica* **61**, 33-36.

GENOTYPES RESISTANT TO NEW STRAINS OF *BARLEY MILD MOSAIC VIRUS* (BAMMV-TEIK, BAMMV-SIL)

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Summary

Barley genotypes mainly derived from East Asia were tested for their reaction to the *rym5* resistance-breaking strains BaMMV-Teik and BaMMV-Sil by mechanical inoculation or in an infested field, respectively. 76 out of 87 investigated accessions were resistant to both strains and moreover according to available information, 45 of these are also resistant to the other barley mosaic viruses up to now known in Germany, i.e. BaMMV (original strain), BaYMV-1 and BaYMV-2. One of these genotypes is 'Taihoku A' carrying the resistance gene *rym13.* Analysis of a DH-population suggested that resistance of ´Taihoku A´ to BaMMV-Teik is inherited in a monogenic manner and that resistance to BaMMV-Teik is also encoded by *rym13*. Using bulked segregant analysis additional AFLP markers and a closely linked SSR were developed for this locus.

Introduction

Barley mild mosaic virus (BaMMV) and *Barley yellow mosaic virus* (BaYMV) cause one of the most important diseases of winter barley in Europe and East Asia. Besides one BaMMV-strain and two BaYMV-strains (BaYMV-1, BaYMV-2) known in Europe since the 1980s (Huth and Lesemann, 1978) and 1990s (Huth, 1989), recently new resistance-breaking strains of BaMMV being able to infect cultivars carrying the resistance gene *rym5* were detected, e.g. in France (Hariri *et al*., 2003, BaMMV-Sil) and in Germany (Habekuß *et al*., 2008, BaMMV-Teik). Ratti *et al*. (2005) provided evidence for the presence of such isolates in Spain and Italy. Because of transmission by the soil-borne plasmodiophorid *Polymyxa graminis,* growing of resistant cultivars is the only method to ensure winter barley cultivation in growing area of infested fields. Therefore, as a prerequisite for efficient breeding for resistance to BaMMV-Teik and BaMMV-Sil, genetic resources of barley were evaluated for resistance to these new strains of BaMMV.

Materials and Methods

87 barley accessions which mainly derived from East Asia were tested by artificial inoculation for their reaction to BaMMV-Teik in a growth chamber in Quedlinburg. At the 3- to 5-leaf stage 10 plants per genotype were inoculated two times at an interval of 5 to 7 days. For the preparation of inoculum portions of 10 g of infected leaf material of the cv. 'Tokyo' were homogenised in K_2HPO_4 buffer (1:10; 0.1 M; pH 9.8) under cold conditions (4°C) and carborundum powder (mesh 300) was added to the plant sap (0.5 g / 25 ml sap). The cultivation of the plants was carried out in a growth chamber at the following conditions: 16 / 12 °C day / night and 12 h light (10 klx). The number of plants with mosaic symptoms was scored 4 to 5 weeks after the first inoculation and DAS-ELISA was carried out using polyclonal antisera.
In parallel these accessions were grown in a field in Sillery (France) infested with BaMMV-Sil during the growing seasons 2005/2006 and 2007/2008. 30 seeds per line were sown in two replications in September. From February until April symptom expression was scored and DAS-ELISA was performed. Furthermore, 154 doubled haploid lines of the cross 'Taihoku A' (*rym13*) x 'Plaisant' (susceptible) were tested for resistance to BaMMV-Teik using mechanical inoculation as described above followed by DAS-ELISA carried out for each single plant. In order to develop closer linked markers for *rym13* bulked segregant analysis using AFLPs and SSRs was carried out (for details cf. Humbroich, 2007).

Results and Discussion

Out of the 87 accessions tested, 76 genotypes turned out to be resistant to BaMMV-Teik after mechanical inoculation. Out of these, 45 genotypes were also resistant to the original BaMMV strain as well as to BaYMV-1 and BaYMV-2 (Table 1).
In the field tests carried out at Sillery no differences in the reaction of these genotypes were detected in comparison to the BaMMV-Teik results obtained in the growth chamber experiments. All genotypes being resistant to BaMMV-Teik were also resistant to BaMMV-Sil. In summary 9 types of reaction to the different members of the *Barley yellow mosaic virus* complex were observed (Table 1).

Table 1. Resistance reaction of barley genotypes to different strains of BaMMV and BaYMV.

Resistance Reaction to				Number of genotypes
BaMMV-Teik[1]	BaMMV (orig.)[2]	BaYMV-1[2]	BaYMV-2[2]	
r	r	r	r	45
r	r	r	s	5
r	r	s	s	1
r	s	s	s	4
r	s	s	r	1
r	s	r	r	8
r	uk	uk	uk	12
?	r	r	r	1
s	r	r	r	3
s	s	r	r	2
s	s	s	s	3
s	uk	uk	uk	2
				87

[1] reaction in growth chamber test r=resistant uk=unknown

[2] reaction according to Ordon, 1992 s=susceptible ? =unclear reaction

Those twelve accessions which turned out to be resistant to BaMMV-Teik and have not been tested for resistance to BaMMV (original), BaYMV-1 and -2 will be evaluated in the growing season 2008/2009 as well as those accessions with doubtful results. Besides this, tests for allelism will be carried out to get more detailed information on the genetics of resistance of these accessions.

In the present studies it turned out that ´Taihoku A´ carrying the resistance gene *rym13* located on chromosome 4H (Werner *et al.*, 2003) is also resistant to BaMMV-Teik and BaMMV-Sil. Therefore, in order to get information on the genetic basis of the resistance against BaMMV-Teik a DH-population comprising 154 lines was tested for resistance to this virus strain. The results of the segregation analysis (67s : 87r, χ^2=2.597) suggested that resistance to BaMMV-Teik is also inherited in a monogenic manner and is also encoded by *rym13* as no recombinants concerning the reaction to the different virus strains were observed.

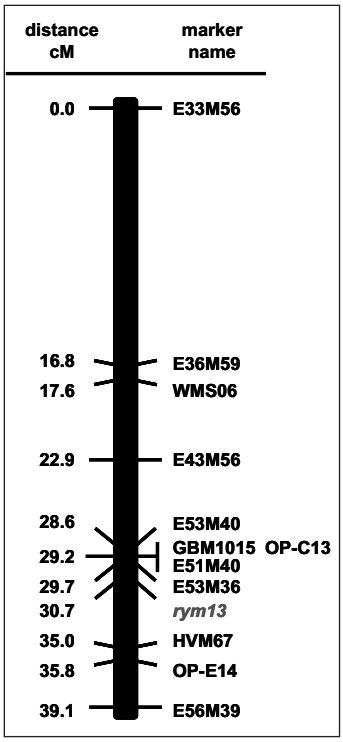

Fig. 1. Genetic map of chromosome 4HL including *rym13* (Humbroich, 2007).

Bulked segregant analysis using AFLPs resulted in the map of *rym13* shown in Figure 1 (cf. also Werner *et al*., 2003). The AFLP marker E53M36 with a distance of 1.0 cM to *rym13* is the closest linked marker, followed by the co-segregating markers E51M40, OP-C13 and GBM1015 with a genetic distance of 1.5 cM. Further attempts aim at converting these AFLPs into easy to handle STS markers, which will facilitate the incorporation of *rym13* into adapted breeding lines leading to cultivars with resistance to all strains of BaMMV/BaYMV known in Europe so far.

Acknowledgements

Thanks are due to the “Arbeitsgemeinschaft industrieller Forschungseinrichtungen ‘Otto von Guerricke’ e.V.” (AIF) for financial support (KF 0073502 MD5).

References

Habekuß, A., Kühne, T., Krämer, I., Rabenstein, F., Ehrig, F., Ruge-Wehling, B., Huth, W. and Ordon, F. (2008). Identification of *Barley mild mosaic virus* Isolates in Germany Breaking rym5 Resistance. *J. Phytopathology* **156**, 36-41.

Hariri, D., Meyer, M. and Prud'homme, H. (2003). Characterization of a new *Barley mild mosaic virus* pathotype in France. *Eur. J. Plant Pathol.* **109**, 921-938.

Humbroich, K. (2007). Identification and mapping of resistance genes against soil-borne viruses in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.). Dissertation, Universität Gießen, 109 pp.

Huth, W. (1989). Ein weiterer Stamm des *Barley yellow mosaic virus* (BaYMV) gefunden. *Nachrichtenbl. Deut. Pflanzenschutzd.* 41, 6-7.

Huth, W. and Lesemann, D. E. (1978). Eine für die Bundesrepublik neue Virose der Wintergerste. *Nachrichtenbl. Deut. Pflanzenschutzd.* **30**, 184-185.

Ordon F. (1992). Genetische Analyse der Resistenz exotischer Gersten gegen bodenbürtige, mosaikinduzierende Viren. Dissertation, Universität Gießen, 125 S.

Ratti, C., Rubies-Autonell, C., Lanzoni, C., Pisi, A. and Achon, M.A. (2005). VPg sequence analyses of Spanish and Italian BaMMV isolates revealed high identity with *rym5* resistant breaking strain. In: *Book of Abstracts of 6th Symposium International Working Group on Plant Viruses with Fungal Vectors*. Bologna (Italy), 5-7 September 2005, p. 61.

Werner, K., Rönicke, S., Le Gouis, J., Friedt, W. and Ordon, F. (2003). Mapping of a new BaMMV-resistance gene derived from the variety 'Taihoku A'. *J. Plant Dis. Protect.* **110**, 304-311.

RESISTANCE TO *SOIL-BORNE CEREAL MOSAIC VIRUS* IN DURUM WHEAT LINES DERIVED FROM THE CROSS "NEODUR x CIRILLO"

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Summary

A population consisting of 160 F_8 recombinant inbred lines (RILs) of durum wheat (*Triticum durum* Desf.) obtained from a cross between cvs. "Neodur" and "Cirillo" was grown during the 2007-08 season in a field with natural inoculum sources of *Soil-borne cereal mosaic virus* (SBCMV) near Bologna, and evaluated on various dates for symptom severity and ELISA absorbance. Results indicated that cv. Neodur carries at least three major SBCMV-resistance genes. The study, moreover, indicated the presence of a sizeable and genetically controlled temporal change in the relative degree of resistance of the lines in terms of both symptom severity and ELISA absorbance causing marked changes in resistance rank order among lines.

Introduction

Most of the durum wheat cultivars marketed in Italy are susceptible to SBCMV, and none was found completely resistant (Rubies-Autonell *et al*., 2006; Vallega *et al*., 2006). The same was observed for a large number of durum wheat cvs. from other countries (Ratti *et al*., 2006). Interestingly, a high proportion of the few SBWMV-resistant cvs. identified (including cvs. Ares, Neodur, Iride, Svevo, Saragolla, Dylan, Tiziana and Levante, all marketed in Italy) derives from cv. "Edmore", bred about thirty years ago in the U.S. (Vallega *et al*., 2004; Ratti *et al*., 2006). It therefore appears reasonable to postulate that the latter cv. and its SBCMV-resistant derivatives carry a major gene or gene-block, that is transmitted to the progeny in a high frequency, even in the absence of a conscious selection pressure for resistance. To test this hypothesis, 160 F_8 RILs obtained by single-seed-descent from a cross between cvs. "Neodur" (a highly resistant Edmore derivative) and "Cirillo" (highly susceptible) were grown in a field with natural inoculum sources of SBCMV near Bologna.

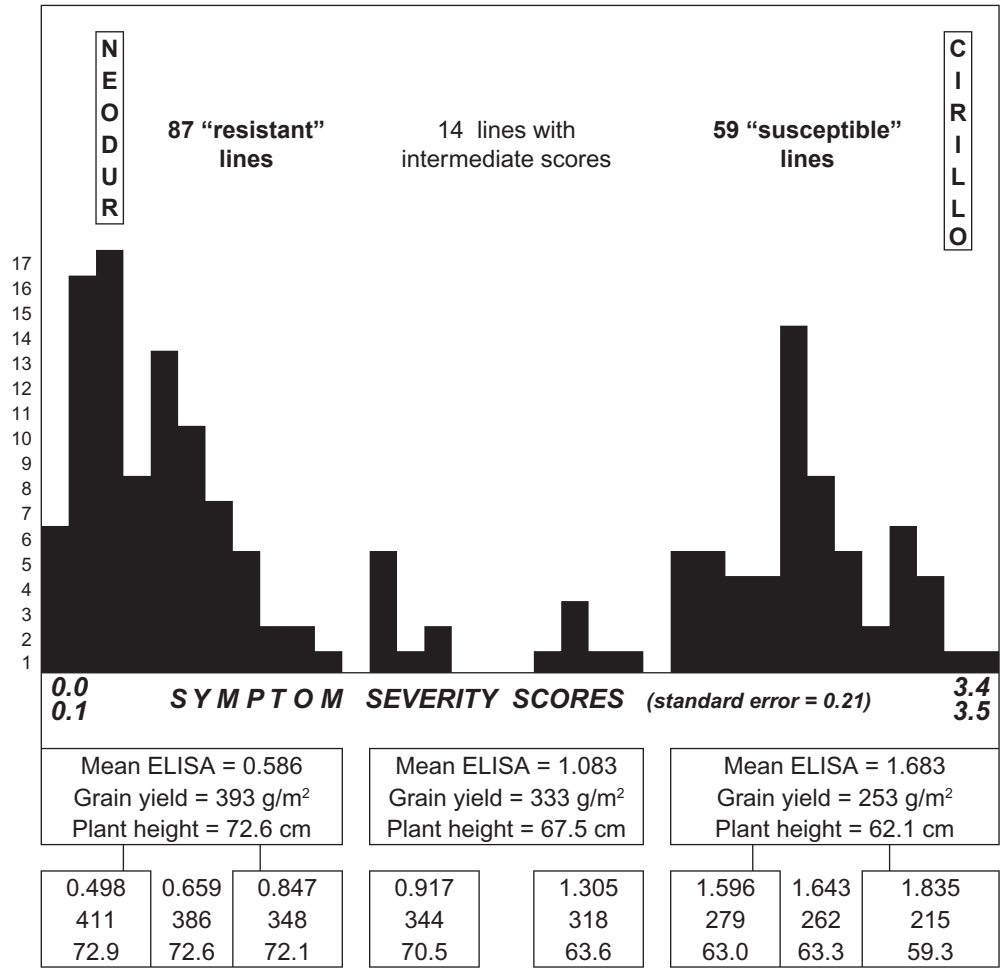
Materials and Methods

Lines and parental cvs. were grown during the 2007-08 season in 2.4 m^2 solid-seeded plots distributed according to a randomized block design with 3 replicates, and evaluated for symptom-severity (March 11, March 27 and April 15), ELISA absorbance (March 11 and April 15), grain yield, test weight, kernel weight, and plant height. Symptom severity was scored on a 0-4 scale) and DAS-ELISA was determined on extracts from a bulk of the youngest fully expanded leaf of 15 plants per plot (Ratti *et al*., 2006). Minimum number of genes in the population was quantitatively estimated by the method of Wright (1968).

Results and Discussion

Symptom scores and ELISA values were significantly correlated (r=0.757 on March 11; r=0.787 for April 15; $P \leq 0.001$), and both parameters showed negative associations ($P \leq 0.001$) with each of the four agronomic traits examined, particularly with grain yield and plant height (r from -0.485 to -0.688 for ELISA and symptoms, depending on the observation date).

Fig. 1. Number of lines derived from the durum wheat cross "Neodur x Cirillo" showing different SBCMV symptom severity scores (0 - 4 scale; means of March 27 and April 15, 2008), and mean ELISA value, grain yield (g/m^2) and plant height (cm) of the lines corresponding to the eight major frequency peaks.



Symptom-severity score frequency distributions showed a markedly greater proportion of resistant vs. susceptible lines on all three observation dates (93/67, 96/64 and 96/64) and 8 major peaks (Fig. 1). Grain yield, plant height and ELISA-absorbance means for the lines contributing to each of the 8 symptom frequency-peaks were highly differentiated, and closely matched the values expected for the corresponding symptom score peaks. The ELISA value distributions too revealed a greater proportion of resistant lines (89/71) and 7 distinct major peaks (data not shown). Most of the major frequency peaks obtained for each of the two resistance parameters were bi-modal. Segregation distortions analogous to that causing a preponderance of SBCMV-resistant types have been often reported for RILs, and may be attributed to various factors, particularly to genetic interactions and – in the case of materials obtained by SSD, which cumulate the effects of

multiple generations - to selective advantages of the corresponding genes or gene-blocks (Song *et al*., 2006).

Fig. 2. Correlation between SBCMV-symptom scores recorded March 11 and April 15 for 160 lines from "Neodur x Cirillo"

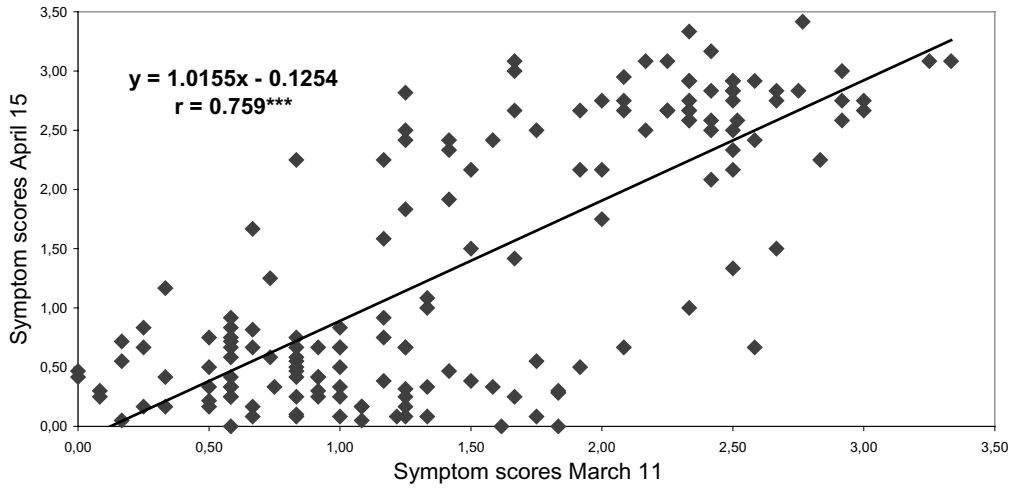
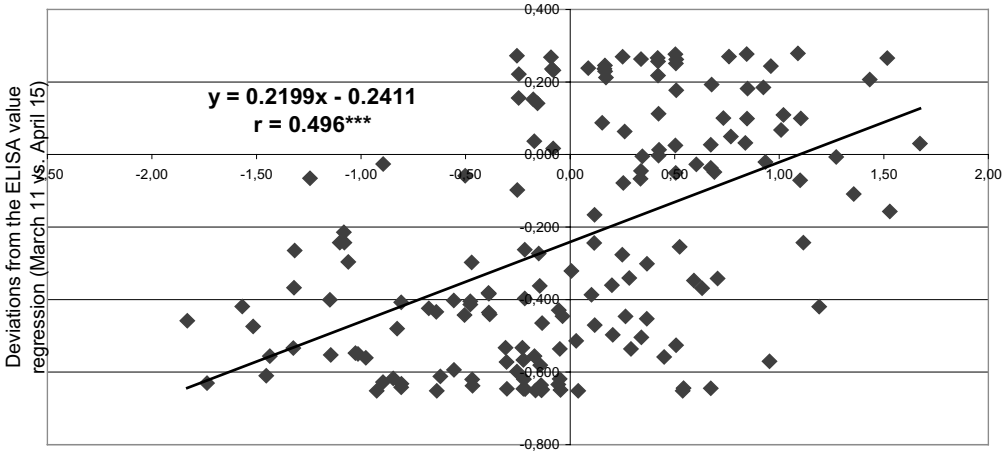


Fig. 3. Correlation between deviations from the symptom score regression (March 11 vs. April 15) and deviations from the ELISA value regression (March 11 vs. April 15), for 160 lines from "Neodur x Cirillo".



The number of genes (2.8, 3.4, and 3.1 for March 11, March 27 and April 15, respectively) estimated by the formula of Wright (1968) approximately corresponds to that which may be envisaged by the major peaks in the frequency distributions. Nonwithstanding the segregation distortion, it may be concluded that cv. Neodur

contributed at least three - possibly linked - major genes accounting for the 8 major frequency peaks and their seemingly bi-modal form.

Symptom scores became increasingly polarized during the course of the season, and numerous lines showed a marked decrease in symptom severity on the last observation date (Fig. 2). As largely expected, the symptom scores recorded March 11 and April 15 were closely correlated ($P \leq 0.001$), and the same applied between ELISA values recorded on the same dates. Interestingly, the correlation between the deviations from each of the two regression equations (Fig. 3) was significant too ($r = 0.496$; $P \leq 0.001$), thus confirming that the relative degree of resistance of the lines - independently measured in terms of symptomatology and ELISA – indeed changed from March 11 to April 15, causing substantial modifications in ranking order among lines. This temporal change may be attributed to a diverse duration of the efficacy of the resistance genes identified, or to genes controlling morphological or phenological plant traits affecting the onstart and/or progress of SBCMV infection. Resistance rankings between cvs. have been reported to change during the season (Vallega et. al., 2006; Rubies-Autonell *et al*., unpublished); the present experiment offered the opportunity to validate the phenomenon in a common genetic background.

A relatively more complex SBCMV-resistance inheritance mode as well as the presence of a quantitative trait locus (QTL) responsible for a major SBCMV-response has been recently reported in RILs derived from a cross between durum wheat cvs. “Meridiano” and “Claudio” (Maccaferri *et al*., 2008). Presently, the “Neodur x Cirillo” population is being profiled to identify the QTLs associated with its specific SBCMV-resistance genes, and to elucidate the nature of the genes causing a temporal change in resistance and of that originating the inheritance pattern distortion.

References

Maccaferri, M., Ratti, C., Rubies-Autonell, C., Tuberosa, R., Demontis, A., Massi, A., Stefanelli, S., Vallega, V. and Sanguineti, M.C. (2008). Mapping genetic factors for resistance to *Soil-borne cereal mosaic virus* (SBCMV) in durum wheat. In: *“Proceedings of the 11th International Wheat Genetics Symposium”* (R. Appels, *et al*. Ed.), pp. 1-3.

Ratti, C., Rubies-Autonell, C., Maccaferri, M., Stefanelli, S., Sanguineti, M.C. and Vallega, V. (2006). Reaction of 111 cultivars of *Triticum durum* Desf. from some of the world’s main genetic pools to *Soil-borne cereal mosaic virus. J. Plant Dis. Prot.* **113**, 145-149.

Rubies-Autonell, C., Ratti, C., Pisi, A. and Vallega, V. (2006). Behaviour of durum and common wheat cultivars in regard to *Soil-borne cereal mosaic virus* (SBCMV): results of fifteen trials. In: *“Proceedings of the 12th Congress of the Mediterranean Phytopathological Union”* (N. Paplomatas Ed.), pp. 100-102.

Song, X.L., Sun X.Z. and Zhang, T.Z. (2006). Segregation distortion and its effect on genetic mapping in plants. *Chin. J. Agricult. Biotech*. **3**, 163-169.

Vallega, V., Rubies-Autonell, C., Ratti, C. and Bianchi, L. (2004). Comportamento di cultivar di frumento duro e tenero rispetto al virus del mosaico comune del frumento (SBWMV): risultati di sei anni di prove. In: *"Proceedings of the Giornate Fitopatologiche"* (A., Brunelli Ed.), Vol. **2**, pp. 385-392.

Vallega, V., Rubies-Autonell, C. and Ratti, C. (2006). Resistance to accumulation of *Soil-borne cereal mosaic virus* in eight cultivars of *Triticum durum* Desf. *Parasitica* **62**, 79-96.

Wright, S. (1968). Evolution and Genetics of Populations. Vol. **1**. Genetic and Biometric Foundations. Chicago, USA: University of Chicago Press. 469 pp.

REACTION OF THIRTY-FOUR DURUM WHEAT CULTIVARS TO *SOIL-BORNE CEREAL MOSAIC VIRUS* IN 2007.

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Summary

Thirty-four cultivars of durum wheat (*Triticum durum* Desf.) were grown during 2006/07 in a field with *Soil-borne cereal mosaic virus* (SBCMV) near Bologna, and evaluated for resistance to this virus on the basis of symptom severity, DAS-ELISA readings and agronomic performance. Cultivars Asdrubal, Dario, Dylan, Hathor and Saragolla showed slight or no SBCMV symptoms, and extracts from their leaves gave relatively low ELISA values. Cultivars Meridiano, Iride, Neodur, Levante and Duilio – all of which had shown high levels of SBCMV resistance in a number of previous trials characterized by a high disease pressure - had mild symptoms and high grain yields y*et al*so high ELISA values. Regression analysis indicated that the most susceptible cultivars suffered a mean grain yield loss of 54% as well as notable reductions in plant height (21%) and 1000-kernel weight (17%).

Introduction

SBCMV has been reported from various European countries (Jezewska, 1995; Clover *et al*., 1999; Vaianopoulos *et al*., 2005; Budge *et al*., 2008), and is widespread in Italy (Canova and Quaglia, 1960; Vallega and Rubies-Autonell, 1989; Ratti *et al*., 2005), where it often causes yield reductions of about 50-70% on susceptible cvs. of common wheat (*Triticum aestivum* L.) and durum wheat (Toderi, 1969; Rubies-Autonell *et al*., 2003; Vallega *et al*., 1997, 1999, 2006), sometimes as a result of mixed infections with *Wheat spindle streak mosaic virus* (Rubies-Autonell and Vallega, 1987; Vallega *et al*., 2003). Most of the durum wheat cultivars marketed in Italy are susceptible or moderately susceptible to SBCMV (Rubies-Autonell *et al*., 2006), and the same applies to durum wheat cvs. bred in other countries (Ratti *et al*., 2006).

Materials and Methods

Thirty four durum wheat cvs. were grown during 2006/07 in a field with SBCMV at Cadriano, near Bologna; 18 of these cultivars (marked with asterisks in Table 1 had never been tested for SBCMV resistance before. The cultivars were grown in 10 m^2 solid-seeded plots, distributed according to a randomized block design with three replicates. Symptom severity was evaluated Feb. 16, Feb. 21, March 12 and April 2 using a 0-4 scale (Vallega and Rubies-Autonell, 1985). DAS (Double Antibody Sandwich) ELISA was performed as reported previously (Ratti *et al*., 2006) on extracts from a bulk of the youngest fully expanded leaf of 15 plants per plot collected Feb. 21 and March 12 (2007). Grain yield, thousand-kernel weight, test weight, heading date and plant height at maturity were also determined. Grain yield data should be interpreted with caution, due to severe and differential bird damage at maturity.

Table 1. Mean symptom severity, mean ELISA value and agronomic performance for 34 cultivars of durum wheat grown in a field with SBCMV at Cadriano (Bologna) during the 2006/07 season.

Cultivar	Mean symptom score (0-4)[a]	Mean ELISA value[b]	Heading (days from April 1)	Plant height (cm)	Thousand kernel weight (g)	Test weight (g)	Grain yield (13% humidity)
Achille *[c]	3.3 a[d]	1.516 af	38.7 ac	61.7 op	32.6 fh	71.1 ad	1.24 ij
Anco Marzio	3.0 ab	1.631 ad	32.7 gl	70.0 kn	33.3 eh	67.8 bj	1.42 hj
Ariosto *	0.8 lp	1.444 af	35.0 dh	87.0 ae	40.6 ab	70.2 af	2.43 ch
Asdrubal *	1.2 hm	0.612 hj	28.0 no	88.7 ac	34.7 dg	72.8 a	4.47 a
Capri *	3.1 ab	1.653 ad	39.0 ab	72.3 in	31.1 gi	68.2 ai	1.62 gj
Casanova *	1.8 eh	1.777 ab	31.3 in	80.7 ci	39.5 ac	67.9 bi	2.36 dh
Catervo *	1.4 gm	1.368 bf	35.0 dh	78.3 ek	36.2 bf	64.4 hj	1.84 fj
Chiara *	1.4 gm	1.406 af	34.7 di	73.3 hn	37.9 bd	67.6 bj	2.91 cf
Claudio	2.5 bd	1.736 ac	34.0 ej	85.0 bf	36.6 bf	72.6 a	2.45 ch
Creso	1.6 ej	1.114 eg	36.3 bf	74.7 gn	39.2 ad	70.6 ae	2.39 dh
Dario *	0.3 oq	0.389 ik	30.7 jo	94.0 a	35.4 cg	69.8 af	2.75 cf
Duilio	1.0 in	1.226 df	34.0 ej	81.7 ch	40.6 ab	69.7 af	3.29 bd
Dylan	0.4 nq	0.305 jk	34.7 di	92.7 ab	37.4 be	71.5 ac	4.18 ab
Grazia	3.2 a	1.685 ad	37.0 ae	66.7 no	29.2 hi	69.5 af	1.07 j
Hathor *	0.1 pq	0.086 k	38.7 ac	71.3 jn	38.3 ad	67.1 cj	1.89 fj
Iride	0.7 mq	1.045 fh	29.0 mo	81.0 ci	35.0 cg	68.9 ah	3.47 bc
Isildur *	3.0 ab	1.732 ac	40.0 a	56.7 p	27.2 i	66.5 dj	1.57 gj
K26 *	1.5 fl	1.551 ae	35.7 bg	72.3 in	36.3 bf	68.9 ah	1.86 fj
Latinur *	1.7 ei	1.433 af	34.0 ej	69.3 lo	39.2 ad	67.3 bj	2.78 cf
Levante	1.2 hm	1.036 fh	34.0 ej	87.3 ad	34.8 dg	69.4 af	3.18 be
Meridiano	1.1 hm	1.095 eg	31.7 hm	85.7 af	35.3 cg	70.5 ae	4.15 ab
Neodur	0.9 ko	1.607 ad	35.3 cg	82.0 ch	38.5 ad	71.7 ac	3.33 bd
Neolatino *	1.1 hm	1.382 bf	29.3 lo	83.3 cg	38.6 ad	71.9 ab	3.28 bd
Normanno	1.3 hm	1.245 cf	34.0 ej	81.3 ch	39.3 ad	69.1 ag	2.57 cg
Orfeo *	1.5 ek	0.757 gi	36.3 bf	78.3 ek	42.7 a	63.3 j	1.21 ij
Orobel	3.0 ab	1.728 ac	39.0 ab	76.7 fm	32.3 fh	65.5 fj	1.40 hj
Pr22d40 *	3.3 a	1.779 ab	38.0 ad	58.3 p	29.6 hi	68.1 ai	1.55 gj
Pr22d89 *	2.2 ce	1.699 ad	34.7 di	75.3 gn	34.9 cg	71.7 ac	2.30 dh
Saragolla *	0.0 q	0.187 jk	30.3 ko	79.7 dj	34.9 cg	69.7 af	4.78 a
Sfinge *	1.2 hm	1.295 bf	27.7 o	78.0 el	37.9 bd	66.2 ej	3.12 ce
Simeto	2.8 ac	1.892 a	33.3 fk	68.3 mo	37.7 be	64.6 gj	1.97 fj
Solex	1.0 jn	1.060 fg	33.3 fk	81.7 ch	39.5 ac	70.1 af	2.70 cf
Vendetta	2.1 df	1.617 ad	32.7 gl	74.3 gn	37.1 be	63.7 ij	2.13 ei
Virgilio	2.0 dg	1.571 ae	33.3 fk	82.7 cg	36.0 bf	67.4 bj	2.35 dh
MEAN	**1.7**	**1.284**	**34.2**	**77.4**	**36.2**	**68.7**	**2.53**

[a] Symptom severity was scored Feb. 16, Feb. 21, March 12 and April 2; [b] DAS-ELISA was performed on extracts from 15 leaves collected Feb. 21 and March 12; [c] cultivars assayed for the first time are marked with asterisks; [d] within columns, means followed by the same letters are not significantly different (P= 0.05) according to Duncan's multiple range test.

Results and Discussion

Cultivars Saragolla and Hathor showed slight or no SBCMV symptoms throughout the season and extracts from their leaves gave relatively low ELISA values (0.187 and 0.086, respectively). Cultivars Asdrubal, Dario and Dylan too showed mild symptoms (≤ 1.2) and relatively low ELISA values (≤ 0.612). None of the above five cvs. had been assayed before, except "Dylan", classified as highly resistant in previous trials. Quite unexpectedly, cultivars Meridiano, Iride, Neodur, Levante and Duilio – all of which had shown high levels of SBCMV resistance in a number of previous trials characterized by a high disease pressure - presented in 2007 low symptom score means (0.7 – 1.2) and high grain yields (3.18 – 4.15 t/ha), y*et al*so relatively high ELISA values (1.046 – 1.607). This was the first time we found contrasting results between ELISA values in different seasons. Six of the cultivars assayed for the first time (Ariosto, Catervo, Chiara, K26, Neolatino and Sfinge) also showed mild symptoms yet high ELISA values, suggesting that these cultivars may express a notably higher degree of resistance in more typical seasons. Evidently, the resistance factors carried by these cvs. can be at least partially overridden under certain environmental conditions, perhaps as a consequence of prolonged cold spring temperatures, as was the case during 2007.

Table 2. Simple correlation coefficients between mean symptom severity, mean ELISA value and agronomic characters among 34 durum wheat cvs. grown in a field with SBCMV at Cadriano (Bologna) during 2006/07.

	ELISA value	Heading date	Plant height	Kernel weight	Test weight	Grain yield
Symptom severity	0.772**	0.514**	-0.704**	-0.614**	-0.237 n.s.	-0.719**
ELISA value	---	0.273 n.s.	-0.491**	-0.276 n.s.	-0.184 n.s.	-0.544**
* = significant at P = 0.05; ** = significant at P = 0.01.						

Among the 34 cvs assayed, the correlation between ELISA values and symptom scores was highly significant, and the same was found for the correlations between each of these two parameters and heading date, plant height, thousand-kernel weight and even grain yield, despite severe differential bird damage (Table 2). Regression analysis allowed to estimate the effects of SBWMV on cvs. with different disease severity scores (Table 3). As in all previous trials, cultivars with relatively mild symptoms were found to suffer lesser yet appreciable grain yield losses: about 14% in the case of cvs. with symptom severity scores below 1.0, and about 26% in cvs. with mean symptom severity scores between 1.0 and 2.0.

Table 3. Agronomic performance and estimated mean effects of SBCMV on 34 durum wheat cultivars with different disease severity grown in a field with SBCMV at Cadriano (Bologna) during 2006/07.

Disease severity score	No. of cvs.	Grain yield		Plant height reduction		Kernel weight reduction		Heading delay
		t/ha	% loss	cm	% loss	g	% loss	days
0.00 – 1.00	9	3.20	14 %	83.5	5 %	37.8	5 %	2.1
1.01 – 2.00	14	2.75	26 %	79.6	10 %	37.7	5 %	1.6
2.01 – 3.00	7	1.89	49 %	72.3	18 %	34.2	14 %	3.8
3.01 – 3.30	4	1.37	63 %	64.8	26 %	30.6	23 %	6.8

References

Canova, A. and Quaglia, A. (1960). Il mosaico del frumento. *Informatore Fitopatologico* **10**, 206-208.

Budge, G.E., Ratti, C., Rubies-Autonell, C., Lockley, D., Bonnefoy, M., Vallega, V., Pietravalle, S. and Henry, C. M. (2008). Response of UK winter wheat cultivars to *Soil-borne cereal mosaic* and *Wheat spindle streak mosaic viruses* across Europe. *Eur. J. Plant Pathol.* **120,** 259-272.

Clover, G.R.G., Wright, D.M. and Henry, C.M. (1999). Occurrence of *Soil-borne wheat mosaic virus* in the UK. In: *"Proceedings of the Fourth Symposium of the International Working Group on Plant Viruses with Fungal Vectors"* (J.L. Sherwood and C.M. Rush, Eds.), pp.105-108.

Jezewska, M. (1995). Detection of Polish isolate of *Wheat soil-borne mosaic virus* in cereal seeds. *Phytopathogia Polonica* **10**, 61-67.

Ratti, C., Pisi, A., Vallega, V. and Rubies-Autonell, C. (2005). Molecular characterization of Italian *soil-borne cereal mosaic virus* (SBCMV) isolates. *Parasitica* **61**, 11-16.

Ratti, C., Rubies-Autonell, C., Maccaferri, M., Stefanelli, S., Sanguineti, M.C. and Vallega, V. (2006). Reaction of 111 cultivars of *Triticum durum* Desf. from some of the world's main genetic pools to *Soil-borne cereal mosaic virus*. *J. Plant Dis. Protection* **113**, 145-149.

Rubies-Autonell, C. and Vallega, V. (l987). Observations on a mixed *Soil-borne wheat mosaic virus* and *Wheat spindle streak mosaic virus* infection in durum wheat. *J. Phytopathology* **119**, 111-121.

Rubies-Autonell, C., Vallega, V. and Ratti, C. (2003). Reactions of cultivars of common wheat (*Triticum aestivum* L.) to *Soil-borne wheat mosaic virus* in northern Italy during 1996-97. *J. Plant Dis. Protection* **110**, 332-336.

Rubies-Autonell, C., Ratti, C., Pisi, A. and Vallega, V. (2006). Behaviour of durum and common wheat cultivars in regard to *Soil-borne cereal mosaic virus* (SBCMV): results of fifteen trials. In: *"Proceedings of the 12th Congress of the Mediterranean Phytopathological Union"* (N. Paplomatas Ed.), pp. 100-102.

Toderi, G. (1969). Osservazioni agronomiche sul mosaico del grano trasmissibile attraverso il terreno. *Rivista di Agronomia* **3**, 178-182.

Vaianopoulos, C., Legreve, A., Moreau, V., Steyer, S., Maraite, H. and Bragard, C. (2005). Occurrence of bymo- and furoviruses on wheat in Belgium. *Parasitica* **61**, 47-54.

Vallega, V. and Rubies-Autonell, C. (1985). Reactions of Italian *Triticum durum* cultivars to *Soil-borne wheat mosaic*. *Plant Dis.* **69**, 64-66.

Vallega, V. and Rubies-Autonell, C. (1989). Ulteriori indagini sulla diffusione del virus del mosaico comune in Italia. *Informatore Fitopatologico* **39**, 57-58.

Vallega, V., Rubies-Autonell, C., Ratti, C. and Turina, M. (1997). Reactions of 36 common wheat cultivars grown in Italy to *Soil-borne wheat mosaic virus* during 1995-1996. In: *"Proceedings of the Tenth Congress of the Mediterranean Phytopathological Union"* (P.A. Signoret and H. Lapierre, Eds.), pp. 627-631.

Vallega, V., Rubies-Autonell, C., Turina, M., Ratti, C. and Contoli, S. (1999). Reactions of durum wheat cultivars to infections by SBWMV grown in northern Italy during 1995-96. *J. Plant Dis. Protection* **106**, 284-290.

Vallega, V., Rubies-Autonell, C. and Ratti, C. (2003). Reaction of durum wheat cultivars to mixed SBWMV and WSSMV infection in central Italy. *Phytopathologia Mediterranea* **42**, 177-182.

Vallega, V., Rubies-Autonell, C. and Ratti, C. (2006). Resistance to accumulation of *Soil-borne cereal mosaic virus* in eight cultivars of *Triticum durum* Desf. *Parasitica* **62**, 79-96.

REACTION OF SEVENTY-ONE CULTIVARS OF *TRITICUM DURUM* DESF. TO *WHEAT SPINDLE STREAK MOSAIC VIRUS* IN CENTRAL ITALY

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Summary

Field trials were carried out over three seasons in a field near Rome (Italy) with natural inoculum sources of *Wheat spindle streak mosaic virus* (WSSMV) to evaluate - using DAS (Double Antibody Sandwich) ELISA - the WSSMV-resistance of 71 cultivars of durum wheat (*Triticum durum* Desf.) marketed in Italy. Different sets of cvs. were assayed in each season, and the ELISA absorbance values obtained for single cvs. in each trial were indexed to allow comparisons between results obtained in different years. Cultivar Claudio, assayed over three seasons, showed the lowest overall mean ELISA index (0.5%), and thus appears – at present - the safest choice for soils with WSSMV in Italy. Relatively low ELISA indexes were observed also for cvs. Colorado, Rusticano, Provenzal, Tiziana, Italo, Ofanto and Dupri.

Introduction

Wheat spindle streak mosaic virus (WSSMV) was first detected in Canada (Slykhuis, 1970), and a similar virus denominated *Wheat yellow mosaic virus* (WYMV) - also transmitted by *Polymyxa graminis* Led. - has been identified in Japan (Inouye, 1969; Clover and Henry, 1999). Both WSSMV and WYMV often co-infect wheat with either *Soil-borne cereal mosaic virus* (SBCMV), *Soil-borne wheat mosaic virus*, or *Chinese wheat mosaic virus* (Chen *et al*., 2000; Lommel *et al*., 1986; Bonnefoy *et al*., 1994; Rubies-Autonell and Vallega, 1987). In Europe, WSSMV was first detected in France (Signoret *et al*., 1977) and subsequently in Belgium, Germany, Italy and Poland (Proeseler and Stanarius, 1983; Vaianopoulos, *et al*., 2006; Jezewska and Trzmiel, 2007). In Italy, WSSMV was first identified in a durum wheat field near Rome in 1985 and subsequently at about 20 other sites throughout the northern and central and regions of the country, either alone or in mixed infection with SBCMV (Rubies-Autonell and Vallega, 1987; Vallega *et al*., 2003). Despite the serious losses caused by WSSMV, very few cvs. of durum wheat and common wheat (*Triticum aestivum* L.) have been thus far assayed for resistance to this virus (Miller *et al*., 1992;

Bonnefoy *et al*., 1994; Vallega *et al*., 2003).

Materials and Methods

The trials, conducted in a field near Rome with natural inoculum sources of both WSSMV and SBCMV, comprised 43 cvs. in 1998/99, 44 cvs. in 2003/04 and 20 cvs. in 2006/07. Nine of these cvs. were grown over three seasons, 18 over two seasons, and 44 in one season only. The cvs. were grown in 10 m^2 solid-seeded plots, distributed in the field according to a randomized block design with either two (1999) or three (2004 and 2007) replicates. DAS (Double Antibody Sandwich) ELISA was performed as reported previously (Ratti *et al*., 2006) on extracts from a bulk of the second or third youngest leaves of 10 or 15 plants / plot collected March 18 (1999), March 16 (2003) and February 27 (2007).

Table 1. WSSMV ELISA values and WSSMV ELISA indexes for 71 cvs. of *Triticum durum* Desf. tested near Rome (Italy) in 1999, 2004 and 2007, and their known reaction to SBCMV.

Cultivar	WSSMV ELISA value[a]			WSSMV ELISA index[b]				SBCMV reaction[c]
	1999	2004	2007	1999	2004	2007	MEAN	
Tiziana	…	0.001c[d]	…	…	0.1	…	0.1	R
Claudio	0.004f	0.000c	0.018c	0.6	0.0	0.9	0.5	MS
Italo	0.010f	…	…	1.3	…	…	1.3	MR
Ofanto	0.032ef	…	…	4.4	…	…	4.4	MS
Dupri	0.039df	…	…	5.4	…	…	5.4	R
Colorado	0.114bf	0.031bc	…	15.6	6.8	…	11.2	R
Rusticano	0.037df	0.081ac	…	5.1	17.7	…	11.4	R
Provenzal	0.054df	0.075ac	…	7.4	16.4	…	11.9	R
Arcobaleno	0.046df	0.106ac	…	6.2	23.0	…	14.6	S
San Carlo	0.000f	…	0.690bc	0.0	…	35.1	17.5	R
Tresor	0.134bf	…	…	18.4	…	…	18.4	MR
Svevo	0.039df	0.124ac	0.674bc	5.3	27.0	34.3	22.2	R
Solex	0.091cf	0.154ac	…	12.4	33.5		22.9	R
Vinci	…	…	0.464bc	…	…	23.6	23.6	MS
Iride	0.000f	0.185ac	0.677bc	0.0	40.3	34.4	24.9	R
Avispa	…	0.118ac	…	…	25.6	…	25.6	R
Vetrodur	…	0.128ac	…	…	27.7	…	27.7	S
Elios	0.210bf	…	…	28.8	…	…	28.8	S
Creso	0.110bf	0.190ac	0.598bc	15.1	41.2	30.4	28.9	MS
Fortore	0.221bf	…	…	30.3	…	…	30.3	S
Ixos	0.227bf	…	…	31.2	…	…	31.2	MS
Pietrafitta	…	0.146ac	…	…	31.7	…	31.7	R
Canyon	…	0.295ac	0.021c	…	64.1	1.1	32.6	R
Cannizzo	…	0.159ac	…	…	34.5	…	34.5	S
Marco	…	0.162ac	…	…	35.3	…	35.3	MS
Quadrato	…	0.165ac	…	…	35.8	…	35.8	MR
Grazia	0.344af	0.279ac	0.023c	47.2	60.7	1.2	36.3	S
Settedue	…	0.168ac	…	…	36.5		36.5	unknown
Giemme	0.267af	…	…	36.7	…	…	36.7	MS
Ceedur	0.270af	…	…	37.0	…	…	37.0	R
Flaminio	0.272af	…	…	37.4	…	…	37.4	MR
Duilio	0.272af	0.116ac	0.979ac	37.3	25.1	49.8	37.4	R
Nefer	0.150bf	0.275ac	…	20.6	59.7	…	40.1	R
Mongibello	0.295af	…	…	40.4	…	…	40.4	MR
Simeto	0.053df	0.379ac	0.683bc	7.2	82.4	34.7	41.5	MS
Lesina	…	0.193ac	…	…	41.9	…	41.9	unknown
Orobel	…	0.243ac	0.689bc	…	52.7	35.0	43.9	MS
Gargano	0.348af	0.188ac	…	47.7	40.8	…	44.3	S
Preco	…	0.204ac	…	…	44.3	…	44.3	R
Baio	0.420af	0.148ac	…	57.7	32.1	…	44.9	R
Platani	0.285af	0.262ac	…	39.1	56.9	…	48.0	MS
Bronte	0.351af	…	…	48.2	…	…	48.2	S
Cappelli	…	…	0.959ac	…	…	48.8	48.8	unknown
Meridiano	…	0.261ac	0.810ac	…	56.6	41.2	48.9	R
Gianni	0.114bf	0.393ac	…	15.6	85.4	…	50.5	R
Neodur	0.523ad	0.166ac	…	71.8	36.1	…	54.0	R

Table 1 (contd.)

Cultivar	WSSMV ELISA value[a]			WSSMV ELISA index[b]				SBCMV reaction[c]
	1999	2004	2007	1999	2004	2007	MEAN	
Poggio	0.406 af	…	…	55.7	…	…	55.7	S
Parsifal	0.409 af	…	…	56.1	…	…	56.1	R
Dylan	…	…	1.116 ac	…	…	56.7	56.7	R
Vesuvio	…	0.264 ac	…	…	57.4	…	57.4	S
Valbelice	0.509 ae	0.208 ac	…	69.8	45.2	…	57.5	S
Ionio = Ares	0.471 af	0.247 ac	…	64.6	53.6		59.1	R
Portorico	…	0.275 ac	…	…	59.7	…	59.7	MS
Derrick	…	0.277 ac	…	…	60.2	…	60.2	MS
Normanno	…	…	1.193 ac	…	…	60.7	60.7	R
Saadi'	0.444 af	…	…	61.0	…	…	61.0	S
Valnova	0.478 af	0.278 ac	…	65.6	60.5	…	63.0	S
Torrebianca	…	0.310 ac	…	…	67.4	…	67.4	MR
Vendetta	…	…	1.333 ab	…	…	67.8	67.8	MR
Colosseo	0.418 af	0.362 ac	1.348 ab	57.4	78.6	68.5	68.2	MS
Karalis	…	…	1.357 ab	…	…	69.0	69.0	unknown
Varano	0.522 ae	…	…	71.6	…	…	71.6	S
Verdi	…	0.331 ac	…	…	72.0	…	72.0	MR
Lloyd	0.526 ad	…	…	72.1	…	…	72.1	R
Nerone	0.544 ac	…	…	74.6	…	…	74.6	S
Valsalso	…	0.353 ac	…	…	76.7	…	76.7	R
Giotto	…	0.359 ac	…	…	77.9	…	77.9	MR
Cirillo	0.594 ab	…	…	81.6	…	…	81.6	S
Saragolla	…	…	1.913 a	…	…	97.3	97.3	R
Ciccio	0.729 a	0.443 ab	1.967 a	100.0	96.2	100.0	98.7	MS
Vitomax	…	0.460 a	…	…	100.0	…	100.0	R
MEAN	**0.265**	**0.217**	**0.876**					
MIN	**0.000**	**0.000**	**0.018**					
MAX	**0.729**	**0.460**	**1.967**					

[a]DAS-ELISA was performed on extracts from 10-15 leaves collected March 18 (1999), March 16 (2003) and Feb. 27 (2007) from two (1999) or three (2004 and 2007) field replicates; [b]percent of the ELISA value recorded on the most susceptible cv. in each season; [c]from Rubies-Autonell *et al*., 2006 and unpublished; [d]within columns, means followed by the same letters are not significantly different (P= 0.05) according to Duncan's multiple range test.

Results and Discussion

The ELISA absorbance values obtained for each cv. in different years are presented in Table 1, where ELISA values are given also as percentage of the value obtained for the most susceptible cv. in each season. Statistically significant differences (P = 0.05) between cvs. were found in each of the three trials. Correlations between ELISA values in different seasons, however, were relatively low; significant for the 23 cvs. tested both in 1999 and 2004 (r = 0.483*) as well as for the 10 cvs tested both in 1999 and 2007 (r = 0.710**), but not for the 12 cvs. tested both in 2004 and 2007 (r = 0.527).

Cultivar Claudio, assayed over three seasons, showed the lowest overall mean

ELISA index (0.5%), and therefore appears – at present - the safest choice for soils with WSSMV in Italy. Relatively low ELISA indexes were observed also for cvs. Colorado (11.2%), Rusticano (11.4%) and Provenzal (11.9%) – tested in two seasons – and for cvs. Tiziana (0.1%), Italo (1.3%), Ofanto (4.4%) and Dupri (5.4%), assayed one season only. Experiments carried out in 1989 on only eight cvs. suggested that resistance to WSSMV and resistance to SBCMV may be correlated (Rubies-Autonell and Vallega, 1991), but this hypothesis has been disproved by our subsequent results on 67 cvs. tested for resistance to both viruses (Table 1).

References

Bonnefoy, M., Boursereau, J. and Chesneau, R. (1994). Comportment des variétés face aux virus de la mosaique du blé et de la mosaique jaune du blé. In *"Proceedings Reunion sur Mosaiques des céréales trasmises par Polymyxa graminis Led."* (ITCF Ed.), pp. 57-62.

Chen, J., Chen, J.-P., Yang, J., Cheng, Y., Diao, A., Adams, M.J. and Du J. (2000). Differences in cultivar response and complete sequence analysis of two isolates of *Wheat yellow mosaic bymovirus* in China. *Plant Pathology* **49**, 370-374.

Clover, G. and Henry, C. (1999). Detection and discrimination of *Wheat spindle streak mosaic virus* and *Wheat yellow mosaic virus* using multiplex RT-PCR. *Eur. J. Plant Pathol.* **105**, 891-896.

Inouye, T. (1969). Filamentous particles as the causal agent of yellow mosaic disease of wheat. *Nogaku Kenyu* **53**, 61-68.

Jezewska, M. and Trzmiel, K. (2007). First report on the occurrence of *Wheat spindle streak mosaic virus* in triticale in Poland. *Phytopatologia Polonica* **44**, 51-53.

Lommel, S.A., Willis, W.G. and Kendall T.L. (1986). Identification of *Wheat spindle streak mosaic virus* and its role in a new disease of winter wheat in Kansas. *Plant Dis.* **70**, 964-968.

Miller, N.R., Bergstrom, G.C. and Sorrells, M.E. (1992). Effect of *Wheat spindle streak mosaic virus* on yield of winter wheat in New York. *Phytopathology* **82**, 852-857.

Proeseler, G. and Stanarius, A. (1983). Nachweis des Weizenspindelstrichelmosaikvirus (*Wheat spindle streak mosaic virus*) in der DDR. *Arch. Phytopath. Pflanzenschutz* **19**, 345-349.

Ratti, C., Rubies-Autonell, C., Maccaferri, M., Stefanelli, S., Sanguineti, M.C. and Vallega, V. (2006). Reaction of 111 cultivars of *Triticum durum* Desf. from some of the world's main genetic pools to *Soil-borne cereal mosaic virus*. *J. Plant Dis. Protect.* **113**, 145-149.

Rubies-Autonell, C. and Vallega, V. (1987). Observations on a mixed *Soil-borne wheat mosaic virus* and *Spindle streak mosaic virus* infection in durum wheat. *J. Phytopathology* **119**, 111-121.

Rubies-Autonell, C. and Vallega, V. (1991). Studies on the development and interaction of *Soil-borne wheat mosaic virus* and *Spindle streak mosaic virus*. In *"Biotic interactions and soil-borne diseases"* (A.B.R. Beemster, G.J. Bollen, M. Gerlach, M.A. Ruissen, B. Schippers and A. Tempel Eds.), pp. 107-112. Elsevier Scientific Publishers, Amsterdam.

Rubies-Autonell, C., Ratti, C., Pisi, A. and Vallega, V. (2006). Behaviour of durum and common wheat cultivars in regard to *Soil-borne cereal mosaic virus* (SBCMV): results of fifteen trials. In *"Proceedings of the 12th Congress of the Mediterranean Phytopathological Union"* (N. Paplomatas Ed.), pp. 100-102.

Signoret, P.A., Alliot, B. and Poinso, B. (1977). Présence en France du *Wheat spindle streak mosaic virus*. *Ann. Phytopathologie* **9**, 377-379.

Slykhuis, J.T. (1970). Factors determining the development of Wheat spindle streak mosaic caused by a soilborne virus in Ontario. *Phytopathology* **60**, 319-331.

Vaianopoulos, C., Legreve, A., Lorca, C., Moreau, V., Steyer, S., Maraite, H. and Bragard C. (2006). Widespread occurrence of *Wheat spindle streak mosaic virus* in Belgium. *Plant Dis.* **90**, 723-728.

Vallega, V., Rubies-Autonell, C. and Ratti, C. (2003). Reaction of durum wheat cultivars to mixed SBWMV and WSSMV infection in central Italy. *Phytopath. Medit.* **42**, 177-182.

INVESTIGATION OF FUROVIRUS VIRULENCE IN WINTER BARLEY

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Summary

The ability of the furoviruses *Soil-borne cereal mosaic virus* (SBCMV) and *Soil-borne wheat mosaic virus* (SBWMV) to infect different genotypes of winter barley was investigated under natural and controlled conditions. SBCMV was not found in field grown plants but could be detected with low incidence and concentration in seedlings which were cultivated in a growth chamber and inoculated via infested soil or mechanically by leaf rubbing. In contrast, the German isolate of SBWMV was able to infect many but not all of the tested genotypes under field conditions. It has to be considered as a new pathogen for winter crops of wheat, rye and triticale in Europe with a potential negative impact on winter barley also.

Introduction

Cereal crops can be affected by several soil-borne viruses, which are all transmitted by the obligate root parasite *Polymyxa graminis* Ledingham. The furoviruses are economically most important particularly for winter crops of wheat, rye and triticale (Kühne, 2009), whilst the production of winter barley so far is being threatened exclusively by the bymoviruses *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) (Huth and Lesemann, 1978; Huth and Adams, 1990). However, according to McKinney (1948) this species can become infected under natural conditions by SBWMV also. In Europe where SBCMV is widely distributed and has been known already for several decades, SBWMV was detected only recently (Koenig and Huth, 2003). The infested field is located in Southwest Germany in the area of Heidelberg (Heddesheim, Baden-Württemberg). Based on our experience with other soil-borne viruses, further dissemination of SBWMV must be expected and the existence of additional still undetected contaminated areas cannot be ruled out. Therefore, the reaction of different barley genotypes to both SBWMV and SBCMV was studied in more detail.

Material and Methods

Over a three year period 50 varieties and genebank accessions of winter barley were grown in the SBWMV-infested field near Heddesheim. All 16 currently known genes conferring resistance to BaYMV and/or BaMMV were present in this assortment. Selected varieties were additionally field tested for reaction against SBCMV at two locations in Saxony-Anhalt (Gödnitz and Walternienburg). At the same time, transmission experiments using either naturally contaminated soil or mechanical virus inoculation were performed in a growth chamber at 17 °C. Plants were analysed for virus infection by DAS-ELISA using poly- and monoclonal antibodies (Rabenstein *et al.*, 2005), IC-RT-PCR and immunosorbent electron microscopy including decoration.

Results and Discussion

From 2005 to 2008 a collection of 50 genotypes of winter barley (cultivars, genetic resources) was cultivated under natural conditions in SBWMV-infested soil. During the spring the plants were repeatedly analysed for the presence of the virus. Infected plants displayed clear symptoms on leaves; particles were visible in the electron microscope and PCR amplicons of corresponding size could be visualised in agarose gels (Fig. 1). Selected results are presented in Table 1. Concluding from the percentage of infected plants per genotype and the mean extinction values of ELISA analyses, the genotypes clearly differ in their reaction to SBWMV. This behaviour obviously did not correlate with resistance properties to BaMMV and BaYMV. In a next step 5 varieties having proved to be susceptible to SBWMV were tested in parallel experiments against SBWMV in Heddesheim and SBCMV in Gödnitz and Walternienburg. Susceptible lines of winter rye, winter triticale and winter wheat served as controls. While SBWMV was clearly identified again in both barley and control plants, the SBCMV could be detected in wheat, rye and triticale but in none of the barley plants (Table 2).

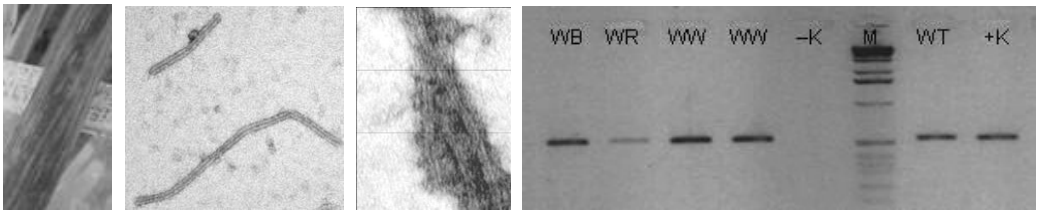

Fig.1.Detection of SBWMV in field plants of winter barley variety ‘Maris Otter’ in the region near Heddesheim (From left to right: Leaf symptoms, non decorated particles, decorated particles, virus specific amplicons in winter barley (WB), winter rye (WR), winter wheat (WW) and winter triticale (WT), -K – healthy plant, +K – lyophilized infected plant.

Table 1. Reaction of different *Hordeum vulgare* accessions resistant to barley bymoviruses in soil infested with SBWMV (Heddesheim)

***Hordeum vulgare* accessions with resistance genes to bymoviruses**	***rym* - genes**	**SBWMV infection (% infected plants and virus extinction)**							
		March 08		**March 07**		**April 07**		**April 06**	
		%	$E_{(405)}$	%	$E_{(405)}$	%	$E_{(405)}$	%	$E_{(405)}$
Mokusekko 3	**1**	60	**0.7**	0	0	0	0	70	**1.3**
Mihori Hadaka 3	**2**	70	**0.7**	0	0	30	**0.3**	100	**1.5**
Ea52	**3**	10	**0.3**	10	0.1	0	0	0	0
Ischuku Shirazu	**3**	0	0	10	0	0	0	n.t.	n.t.
Resistant ym No1	**5**	0	0	20	**0.1**	30	**0.1**	n.t.	n.t.
Misato Golden	**5+6**	n.t.	n.t.	30	**0.1**	50	**0.4**	n.t.	n.t.
HHOR3365	**7**	10	0,1	0	0,0	0	0	20	0.1
10247	**8**	0	0	30	**0.2**	30	**0.2**	n.t.	n.t.
Bulgarian 347	**9**	0	0	0	0	0	0	70	**0.2**
Hiberna	**10**	0	0	0	0	0	0	50	**0.3**
Russia 57	**11**	0	0	0	0	0	0	40	0.1
Muju covered 2	**12**	50	**0.7**	30	**0.2**	30	**0.3**	100	**2.2**
Taihoku A	**13**	10	**0.2**	0	0	0	0	20	**0.2**
BAZ-4006*	**14**	0	0	0	0	0	0	70	**0.2**
Chikurin Ibaraki 1	**3+15**	n.t.	n.t.	90	**0.8**	60	**0.4**	50	**0.1**
BAZ-5061*	**16**	60	**0.3**	0	0	60	**0.3**	60	**0.1**

** Rym14* and *Rym16* are dominant genes; n.t. – not tested

Surprisingly, seedlings of the variety 'Uschi' became weakly but systemically infected by SBCMV when they were cultivated in infested soil from Walternienburg in a growth chamber at 17 °C. This did not happen with soil from Gödnitz. The observed rate of about 30 % virus containing plants rapidly dropped down to about 10 % when the intensive growth of the plantlets started in early spring (Fig. 2). Mechanical inoculation of barley, wheat, triticale and rye with leaf sap from SBWMV-infected barley plants resulted in high infection rates of test plants. With the exception of barley similar infection rates were obtained with a SBCMV-containing inoculum (Fig. 3.).

Table 2. Virulence of different furovirus populations to cereal crops under field conditions.

Genotypes		**DAS-ELISA (E_{405})**		
Culture	Cultivar	SBWMV	SBCMV	
		Heddesheim	Gödnitz	Walternienburg
Winter barley	Uschi	**0,4**	0,0	0,0
	Mokusekko 3	**0,3**	0,0	0,0
	Mihori Hadaka 3	**1,5**	0,0	0,0
	Muju covered 2	**2,0**	0,0	0,0
	Taihoku A	**0,4**	0,0	0,0
Winter rye		**0,5**	**2,8**	**2,5**
Winter triticale	susceptible lines	**2,0**	**2,7**	**2,2**
Winter wheat		**1,5**	**1,7**	**1,9**

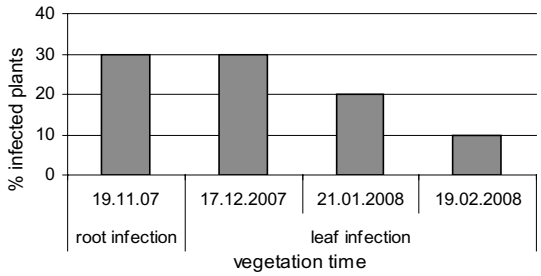

Fig. 2. Time course of SBCMV infection in winter barley ('Uschi') during the cultivation period November – February.

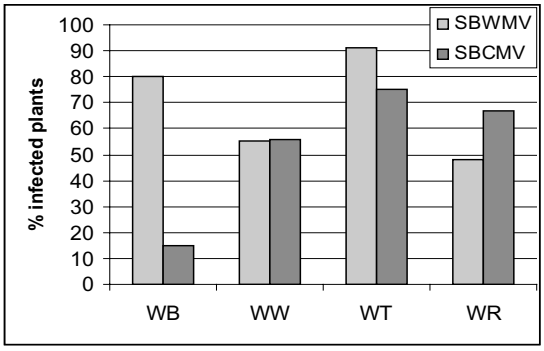

Fig. 3. Infection rates of SBCMV and SBWMV in winter barley (WB), winter wheat (WW), winter triticale (WT) and winter rye (WR) after mechanical virus transmission

In summary, SBWMV but not SBCMV can infect barley with high virulence under field conditions in Germany (Kastirr *et al.,* 2006). This has to be taken into consideration for the development of new varieties in future. There is a pronounced variation in susceptibility in the gene pool, which offers a good opportunity to select appropriate resistance sources.

References

Huth W. and Lesemann D.E. (1978). Eine für die Bundesrepublik neue Virose an Wintergerste. *Nachrichtenbl. Deut. Pflanzenschutzd*. **30**, 184-185.

Koenig R. and Huth W. (2003). Natural infection of wheat by the type strain of *Soil-Borne wheat mosaic virus* in a field in Southern Germany. *Eur. J. Plant Pathol.* **109**, 191-193.

Kastirr, U., Wortmann, H. and Ehrig, F. (2006). Untersuchungen zum Infektionsverlauf und zur biologischen Differenzierung von bodenbürtigen Viren in Roggen, Triticale und Weizen. *Ges. Pflanzen* **58**, 231-238.

Kühne, T. (2009). Soil-borne viruses affecting cereals – known for long but still a threat. *Virus Res.* (in press).

McKinney H.H. (1948). Wheats immune from *Soil-borne mosaic viruses* in the Field, susceptible when inoculated manually. *Phytopathology* .**38**, 1003-1013.

Rabenstein F., Mühlheim H., Kastirr U. and Kühne T. (2005). Monoclonal antibodies for differentiation between *Soil-borne cereal mosaic virus* and *Soil-borne wheat mosaic virus.* In: *Proceedings of the Sixth Symposium of the International Working Group on Plant Viruses with Fungal Vectors*, ed. Rush, C.M., 2005, Bologna, Italy, 53-56.

THE ACCUMULATION OF *BEET NECROTIC YELLOW VEIN VIRUS* IN ROOTS OF MECHANICALLY INOCULATED, PARTIALLY RESISTANT SUGAR BEET SEEDLINGS IS GREATLY INCREASED BY A SINGLE U/C NUCLEOTIDE SUBSTITUTION CHANGING ALANINE TO VALINE IN THE VIRAL P25 PROTEIN

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Summary

The European A type *Beet necrotic yellow vein virus* (BNYVV) isolates E12 and S8 served as starting material for studying the importance of different BNYVV genome portions for the virulence of the virus in partially resistant sugar beet cultivars. The two isolates originated from areas where resistance-breaking had (E12) or had not (S8) been observed, respectively. Their RNAs 1 as well as their RNAs 2 are closely related (99.9% sequence identity for each RNA species), but somewhat more distantly related to RNA 1 and 2 of B type, P type and East Asian A type isolates. Subisolates containing only RNAs 1 and 2 of the two isolates were obtained by serial local lesion passages; biologically active cDNA clones were produced for their RNAs 3. The latter differed in their coding sequences for the amino acids in positions 67, 68 and 129 of P25, the major RNA 3-encoded protein. RNA 1+2/RNA 3 pseudorecombinants were introduced in the absence of the vector *Polymyxa betae* into young sugar beet seedlings by means of a 'vortexing' procedure. The origin of RNAs 1+2 in the pseudorecombinants had no appreciable influence on the amount of virus accumulating in the roots of the beets which carried either only the *Rz1* or both the *Rz1* and the *Rz2* resistance genes. The E12 RNA 3 coding for $V_{67}C_{68}Y_{129}$ P25, however, enabled a much higher virus accumulation than the S8 RNA 3 coding for $A_{67}H_{68}H_{129}$ P25. Further studies with mutated RNAs 3 revealed that only the G<u>U</u>U codon for valine in position 67 of P25 (as opposed to the GCU codon for alanine) was responsible for this increased virus accumulation in the two partially resistant varieties.

Introduction

Several authors have suspected a correlation between the composition of the highly variable amino acid tetrad in positions 67 – 70 of the *Beet necrotic yellow vein virus* (BNYVV) RNA 3-encoded P25 and the pathogenicity of the

virus for partially resistant sugar beet varieties. Their conclusions were based on experiments either with plantlets grown in various soils (Liu and Lewellen, 2007; Acosta-Leal and Rush, 2007) or with sugar beet leaves inoculated mechanically with various BNYVV isolates (Chiba *et al*., 2008). In order to be able to study the pathogenicity of BNYVV isolates or pseudorecombinants for sugar beet roots in the absence of additional effects which may be exerted by the vector *Polymyxa betae* and possibly by additional pathogens in soil samples, we have developed a mechanical inoculation procedure in which young sugar beet seedlings are 'vortexed' in a highly concentrated virus inoculum. To investigate the influence of various parts of the BNYVV genome on viral pathogenicity, biologically active RNA 3 cDNA clones were produced for two BNYVV isolates coming from areas where resistance-breaking had or had not been observed, respectively; wild type or mutated forms of these RNAs 3 were recombined with RNAs 1 and 2 of these isolates obtained by serial single lesion passages. A detailed account of this work has been given elsewhere (Koenig *et al*., 2009*).*

Materials and Methods

The E12 and S8 isolates of BNYVV came from areas in Sweden and Spain where resistance-breaking had or had not been observed, respectively. They served as starting material to obtain in nine successive local lesion passages on *Chenopodium quinoa* subisolates which contained only their RNAs 1+2. Together with biologically active cDNA clones of their wild type RNAs 3 or mutated forms of them these subisolates were used to produce different RNA 1+2/RNA3 pseudorecombinants in two passages on *C. quinoa*. For the first passage 120 local lesions of the only RNA 1+2-containing S8 or E12 subisolates were ground in a mortar with 2.8 ml of 50 mM Na/K phosphate buffer pH 7.2 and mixed with 400 µl of an aqueous solution containing c. 400 µg of one of the RNA 3 cDNA plasmids. These mixtures were rubbed on the leaves of *C. quinoa* which had been in the eight to ten leaf stage and from which all but four leaves on each plant were removed. After ca. 10 to 14 days bright yellow and pale green lesions were readily distinguished on these leaves. Only the bright yellow lesions were used to prepare the inoculum for the second passage on *C. quinoa*, because only they contain the plasmid-derived RNA 3. After about 10 days many yellow lesions appeared on the inoculated leaves of this second passage. Only those areas from each leaf which were entirely covered with local lesions were used to prepare the inoculum for sugar beet seedling inoculation (Fig. 1A; Koenig and Stein, 1990). The sap expressed from this local lesion material was mixed with three volumes of 50 mM phosphate buffer pH 7.2 and was used immediately for the inoculation of seven day old sugar beet seedlings which were placed (twenty at a time, Fig. 1B, C and D) in a centrifuge tube (diameter 2.2 cm, height ca. 9 cm) together with 90 mg carborundum and 4 ml of the diluted plant sap. The tubes were 'vortexed' twice for 30 seconds with a 60 second interval at a medium speed (Vortex Genie, speed setting 3) (Fig. 1D). The inoculated plantlets were

left for further five minutes in the inoculation mix. and were then washed in a beaker containing c. 1 l of a nutrient solution consisting of 0.5 g/l FERTY® 3 green (http://www.plantafert.com/frameset1280.htm) (Fig. 1E). For hydroculture the plantlets were grown in the same nutrient solution in dark plastic boxes (c. 34 x 12 x 9 cm). The boxes were covered with plastic lids containing 39 holes into which the inoculated seedlings of the two cultivars were planted in an alternate arrangement by means of rings of rubber foam (Fig. 1E and F). One hole in each box was not used for plantlets, but rather for refilling the boxes with the nutrient solution every three to four days. The plantlets were harvested after about 18 to 20 days (Fig. 1F). The roots of each variety and box (19 plantlets each) were homogenized together in 30 times their weight of ELISA sample buffer (v/w). For each pseudorecombinant at least five hydroculture boxes were used and the average ELISA readings were determined for the plantlets harvested from each of these five boxes.

Results and Discussion

The almost complete sequences were determined for RNAs 1 and 2 of the E12 and S8 isolates. These first almost complete sequences to be reported for European A type RNA 1 and RNA 2 proved to closely related (99.9% sequence identity for each RNA species). They were somewhat more distantly related to the corresponding sequences of B type, P type and East Asian A type BNYVV isolates (data not shown). In pathogenicity tests, done with the sugar beet cultivars A containing both the *Rz1* and the *Rz2* resistance gene and B containing only the *Rz1* resistance gene, the origin of RNAs 1+2 in the pseudorecombinants had no appreciable effect on the amount of virus accumulating in the roots of the inoculated beets (data not shown). The E12 RNA 3 coding for $V_{67}C_{68}Y_{129}$ P25, however, enabled in both cultivars a much higher virus accumulation than the S8 RNA 3 coding for $A_{67}H_{68}H_{129}$ P25 (Fig. 2, columns 1 to 4). This was obviously not due to the Y in position 129 of P25, because the
RNA 3 mutant S8 VCHG, in which the coding sequence for the AHHG tetrad had been exchanged by that for a VCHG tetrad, enabled the same high virus accumulation in roots of both beet cultivars roots as did the E12 RNA 3 despite the fact that it has an H rather than a Y in position 129 (Fig. 2, columns 1, 2, 5 and 6). With the mutant E12-ACHG, on the other hand, the same low virus accumulation was observed as with the wild type S8-AHHG-RNA 3 (Fig. 2, columns 3, 4, 7, 8) suggesting that it must be the valine in P25 position 67 (or its G<u>U</u>U codon, respectively) as opposed to the alanine in this position (or its G<u>C</u>U codon) which enables the greatly increased virus accumulation in partially resistant sugar beet cultivars. The higher virus concentrations observed with the V_{67} P25 producing pseudorecombinants in

Fig. 1. Various steps in the mechanical inoculation of sugar beet with BNYVV by means of the 'vortexing' method – for explanations see text.

beets was not due to a possibly higher virus concentration in the inoculum, because by means of ELISA it was found that the virus concentration in the inocula for all four recombinants shown in Fig. 2 was either very similar or in some experiments somewhat lower for the VCHG P25-producing recombinants which tended to form fewer local lesions on *C. quinoa*. Despite of this apparently somewhat lower pathogenicity for *C. quinoa*, the virus accumulation in beet roots was always much higher when RNA 3 with a coding sequence for VCHG was present. These experiments show for the first time that the highly variable amino acid tetrad in

pos. 67 - 70 of BNYVV has a direct influence on BNYVV accumulation in roots of partially resistant beets, also in the absence of the vector *P. betae* and possibly other pathogens in the soil. In a few experiments we planted the inoculated seedlings in soil after about one week of hydroculture. Many, though not all of the plants inoculated with the pseudorecombinant containing E12-VCHG RNA 3 developed strong leaf symptoms, whereas those inoculated with the S8-AHHG RNA 3 did not (Fig. 1G). Our results are in line with observations on field grown beets reported by Acosta-Leal and Rush (2007) who considered $V_{67}E_{135}$ as the signature for resistance breaking variants of BNYVV from the Imperial Valley, as opposed to the wild type motif $A_{67}D_{135}$. The BNYVV RNAs 3 used in our experiments all contained D_{135}. So it remains to be shown whether this amino acid would also contribute to an increased virus accumulation in the roots of partially resistant beet cultivars and to resistance-breaking.

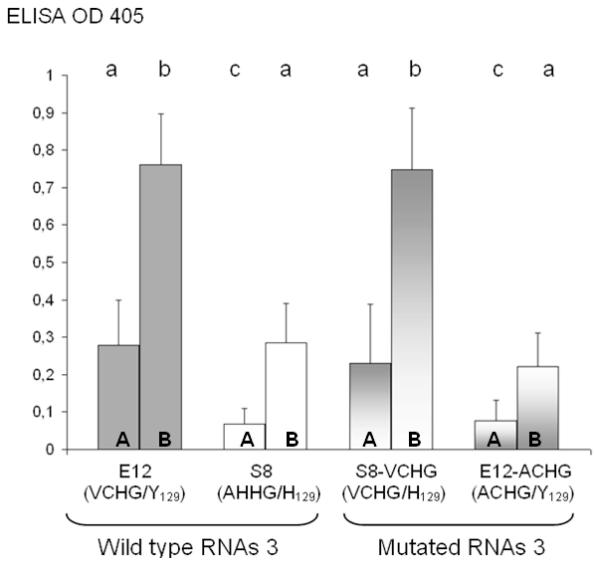

Fig. 2. Average ELISA readings obtained with the rootlets of sugar beet seedlings of cultivars A (*Rz1+Rz2*-mediated resistance) and B (only *Rz1*-mediated resistance) after mechanical inoculation with pseudo-recombinant BNYVV containing RNA 1+2 of the E12 isolate and either wild type RNA 3 of the S8 or E12 isolates or the RNA 3 mutants S8-VCHG or E12-ACHG, respectively. Standard errors are indicated as bars. Means labelled with different lower case letters indicate a significant difference between treatments (Multiple comparison for the effect of cultivar*virus, $P<0.05$, Tukey-Test).

Acknowledgement: The financial support given by the German Federal Ministry of Economics and Technology (AiF Grant 14163 N72, GFP BR42/04) is gratefully acknowledged.

References

Acosta-Leal, R. and Rush, C. M. (2007). *Phytopathology* **97**, 325-330.

Chiba, H., Miyanishi, M., Andika, I.B. and Tamada, T. (2008). *J. Gen. Virol.* **89**, 1314-1323.

Koenig, R. and Stein, B. (1990). *Schriftenreihe Deutsche Phytomedizinische Gesellschaft* 1*, Proceedings of the First Symposium of the International Working Group on Plant Viruses with Fungal Vectors, Braunschweig, Aug 1990,* pp. 87-89. Stuttgart, Ulmer Verlag.

Koenig, R., Loss, S., Specht, J., Varrelmann, M., Lüddecke, P. and Deml, G. (2009). *J. Gen. Virol.* (in press).

Liu, H.-Y. and Lewellen, R. T. (2007). *Plant Dis.* **91**, 847-85.

SOIL DIAGNOSIS BY DETECTION OF *POTATO MOP TOP VIRUS* USING BAIT PLANT BIOASSAY AND RT-PCR-MICROPLATE HYBRIDIZATION

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Summary

For the first time in 25 years, an incidence of 'spraing' (brown rings or arcs) caused by *Potato mop top virus* (PMTV) has affected potatoes (cv. Sayaka) in a field within Tokachi district of Hokkaido, the northernmost island of Japan in November 2005. In order to study the epidemic of PMTV, we developed a soil diagnosis method that consisted of bait plant bioassay for trapping the vector of PMTV, *Spongospora subterranea*, a causal agent of powdery scab of potatoes, and reverse transcription–polymerase chain reaction (RT-PCR)-microplate hybridization (MPH) for detecting PMTV from the roots of bait plants. With our method, PMTV was reliably detected from the soil sampled in the field from which some of the harvested tubers clearly exhibited the typical symptom of spraing. The soil diagnosis of 224 fields located within about 7 km radius from the spraing-occurred field resulted that 137 fields (61.2%) have already been infested by PMTV although tubers harvested from only one of these fields had spraing.

Introduction

Spraing of potato (*Solanum tuberosum*), characterized by brownish arcs and rings on the surface and tuber flesh, is caused by *Potato mop top virus* (PMTV), the type species of the genus *Pomovirus*. Spraing was first recorded by Calvert and Harrison (1966) in Scotland and northern Ireland and then reported from various parts of European countries, south America, China, Canada and recently in the USA. In Japan, the first report of spraing was made by Imoto *et al 1986* on cv. Norin No.1 for autumn crop in Hiroshima in 1980, but no incidence was recorded for 25 years until the second outbreak in Tokachi, Hokkaido, northern Japan in 2005 (Maoka *et al.*, 2006). PMTV is known to be transmitted by the

Plasmodiophoraceae fungus, *Spongospora subterranea* f.sp. *subterranea*, the causal agent of potato powdery scab. To prevent the expansion of spraing-affected areas, we must accurately detail the distribution of PMTV. In this paper, we describe results from a soil survey on the distribution of PMTV using a bait plant bioassay combined with reverse transcription–polymerase chain reaction-microplate hybridization (RT-PCR-MPH).

Materials and Methods

Soil samples were collected from the cropping layer (5 to 10 cm below the surface) in 224 fields in Tokachi, Hokkaido in spring 2006. The collected soil was mixed thoroughly and air-dried at room temperature then stored at 4ºC. Tomato plants (*Solanum lycopersicum* cv. Momotaro) were sown and grown on a commercial medium at 23ºC and 16-hr photoperiod for 3 weeks. Seedlings were transplanted and pregrown in transparent plastic containers (4 plants per container) filled with a nutrient solution [2.5-fold to original description (Merz, 1989)] at 18ºC for 7 days. The nutrient solution was then replaced with a soil suspension prepared by suspending soil (50 g) in 200 mL of the nutrient solution. Seedlings were incubated at 18ºC for 9 days. The plants were then collected, and the roots (50 to 100 mg), which had been submerged in the suspension to ca. 1.5 cm were excised from the plants. Total RNA was extracted from the root pieces using TRIzol reagent (Invitrogen Japan K.K.) and finally RNA was dissolved in RNase-free water (50 µL).

The RT reaction mixture (10 µL) consisted of 2 µL of 2.5 mM dNTPs (Takara Bio Inc.), 1 µL of 12.5 µM random nonamer (Takara Bio Inc.), 1 µL of 0.1 M dithiothreitol (Invitrogen Japan K.K.), 2 µL of 5× RT buffer (Invitrogen Japan K.K.), 0.5 µL of M-MLV RTase (200 U/µL; Invitrogen Japan K.K.), 1.5 or 2.5 µL of RNase free water and 2 or 1 µL of RNA solution. RT reaction was performed using a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems Japan Ltd.) at 30ºC for 10 min, 42ºC for 30 min, 99ºC for 5 min, and they were then stored at 10ºC.

PCR reaction mixture contained 2 µL of 2.5 mM dNTPs (Takara Bio Inc.), 0.5 µL each of 10 µM forward (PMTV-9P; 5'-GCTTGATCCAGAAGTCATTAAGG-3') and reverse (PMTV-9M; 5'-CCTGGAAGCACCAATACTTAACG-3') primers (Hataya *et al.*, 2006), 2.5 µL of 10× PCR buffer (Takara Bio Inc.), 0.125 µL of *Taq* polymerase (5 U/µL; *TaKaRa EX Taq*, Takara Bio Inc.) and 18.375 µL of sterilized distilled water. First strand cDNA (1 µL) was added to the mixture (24 µL), and PCR amplification was performed using a thermal cycler with initial denaturation at 94ºC for 4 min 30 s; 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 1 min, extension at 72ºC for 1 min; and an extension at 72ºC for 4 min. The products were then stored at 10ºC.

RT-PCR products were subjected to hybridization reaction with a digoxigenin-labeled cDNA probe for the target region of PMTV RNA in a microplate basically as described in the literature (Hataya *et al.*, 1994). Absorbance at 405 nm was

measured and the sample was diagnosed as positive (PMTV-infested) when the absorbance value exceeded 0.1.

Results and Discussion

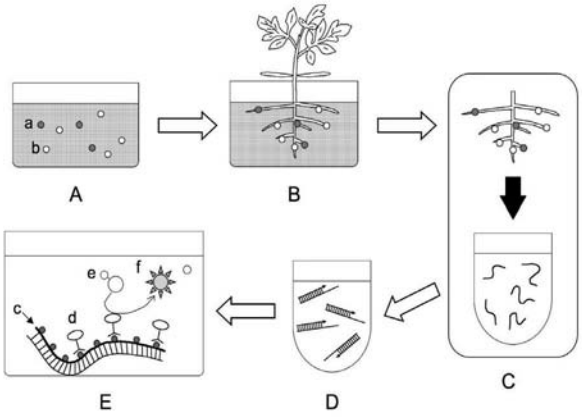

Fig. 1. Diagram of soil diagnostic method for PMTV. (A) Soil sample suspended in nutrient solution (a: PMTV-bearing, b: PMTV-free *Spongospora subterranea*), (B) roots of tomato bait plants immersed in soil suspension, (C) collection of bait plant roots and extraction of total RNA, (D) RT-PCR using primer pair PMTV-9P/PMTV-9M, (E) microplate hybridization (MPH) of RT-PCR products (c: a digoxigenin [DIG]-labeled probe, d: alkaline phosphatase conjugate of anti-DIG IgG, e: *p*-nitrophenyl phosphate, f: *p*-nitrophenol).

Development of soil diagnostic methods for soil-borne plant pathogens is crucial to establish effective control measures. A number of reports have been published that deal with soil-borne plant viruses, however, there is little information on detecting PMTV in agricultural fields. Because the vector, *S. subterranea*, is an obligate plant pathogen (Jones and Harrison, 1969), it needs to be trapped using a host plant species as a bait plant to detect PMTV in soil. In this paper, we established the method for detecting PMTV in field soil using the bait plant (Fig. 1). The method facilitated detection of the vector as well as PMTV by incubating tomato plants for 9 days in hydroponic culture.

Using the new diagnostic method, we surveyed infestation of soil samples within ca. 7-km radius from the spraing-affected field. PMTV was detected from the soil in 137 of 224 fields (61.2%). We simultaneously checked several fields owned by the same farmers, and the results revealed that the ratios of PMTV-positive fields varied for each farmer, and not all the fields owned by a farmer were necessarily PMTV-positive (data not shown). In plots of PMTV-positive and -negative fields on the map (Fig. 2), the positive fields were distributed at random within the surveyed area. Although we found 137 of 224 fields were contaminated with PMTV by our soil diagnostic method, spraing incidence was limited to a single field, and other PMTV-positive fields were still “spraing-free” even though the cultivar Sayaka was planted in some of these fields (Fig. 2). Buundgard *et al.* (2007) showed that PMTV was detected in 73% of 349 soil

samples collected nationwide in Norway using *N. benthamiana* and *N. debneyi* as bait plants; however, the relationship between the detection of PMTV from the field soil and the incidence of spraing was not mentioned. Soil infestation with PMTV may not directly result in spraing incidence. Thus it is still a burning question whether spraing can develop when potato is planted in PMTV-positive fields. Because our method is optimized for qualitative detection of PMTV, it is crucial to estimate the infestation level of PMTV in the field, i.e., the density of *S. subterranea* carrying PMTV, and to clarify the relationship between infestation level and spraing incidence. Random distribution pattern of infested fields rather than a concentric pattern (Fig. 2) and presence of PMTV even in the fields where potato had not been planted for at least 3 years suggests that PMTV entered one field site at least several years before the discovery and then spread throughout the area gradually. In addition to the high sensitivity for detecting viral genomic RNA and the capability of simultaneous detection of multiple samples with the use of a multiwell microplate, this information indicates that the soil diagnostic method is applicable as a tool for the epidemiologic study of PMTV.

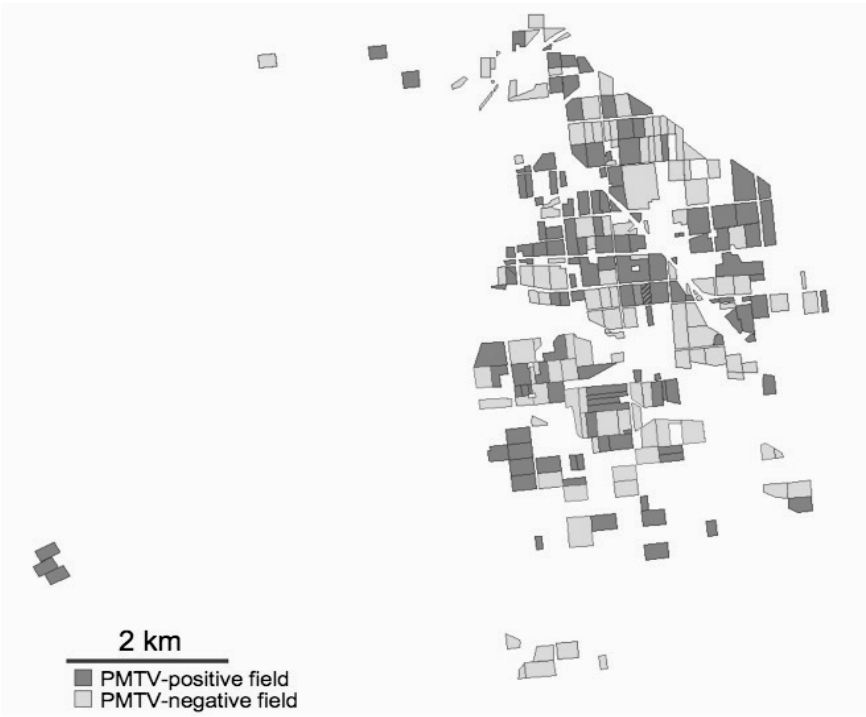

Fig. 2. Map of fields tested for PMTV with the soil diagnostic method. Each polygon represents a tested field. Light-gray and dark-gray polygons represent PMTV-negative (not infested) and PMTV-positive (infested) fields, respectively. Hatched polygon represents the only field where spraing was found.

Acknowledgement

This research was grant-aided by the Agriculture, Forestry and Fisheries Research Council, the Ministry of Agriculture, Forestry and Fisheries (No. 18099).

References

Buundgard, K., Ørstad, K., Valkonen, J.P.T. and Spetz, C. (2007). Distribution and variability of *Potato mop top virus* in Norway. *Proceedings of the The 13th European Association for Potato Research Virology Section Meeting*, 2007. Coylumbridge, Aviemore, Scotland, UK: 77.

Calvert. E.L. and Harrison, B.D. (1966). *Potato mop top, a soil-borne virus*. *Plant Pathology* **15**, 134-139.

Harrison, J.G., Searle, R.J. and Williams NA, 1997. Powdery scab disease of potato - a review. *Plant Pathology*, **46**, 1-25.

Hataya, T., Inoue, A.K. and Shikata, E. (1994). A PCR-microplate hybridization method for plant virus detection. *J. Virol. Methods* **46**, 223-236.

Hataya, T., Nakayama, T. and Maoka, T. (2006). Characterization and molecular diagnosis of *Potato mop top virus* in Japan. *Jap. J. Phytopathology* **72**, 253 (abstract in Japanese).

Imoto, M., Iwaki, M., Tochihara, H., Nakamura, K. and Hanada, K. (1986). The occurrence of *Potato mop top virus* in Japan and some of its properties. *Ann. Phytopathol. Soc. Jpn.* **52**, 752-757 (in Japanese with English abstract).

Jones R.A.C. and Harrison B.D. (1969). The behaviour of *Potato mop top virus* in soil, and evidence for its transmission by Spongospora subterranea (Wallr.) Lagerh. *Ann. Appl. Biol.* **63**, 1-17.

Maoka, T., Nakayama, T. and Hataya, T. (2006). Occurence of *Potato mop top virus* in Tokachi region of Hokkaido. *Jap. J. Phytopathology*, **72**, 253 (abstract in Japanese).

Merz, U. (1989). Infectivity, inoculum density and germination of *Spongospora subterranea* resting spores: a solution-culture test system. *Bull. OEPP* **19**, 585-92.

LACTUCA VIROSA L. EXHIBITS GENETIC VARIATION FOR BIG VEIN SYMPTOM EXPRESSION AND *MIRAFIORI LETTUCE BIG VEIN VIRUS* INCIDENCE

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Summary

Lactuca virosa L. is a wild relative of lettuce that could be an important source of resistance to big vein disease, an economically damaging disease of lettuce. Identification of accessions with resistance to *Mirafiori lettuce big vein virus* (MLBVV), the disease causing agent, may be useful in lettuce breeding. The objectives of this research were to determine the genetic variation for big vein symptom expression and MLBVV accumulation in *L. virosa*. Greenhouse testing was conducted to characterize variation for symptom expression 90 to 100 days after planting (DAP) with 70 *L. virosa* accessions in unreplicated experiments in 2001 and 2003, and with 10 accessions in an experiment with 3 replications conducted in 2004. In 2005, 6 replications of 7 accessions were evaluated for the percentage of symptomatic plants 120 DAP and 180 DAP in a growth chamber experiment. Reverse transcription – polymerase chain reaction or nucleic acid spot hybridization was used to determine MLBVV presence or absence at each reading date. Genetic variation for symptom expression was confirmed among the *L. virosa* accessions, although the majority of tested accessions did not express big vein symptoms. Symptomless infections were discovered, although accumulation of MLBVV to detectable levels appears to be a slow process in *L. virosa*. Genetic variation for the incidence of MLBVV positive plants was identified within symptomless accessions, and suggests that symptom expression and MLBVV incidence may be independent factors contributing to big vein resistance. Regardless, symptomless accessions with low MLBVV incidence were identified, and should be useful for breeding new big vein resistant cultivars.

Introduction

Lactuca virosa L. is an evolutionarily divergent wild relative of *L. sativa* with a geographic distribution centered around the Mediterranean basin of Europe. *L. virosa* is an important source of genetic resistance to numerous viral, fungal, bacterial, and insect pests of lettuce, and could be an important source of complete resistance or potential immunity to lettuce big vein disease (Hayes et al., 2006). Big vein is an economically damaging disease complex of lettuce (*Lactuca sativa* L.) that occurs in lettuce production regions around the world.

The disease causing agent is *Mirafiori lettuce big vein virus* (MLBVV), which is vectored by the soil-borne fungus *Olpidium brassicae* (Lot *et al*., 2002; Roggero *et al*., 2003). While genetic resistance from *L. virosa* offers an effective and economically feasible method of controlling big vein, only partial resistance exists in cultivated lettuce.

Complete resistance to big vein, the consistent and complete absence of symptoms, was described in the *L. virosa* accessions IVT278 and IVT280 (Bos and Huijberts, 1990; Hayes *et al*., 2006). Efforts to introgress resistance from *L. virosa* into lettuce using accession IVT280 has been reported (Hayes and Ryder, 2007). Hybrid breeding populations did not contain individuals with complete resistance, but did have variation for a high level of partial resistance that was likely based on novel alleles derived from *L. virosa*. The failure of this effort may be related to the difficulty of breeding with *L. virosa*, which requires the use of bridge crosses with *L. serriola*, colchicine doubling, or embryo rescue (Maisonneuve *et al*., 1995). More effort is needed to develop lettuce cultivars with complete big vein resistance derived from *L. virosa*. It is not clear whether the inability to introgress resistance from IVT280 into *L. sativa* to date is due to a specific problem with accession IVT280. Additional accessions with complete resistance to big vein are needed to determine this. Furthermore, susceptible *L. virosa* accessions would be useful for determining the biology and genetics of big vein resistance in *L. virosa*. The objective of this research was to determine the genetic variation for big vein symptom expression and MLBVV resistance in *L. virosa*.

Materials and Methods

All greenhouse or growth chamber experiments were performed according to Ryder and Robinson (1995). Inoculum was produced in the greenhouse by growing big vein symptomatic plants in 15 cm pots containing *O. brassicae* infested field soil collected from the USDA-ARS research station in Salinas, CA, a well characterized big-vein infested field. A suspension of greater than 30,000 *O. brassicae* zoospores per ml was prepared by macerating roots of diseased plants in water. Seedlings were germinated in a 2:1 ratio of field soil to sand potting mix and grown for three weeks. Inoculations were conducted by watering these seedlings with the zoospore suspension. Plants were grown at 18°C and symptoms evaluated over an eight week period, approximately 90 – 100 days after planting (DAP). Tissue samples were ground in liquid nitrogen, total nucleic acid was extracted, and extracts were stored at -80°C. Primers and methods for amplification of MLBVV by RT-PCR and for nucleic acid spot hybridization (NASH) were as described previously (Hayes *et al*., 2006). Positive and negative controls consisted of MLBVV infected *L. sativa* and *L. virosa*, and greenhouse grown *L. virosa* and *L. sativa* not exposed to MLBVV, respectively (Hayes *et al*., 2006).

Unreplicated plots of *L. virosa* accessions and *L. sativa* control cultivars were evaluated in 2001 (11 accessions) and 2003 (63 accessions). Up to 12 plants per accession were evaluated, notes on putative symptoms were recorded throughout, and the presence or absence of MLBVV was evaluated using RT-PCR at the end of experiments as previously described (Hayes *et al*., 2006). Big vein resistance was also evaluated in a greenhouse experiment in 2004 with 3 replications of 12 plants. The materials evaluated included *L. virosa* accessions PI271938, SAL012, SAL177, IVT280, and the *L. sativa* cultivars Pavane and Great Lakes 65. In addition, accessions CGN16272, CGN16273, CGN16274, CGN16275, CGN16276, and CGN16277 that were not previously tested were included. Accession CGN16273 was reported to express big vein symptoms (Johan Schut, personnel communication). The percentage of symptomatic plants at the end of the experiment was calculated, and chi-square goodness-of-fit was used to test dependence between *L. virosa* accessions and the percentage of big vein symptomatic plants. Tissue was collected at the end of the experiment to determinate MLBVV presence or absence using NASH.

A growth chamber experiment to evaluate big vein resistance was conducted with up to 6 replications of 10 plants per plot. Only symptoms that were typical of big vein were recorded for each plot and the proportion of symptomatic plants was determined, and tissue sampled at 120 DAP and 180 DAP. Total nucleic acids were extracted, and plants tested for MLBVV by NASH. The proportion of MLBVV positive plants was calculated, and proportion data were transformed to arcsine values, analyzed in proc mixed in SAS as a randomized complete block design with accession as a fixed effect and block as a random effect. More detailed information on materials and methods can be found in Hayes *et al*. (2008).

Results

Two years of unreplicated greenhouse experiments identified 62 asymptomatic accessions of *Lactuca virosa*, and one accession, SAL177, with 16% of plants with typical vein banding symptoms. The susceptible control Great Lakes 65 and the partially resistant cultivar Pavane also exhibited typical vein banding symptoms. Atypical growth habits or putative symptoms were also observed. Leaf crinkling, epinasty, and necrosis were observed on SAL014 in 2001 and 2003, and in SAL094, SAL193, SAL194, SAL195, SAL196, and SAL197 in 2003. Symptomatic leaf samples were taken from 21 plants of these accessions grown in the 2003 experiment to determine the presence of MLBVV using NASH and RT-PCR. MLBVV was only detected in a single plant from SAL195, and was not detected in any of the other lines exhibiting atypical symptoms, strongly suggesting that MLBVV was not the cause of those symptoms.

Genetic variation for vein banding symptoms typical of big vein disease was identified between ten accessions of *L. virosa* and Pavane in a greenhouse

experiment. The percentage of symptomatic plants ranged from 0% (IVT280, PI271938, and SAL012) to 17% (CGN16273), and the difference between accessions was significant ($]^2$, 10 *df* = 19.5; $p < 0.05$). Seven *L. virosa* accessions had at least one symptomatic plant. Symptoms were observed on 9% of plants of the cultivars Pavane, a *L. sativa* cultivar with partial resistance to big vein. The susceptible cultivar, Great Lakes 65, which was not included in the chi-square analysis, had 89% symptomatic plants. Six accessions in addition to Great Lakes 65 were sampled and tested for MLBVV using NASH. *L. virosa* accessions ranged from 0% (SAL012) to 100% (CGN16274) of plants that were positive for MLBVV. Great Lakes 65 had 100% of tested plants positive for MLBVV. Accessions IVT280, SAL177, and SAL012 had varying numbers of samples that resulted in faint signals, and could not conclusively be determined as MLBVV positive in subsequent retesting. However, if these are also considered to be positive results it increases the number of MLBVV positive plants to 92% in SAL177, 75% in IVT280, and 17% in SAL012.

Seven *L. virosa* accessions were further tested in a growth chamber experiment for big vein symptom expression, and genetic variation for the percentage of symptomatic plants was identified. CGN16275 and CGN16277 had significantly greater percentages of symptomatic plants than the remaining accessions at 120 DAP and 180 DAP. Symptomatic plants were not observed in CGN16276 at 120 DAP, but 31% symptomatic plants were observed at 180 DAP. No plants with vein banding symptoms were observed in accessions SAL195, IVT280, PI274378, and SAL012 at 120 DAP or 180 DAP. Variation for the percentage of MLBVV positive plants was found at both testing dates. At 120 DAP, all accessions had MLBVV positive plants and the percentage of positive plants ranged from 8% (SAL012 and CGN16276) to 73% (CGN16725). The percentage of MLBVV positive plants in accessions GCN16275 and CGN16277 was significantly higher than the remaining accessions. By 180 DAP, the percentage of MLBVV positive plants had increased in accession CGN16275, CGN16276, CGN16277, SAL195, and IVT280 while the percentage of MLBVV positive plants decreased in PI274378 and SAL012. This resulted in SAL012 having a significantly lower percentage of MLBVV positive plants than every accession except PI274378, and with PI274378 having a significantly lower percentage of MLBVV positive plants than CGN16275, CGN16276, CGN16277. IVT280, which remained symptomless throughout every experiment, had 51% MLBVV positive plants by 180 DAP.

Discussion

Lactuca virosa has genetic variation for symptom expression, and complete resistance to big vein disease appears to be wide spread in *L. virosa*. This is a significant finding since extensive screening of diverse *L. sativa* accessions has only discovered partial resistance, and complete resistance has not been found

in *L. sativa* (Hayes and Ryder, unpublished).
Symptomless expression of MLBVV infections in susceptible and partially resistant cultivars of lettuce have been widely reported (Roggero *et al*., 2003; Navarro *et al*., 2004; Hayes *et al*., 2006). We have shown in this research that *L. virosa* may also have symptomless infections of MLBVV. It is clear that virus accumulation in *L. virosa* can be a slow process, and previous studies may not have been allowed to continue long enough to detect MLBVV accumulation in *L. virosa*. In this research, extending the length of the experiments to 180 DAP was likely an important factor in discovering MLBVV symptomless infection. Furthermore, it also seems likely that some "faint" positive NASH results observed with some accessions in one experiment were MLBVV positive plants that had not had sufficient time to accumulate clearly detectable quantities of MLBVV. Introgression of big vein resistance from IVT280 into *L. sativa* was not successful in identifying hybrid lines with complete resistance (Hayes and Ryder, 2007). The discovery that IVT280 can have high percentages of MLBVV positive plants despite being asymptomatic may have contributed to the failure of this breeding effort. Additional breeding should be conducted with *L. virosa* accessions that have low percentages of MLBVV positive plants. Big vein symptom expression is known to be environmentally dependant; therefore, it is not known how these *L. virosa* accessions will perform beyond 180 DAP or in environments that are further conducive to big vein symptom expression. Importantly, most lettuce crops require only 60 to 90 days from planting to harvest, and if measurable accumulation can be delayed in *L. virosa-L. sativa* hybrids until after 100 days, this should substantially reduce the potential for big vein disease development.

Genetic variation also exists among *L. virosa* accessions for the incidence of MLBVV infected plants. This has not been previously reported in lettuce or any other *Lactuca* species. The finding of variation for MLBVV accumulation within accessions that are completely asymptomatic suggests that symptom expression and MLBVV resistance may be independent factors in *L. virosa* contributing to big vein resistance. Additional research to determine the rate of MLBVV accumulation in *L. virosa* and *L. virosa-L. sativa* hybrids is needed to further characterize these accessions. More details can be found in Hayes *et al*. (2008).

References

Bos, L. and Huijberts, N. (1990). Screening for resistance to big vein disease of lettuce. *Crop Protection* **9**, 446-452.

Hayes, R.J., Wintermantel, W.M., Nicely, P.A. and Ryder, E.J. (2006). Host resistance to *Mirafiori lettuce big-vein virus* and *Lettuce big-vein associated virus* and virus sequence diversity and frequency in California. *Plant Dis*. **90**, 233-239.

Hayes, R.J. and Ryder, E.J. (2007). Introgression of novel alleles for partial resistance to big vein disease from *Lactuca virosa* into cultivated lettuce. *Hortscience* **42,** 35-39.

Hayes, R.J., Ryder, E.J. and Wintermantel, W.M. (2008). Genetic variation for big vein symptom expression and *Mirafiori lettuce big vein virus* incidence in Lactuca virosa L., a wild relative of cultivated lettuce. *Euphytica* http://dx.doi.org/10.1007/s10681-008-9738-x.

Lot, H., Campbell, R.N., Souche, S., Milne, R.G. and Roggero, P. (2002). Transmission by *Olpidium brassicae* of *Mirafiori lettuce virus* and *Lettuce big vein virus*, and their roles in lettuce big-vein etiology. *Phytopathology* **92,** 288-293.

Maisonneuve, B., Chupeau, M.C., Bellec, Y. and Chupeau, Y. (1995). Sexual and somatic hybridization in the genus *Lactuca*. *Euphytica* 8, 281-285.

Navarro, J.A., Botella, F., Maruhenda, A., Sastre, P., Sánchez-Pina, M.A. and Pallas, V. (2004). Comparative infection progress analysis of *Lettuce big vein virus* and *Mirafiori lettuce* in lettuce crops by developed molecular diagnosis techniques. *Phytopathology* **94**, 470-477.

Roggero, P., Lot, H., Souche, S., Lenzi, R. and Milne, R.G. (2003). Occurrence of *Mirafiori lettuce virus* and *Lettuce big vein virus* in relation to development of big vein symptoms in lettuce crops. *Eur. J. Plant Pathol*. **109,** 261-267.

Ryder, E.J. and Robinson, B.J. (1995). Big-vein resistance in lettuce: Identifying, selecting, and testing resistance cultivars and breeding lines. *J. Amer. Soc. Hort. Sci.* **120**, 741-746.

OCCURRENCE OF SOIL-BORNE CEREAL VIRUSES IN POLAND

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Summary

Four Soil-borne cereal viruses were detected and identified in Poland: *Soil-borne cereal mosaic virus* (SBCMV), *Wheat spindle streak mosaic virus* (WSSMV), *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV). The occurrence of SBCMV was revealed already in 1991. Since its identification, a map of incidence of the virus has been consequently completed. In spite of relatively broad area of occurrence, SBCMV did not cause serious damages, at least in winter wheat, yet. WSSMV was found sporadically and usually accompanied SBCMV. Clear disease symptoms were observed only in Triticale plants. In 2008, two viruses causing barley yellow mosaic were isolated from diseased winter barley plants and identified. The outbreak of the disease was noted in Lower Silesia region. Disease symptoms were observed mainly in barley plants cv. Vanessa. It is the first report on the occurrence of BaYMV and BaMMV in Poland.

Introduction

Soil-borne cereal viruses, vectored by *Polymyxa graminis* Led., are dangerous pathogens for their long persistence in the environment and the lack of efficient means of control, except host resistance. *Soil-borne cereal mosaic virus* (SBCMV) was first virus of this group detected in Poland in 1992 (Je]ewska, 1994). It was isolated from rye and tritticale plants showing symptoms of mild leaf mosaic and stunting. The incidence of SBCMV, previously named SBWMV, was consequently monitored, but it did not expand and its harmfulness was evaluated as moderate. *Wheat spindle streak mosaic virus* (WSSMV) was sporadically detected in wheat plants in symptomless infections. However in 2007 WSSMV was isolated from triticale plants with characteristic leaf mosaic (Je]ewska and Trzmiel, 2007). Since 1994 routine monitoring has been conducted aiming at the detection of possible infections of winter barley with *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV), causal agents of a serious disease, known for important crop losses in several countries, particularly in Germany. However, the viruses were not found until 2008, when appeared with strong symptom expression. The main objective of our investigations was to identify locations (regions) of soil-borne cereal viruses in order to help farmers in proper planting strategy.

Materials and Methods

Plant samples were collected in fields with disease symptoms suggesting infection with viruses. Diagnostics was initially performed by ELISA tests. Commercial kits for DAS-ELISA and TAS-ELISA produced by Loewe (Germany) and Neogen (Great Britain) were used. RNA was isolated from infected plant leaves. Total RNA extraction from 100-120 mg fresh plant tissue was carried out using RNeasy Mini Kit manufactured by Qiagen (Germany), according to the procedure supplied by the producer. RNA extract was reverse transcribed and then amplified by PCR using one step RT-PCR kit from Qiagen. Following pairs of primers were tested for the identification of isolated viruses:

SBCMV	sb40/sb20 and sb11/gsb55	(Koenig and Huth, 2000)
	SBWMVEF/SBWMVUNR	(Clover *et al.*, 2001)
	Pa/Pb and P2/P3	(Kastirr *et al.*, 2002)
	FURO1-F/FURO1-R FUROc1-F/FUROc1-R FUROc2-F/FUROc2-R SBWMVc1-F/SBWMVc1-R SBCMVc1-F/SBCMVc1-R	(Vaïanopoulos *et al.*, 2005)
WSSMV	WSSMV1-F/WSSMV1-R WSSMVc1-F/WSSMVc1-R WSSMVc2-F/ WSSMVc1-R	(Vaïanopoulos *et al.*, 2005)
BaYMV / BaMMV	BaYMV-F/BaYMV-R BaMMV-F/BaMMV-R	(Vaïanopoulos *et al.*, 2003)

1 µl of target RNA was added to RT-PCR components in standard concentrations recommended by manufacturer (with 10 µl final concentration of primers). The reactions were conducted in Whatman Biometra Tpersonal thermocycler. The annealing temperature was optimised by testing a gradient of temperatures in Whatman Biometra Tprofessional gradient thermocycler. PCR products were separated electrophoretically in 1.5% agarose gel and visualised in UV after staining with ethidium bromide.

Results

On a map (Fig. 1) the occurrence of soil-borne cereal viruses in Poland is presented. Each location designated on the map represents also surrounding area where the virus was found, as well.

Fig.1. Occurrence of soil-borne cereal viruses in Poland.

There are 10 locations connected with SBCMV incidence. The distribution of the virus, monitored since 1994, is unequal. Majority of sites where SBCMV was found is situated in the Western part Poland, especially in Wielkopolska and Lower Silesia. However it is noteworthy that at the opposite side of the country, near Chełm, an area seriously infested with the virus was revealed, too. Originally, infections with the virus were observed mainly in winter rye and winter triticale. Since 2003 increasing number of SBCMV infected winter wheat plants was noted. In 2007 SBCMV was detected in 40 plants out of 539 tested (7.4%), but in 2008 the respective results were as follows: 101 and 718 (13.9%). The large diversity of symptoms occurred, from very mild to strong damages, including yellowing, stunting and complete breakdown. In preliminary experiments, performed in field and glasshouse conditions, certain data on the reaction of some winter wheat varieties were collected. Few or no foliar symptoms were seen on varieties: Bogatka, Finezja, Legenda, Smuga, Tonacja and Turkis. Brilliant was the most susceptible to SBCMV infection, showing typical symptoms and the high rate of infected plants.

Diagnostics of SBCMV initially was limited to ELISA tests. Since 2002 it was completed by RT-PCR technique using a range of primers listed above. The most reliable results were obtained with following pairs of primers: FURO1-F/ FURO1-R and FUROc2-F/FUROc2-R. Primers sb40/sb20 and sb11/gsb55 were not useful in the case of samples with low virus RNA concentration, e.g. with stored plant materials. Surprisingly, no expected products were generated when using primers: SBWMVEF/SBWMVUNR, Pa/Pb, P2/P3, FUROc1-F/FUROc1-R, SBWMVc1-F/SBWMVc1-R and SBCMVc1-F/SBCMVc1-R.

WSSMV was found in 4 locations, usually in association with SBCMV. Exceptionally in Winna Góra WSSMV occurred alone, causing typical leaf mosaic in triticale plants. It would be difficult to evaluate the harmfulness of the virus but it seems not causing important crop losses actually. The virus occurred in low concentration but it was easily detected by means of RT-PCR.

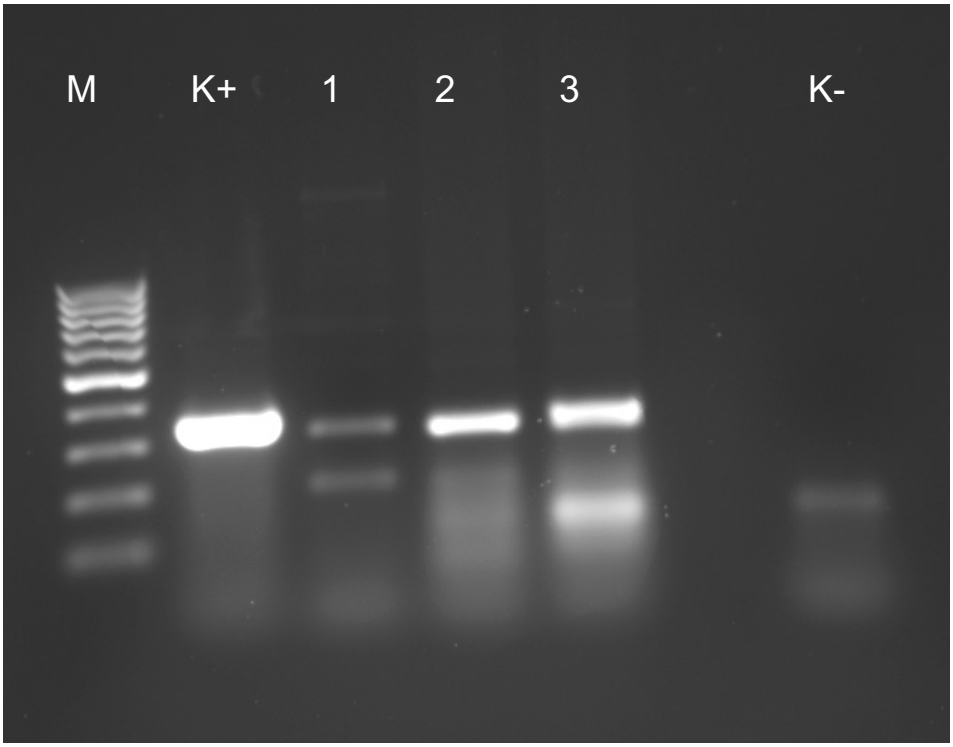

Fig. 2. Detection of BaYMV in winter barley plants by RT-PCR with primers BaYMV-F/BaYMV-R.
M- marker O'GeneRuler 100bp DNA Ladder (Fermentas)
K+ - positive control
1 – winter barley infected with BaYMV
2 – winter barley infected with BaYMV
3 - winter barley infected with BaYMV
K- - negative control (healthy plant)

BaYMV and BaMMV, causal agents of barley yellow mosaic, were detected in Poland for the first time in 2008. There was an outbreak of the disease in Lower Silesia. Symptom expression was very pronounced in sensible winter barley variety Vanessa in the region of Strzegom. In each location where barley yellow mosaic occurred both viruses were detected, in single or mixed infections. 135 barley plants were found infected out of 352 tested. However the proportions of infected plants were different depending on the region; in surroundings of

Strzegom about 80% of assayed samples were infected with at least one of the *Barley yellow mosaic viruses*. In surroundings of Pisarzowice the rate of detection was about 40%, while in the regions of Pisarzowice and Łagiewniki did not exceed 10%. Diagnostics of the viruses was very clear using RT-PCR (Fig. 2).

Discussion

The reason for an unforeseen increase of SBCMV incidence in winter wheat in Poland recently might be favourable climatic conditions. Nevertheless the possibility of new virus isolate appearance should be considered, too. It seems necessary to intensify investigations on the selection of resistant/tolerant wheat varieties. The results of SBCMV detection using different primers might suggest a certain specificity of Polish isolate(s). Detection of BaYMV and BaMMV created new situation for barley producers and breeders in Poland. It is necessary now to select carefully winter barley varieties to be sown in regions with infested soils

Acknowledgements

The authors wish to thank ing. Maria Lubik for excellent technical assistance.

References

Clover, G.R.G., Ratti, C. and Henry, C.M. (2001). Molecular characterisation and detection of European isolates of *Soil-borne wheat mosaic virus*. *Plant Pathol*. **50**, 761-767.

Je]ewska, M. (1994). Identification and some properties of *Wheat soil-borne mosaic virus* isolated in Poland. *Phytopathol. Pol*. **8**, 97-102.

Je]ewska, M. and Trzmiel K. (2007). First report on the occurrence of *Wheat spindle streak mosaic virus* in triticale in Poland. *Phytopathol. Pol*. **44**, 51-53.

Kastirr U., Rabenstein, F. and K]hne, T. (2002). Investigation of the epidemiology of *Soil- borne cereal mosaic virus* in the German country Saxony-Anhalt. *Proc. 5th Symposium IWGPVFV, July 22-25, 2002, Zurich (Switzerland)*, 119-122.

Koenig R. and Huth W. (2000). *Soil-borne rye mosaic* and *European wheat mosaic virus*: two names for a furovirus with variable genome properties which is widely distributed in several cereal crops in Europe. *Arch. Virol*. **145**, 689-697.

Vaïanopoulos C., Legrève A., Barbier A., Steyer S., Maraite H. and Bragard C. (2003). Detection of *Barley yellow mosaic virus* and *Barley mild mosaic virus* by RT-PCR on resistant barley cultivars. *Parasitica* **59**, 67-74

Vaïanopoulos C., Legrève A., Moreau V., Steyer S., Maraite H. and Bragard C. (2005). Occurrence of bymo- and furoviruses on wheat in Belgium. *Parasitica* **61**, 47-54.

IDENTIFICATION OF TWO NEW RNA 5-CONTAINING *BEET NECROTIC YELLOW VEIN VIRUS* GENOTYPES IN THE UK AND IN GERMANY

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Summary

Two new RNA 5-containing *Beet necrotic yellow vein virus* (BNYVV) strains have been detected in the UK and in Germany, respectively, each one on one single field only. They are clearly distinct from the RNA 5-containing P type BNYVV occurring in limited areas in France and the UK. The RNA 5 of the UK FF isolate occupies an intermediate position between European P type and East Asian RNAs 5. RNAs 2, 3, 4 and 5 of the German OW1 isolate are closely related to those of the Chinese Har4 isolate. The detection of this isolate in Germany was especially surprising, because in this country almost exclusively B type BNYVV is found. Neither A types nor the especially damaging P type have so far succeeded to become established in Germany although there must have been many opportunities for them to become introduced from neighbouring countries.

Introduction

BNYVV RNA 5 was first described in Japan (Tamada *et al*., 1989) where it is associated with either A or B type BNYVV (Miyanishi *et al*., 1999) and leads to an increase of the severity of rhizomania symptoms. A deviating form of RNA 5 which is associated with P type BNYVV and differs from the Japanese (and Chinese) forms of RNA 5 by more than 40 nt exchanges and several insertions and deletions has been identified in limited areas in France (Pithiviers), in Kazakhstan and the UK (Koenig *et al*., 1997; Koenig and Lennefors, 2000; Harju *et al*., 2002; Ward *et al*., 2007). In the present paper we summarize the properties of two new RNA 5-containing BNYVV isolates which we have recently identified in the UK and Germany, respectively. Detailed descriptions have been published elsewhere (Ward *et a*l., 2007; Koenig *et al*., 2008).

Materials and Methods

The following regions of the five BNYVV RNA species (GenBank accession numbers in parentheses) were amplified by means of PCR as described by Ward *et al.* (2007): RNA 1 (NC003514) nt 5940 – 6590, RNA 2 (D84411) nt 3187 – 4106, RNA 3 (D84412) nt 300 – 1205, RNA 4 (D84413) nt 302 – 1334, RNA 5 (U78293) nt 1-1348. The nt sequences of the PCR products were analyzed by means of the Invitrogen Vector NTI Advance 10 and the DNAman (Lynnon Bio/Soft) softwares. For visualizing nt differences in sequence alignments the Boxshade software (www.ch.embnet.org/software/BOX_form.html) was used.

Results and Discussion

The BNYVV isolate UK-FF was obtained from sugar beets on one field in Norfolk, UK. It could not be clearly assigned to any of the previously described European BNYVV types. The analysed portions of its RNAs 2 and 4 are closely related to the corresponding portions in the RNAs of the East Asian A type isolate S, whereas those of its RNAs 1 and 3 resemble P type RNA 1 from Kazakhstan and European A type RNA 3, respectively. The P25 encoded on its RNA 3 has an unique TYHG tetrad in the highly variable amino acid positions 67–70. Sequence alignments (Fig. 1) indicate that the UK-FF RNA 5 occupies an intermediate position between P type and East Asian RNAs 5 (Figs. 1 and 2). In some positions it has nts typically occurring in P type RNAs 5 whereas in others it has nts typical for East Asian RNAs 5 (Fig. 1).

P type and most of the East Asian BNYVV RNAs 5 differ not only by nts in specific positions, but also by a number of insertions or deletions in the 5' untranslated regions (5' UTR) and the P26-coding region (Fig. 1). The UK-FF RNA 5 again exhibits intermediate features. Like the typical East Asian RNAs 5, it has unique insertions in the AU-rich region following nt 350 in the 5' UTR and it lacks the GAU insertion following nt 690 in P type RNAs 5. It resembles the P type RNAs 5, however, in having a 6 nt deletion following nt 428 in the 5'UTR and a 9 nt insertion close to the 3' end of its P26 coding region (Fig. 1). Since the similarities and dissimilarities of the UK-FF RNA 5 sequence with either the P type or the East Asian RNA 5 sequences are distributed along its entire sequence, this RNA is probably not the result of a recombination event, but rather of an independent phylogenetic development. The phylogenetic relationships of the P26 coding area on UK-FF RNA 5 are shown in Fig. 2.

In Germany we detected an RNA 5-containing BNYVV genotype (named OW1) in one field in Saxony-Anhalt. The analyzed portions of its RNA 2, 3, 4 and 5 share more than 99% sequence identity with those of the Chinese isolate Har4 (Li *et al*., 2008). The relationships of its RNA 5 to those of East Asian and European BNYVV isolates are shown in Figs. 1 and 2. The discovery of this East Asian BNYVV type in Germany was especially surprising, because in all other parts of

this country almost exclusively B type BNYVV is found despite the fact that the country is surrounded by others where A type BNYVV is widely distributed (Fig. 3). Neither A types nor the especially damaging P type have so far succeeded to become established in Germany although there must have been many opportunities for them to become introduced from neighbouring countries, e.g. by soil adhering to agricultural products, footwear of passers-by, car tires etc. A similar observation that regions which are infested by one BNYVV type are not easily superinfested with other BNYVV types has been made in the Pithiviers area in France where the P type has apparently not spread in the past 15 years into surrounding areas which are infested by A or B types (Richard-Molard, pers. communication). This might be due to a competition between the different virus types or between *Polymyxa* strains which in different areas may differ in their ability to transmit individual BNYVV genotypes. The OW1/Har4 genotype of BNYVV and its vector may have been introduced to Germany a long time ago, e.g. with planting material of ornamentals, trees or others. BNYVV is not widely spread in Saxony-Anhalt. This might have facilitated the establishment of this East Asian genotype in one field. The occurrence of BNYVV in this field has been known for several years, but there have been no indications that the virus has spread to neighbouring regions.

Acknowledgement

This research has been carried out with the financial support of the Federal Ministry of Economics and Technology (BMWi), administered via the Arbeitsgemeinschaft industrieller Forschungsvereinigungen e.V. (AiF) and the Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP).

References

Harju, V.A., Mumford, R.A., Blockley, A., Boonham, N., Clover, G.R.G., Weekes, R. and Henry, C.M. (2002). *Plant Pathol* . **51**, 811.

Koenig, R., Haeberle, A.M. and Commandeur, U. (1997). *Arch. Virol.* **142**, 1499-1504.

Koenig, R., Kastirr, U., Holtschulte, B., Deml, G. and Varrelmann, M. (2009). *Arch. Virol.* (in press; DOI: 10.1007/s00705-00-0234-3).

Koenig, R. and Lennefors, B.-L. (2000). *Arch. Virol.* **145**, 1561-1570.

Li, M., Liu, T., Wang, B., Han, C.G., Li, D.W., and Yu, J.L. (2008). *Virus Genes* **36**, 429-432.

Miyanishi, M., Kusume, T., Saito, M. and Tamada, T. (1999). *Arch. Virol* .**144**, 879-892.

Tamada, T., Shirako, Y., Abe, H., Saito, M., Kiguchi, T. and Harada, T. (1989). *J. Gen. Virol.* **70**, 3399-3409.

Ward, L., Koenig, R., Budge, G., Garrido, C., McGrath, C., Stubbley, H. and Boonham, N. (2007). *Arch. Virol*. **152**, 59-73.

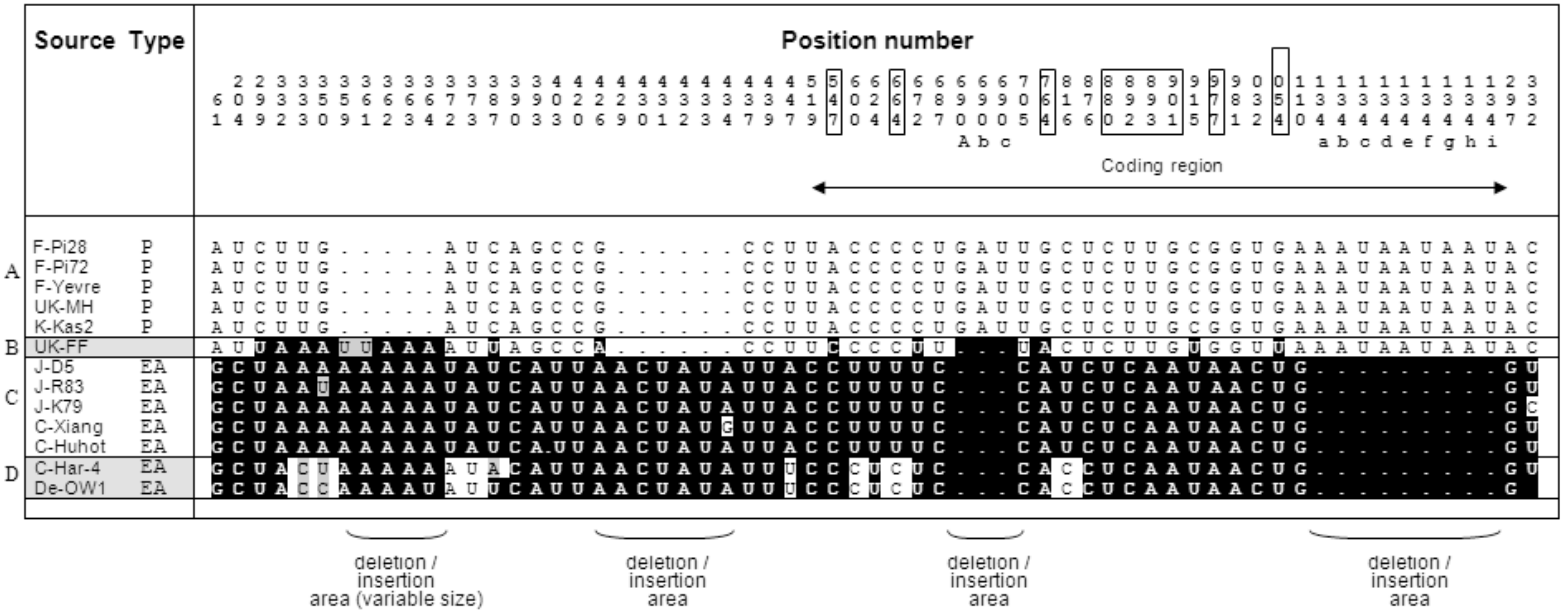

Fig. 1. Sequence alignment of different types of BNYVV RNA 5. Only the nts in those positions are shown which differ in P type and East Asian RNA 5s. Nts typical for the latter are highlighted by white letters on a black background

A -Typical P type RNA 5s from limited areas in France, the UK and Kazakhstan

B - FF RNA 5 with intermediate properties from a single site in the UK

C - Typical East Asian RNA 5s.

D - Slightly deviating East Asian type RNA 5s found in China and at a single site in Germany

The numbering of nts corresponds to that of C-Huhot (accession number AJ236895)

Nts in the P26-coding region which lead to amino acid changes are boxed

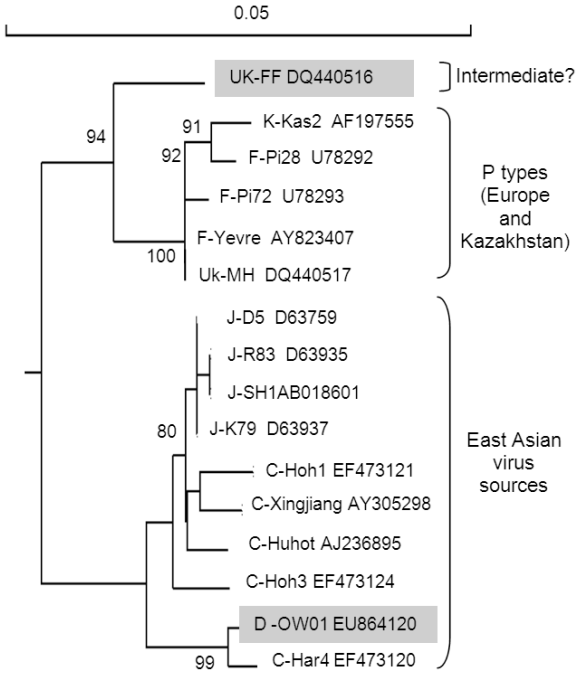

Fig. 2. Phylogenetic tree derived from sequence alignments of the P26 coding regions of BNYVV RNA 5s from various parts of the world. The numbers on the branches give the percent boot strap scores in 1000 trials, only values >85 are shown. The length of the branches can be estimated by means of the scale bar shown on the top of the figure. The letters preceding the hyphen in the virus sources indicate the country of origin, i.e. C - China, F – France, J – Japan, K – Kazakhstan, UK – United Kingdom. The letters or numbers following the hyphens designate the particular isolate, they are followed by the gene bank accession numbers.

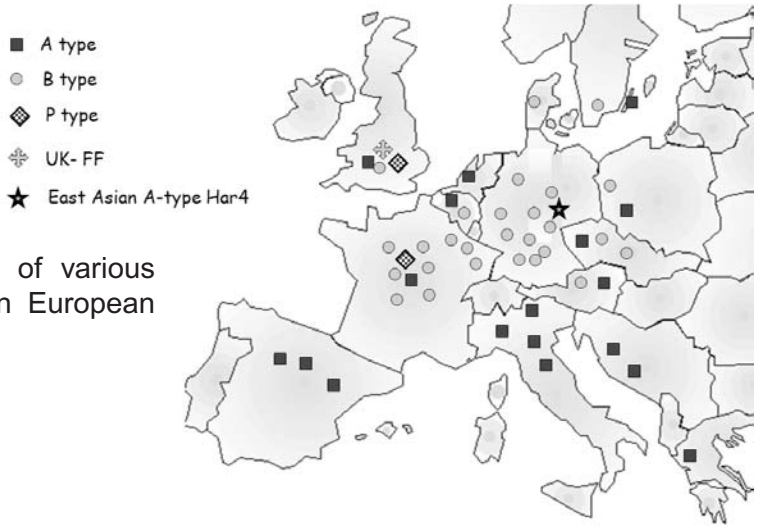

Fig. 3. Occurrence of various BNYVV genotypes in European countries.

POTATO MOP TOP VIRUS RNAS DISPLAY DIFFERENT REQUIREMENTS FOR LONG-DISTANCE MOVEMENT

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Summary

The long-distance movement of the three *Potato mop top virus* (PMTV) genomic RNAs in *Nicotiana benthamiana* was investigated. Movement of infectious clones containing different mutations in the RNA encoding the capsid proteins (CP and CP-RT), RNA-CP, was investigated by Northern and Western blotting. The results show that movement of RNA-CP was inhibited when truncated CP-RT was expressed or when CP was expressed in the absence of CP-RT, whereas the movement of the other two genomic RNAs was unaffected. Heterologous protein interactions were detected between the movement protein TGB1 and CP-RT but not between TGB1 and CP. The results support the novel concept that PMTV RNAs can move long-distance in two different forms either as vRNP complexes or as assembled virus particles. They also support a hypothesis that PMTV virions may contain the supplementary proteins CP-RT and TGB1 at one end.

Introduction

Potato mop top virus (PMTV) is the type species of the genus *Pomovirus,* other members of the genus include *Beet virus Q*, *Beet soil-borne virus* and *Broad bean necrosis virus* (Koenig and Lesemann, 2005). The PMTV genome encodes replicase, movement and capsid proteins on three different RNA species that are encapsidated within tubular rod-shaped particles. Previously we showed that the protein produced on translational readthrough of the coat protein (CP) gene, CP-RT, is associated with one extremity of the virus particles (Cowan et al., 1997), and that the two RNAs, encoding replicase (RNA1) and movement proteins (RNA-TGB), can move long-distance (systemically) in the absence of the third RNA (RNA-CP) that encodes the CP and CP-RT (Savenkov et al., 2003). The observation that the RNA-CP is dispensable in systemic infection is puzzling since PMTV is thought to be acquired by the vector (*Spongospora subterranea*) in the form of virus particles and a domain in the CP-RT is necessary for transmission (Reavy et al., 1998). In this work we examined the roles of the CP and CP-RT polypeptides on RNA movement using infectious clones carrying mutations in the CP and CP-RT coding domains.

Materials and Methods

Plant inoculations and analyses: Nicotiana benthamiana plants were inoculated with recombinant viruses, Western and Northern blot analyses were carried out as described (Savenkov et al. 2003; Yelina et al. 2002). Leaves were sampled at 14 dpi or 28 dpi.

Construction of mutant clones: Standard recombinant DNA procedures were performed by use of a combination of PCR, site-directed mutagenesis, and swapping of restriction fragments. The plasmid pPMTV-2 (Savenkov et al. 2003) was used to generate the mutants and constructs described in this study. The details of the plasmid construction and primer sequences used will be made available by the corresponding author upon request.

Yeast two-hybrid assays. The interaction between Lex A and VP16 fusion proteins was evaluated in *Saccharomyces cerevisiae* strain L40 as described (Cowan et al. 2002).

Results

Analysis of RNA-CP mutants showed that deletions in the CP-RT coding sequence inhibited systemic movement of RNA-CP but not the other two RNAs. A series of deletions were made in the RT-encoding part of a full-length RNA-CP clone. The resulting mutants were prepared so that the integrity of the 3' UTR was conserved (Fig. 1A mutants 1-4). RNA-CP transcripts from each of the mutants were inoculated onto leaves of *N. benthamiana* along with transcripts of RNA1 and RNA-TGB. Inoculated and upper non-inoculated leaves were analyzed by Western and Northern blotting. Analysis revealed that mutations in RNA-CP that resulted in a truncated CP-RT polypeptide did not move to the upper non-inoculated leaves except for mutant 2 (Fig. 1B lanes 1-4), a deletion mutant that spontaneously occurs in nature and in cultures on successive manual passage (Sandgren et al., 2001). RNA1 and RNA-TGB were detected in the upper leaves of all samples (Fig. 1B bottom panel). Western analysis detected CP antigen in inoculated leaves of all mutants but only in the upper leaves of plants inoculated with mutant 2 (data not shown).

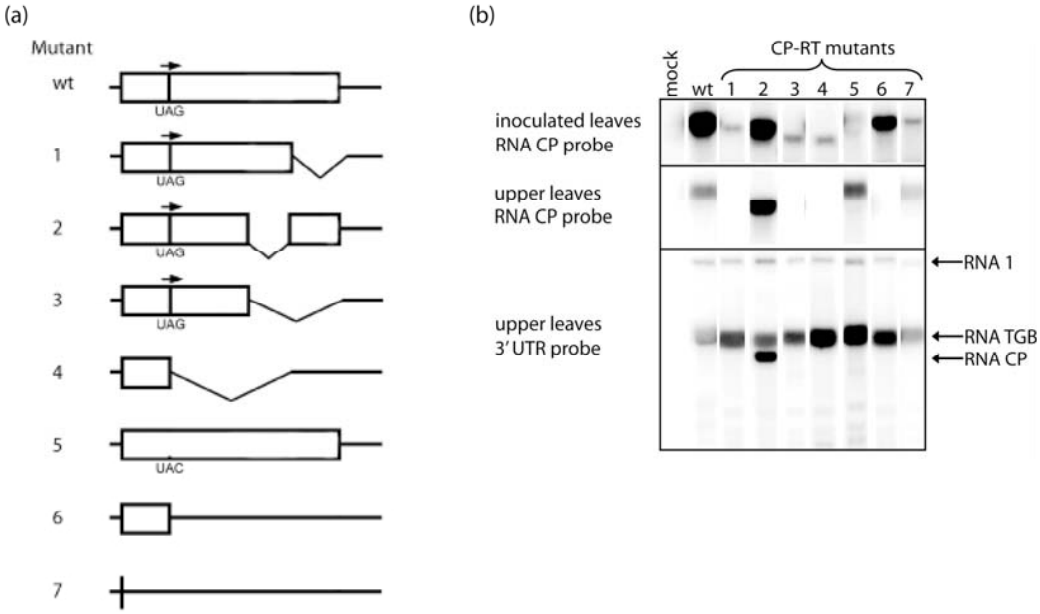

Fig 1 (a). Diagrammatic representation of RNA-CP wild type (wt) and mutants. The boxes represent the encoded proteins, CP (on left) and CP-RT. The arrow indicates read through of the UAG codon to give CP-RT polypeptide. In mutant 5 this codon was mutated to UAC coding for tyrosine which prevents expression of individual CP sub units. In mutant 7, two stop codons were inserted in place of the initiation codon for CP so that no proteins are translated. (b). Northern blots of RNA from leaves inoculated with RNA1, RNA-TGB and RNA-CP or RNA-CP mutants. Blots were hybridised with probes specific for RNA-CP (top and middle panels) or 3'UTR (bottom panel).

Expression of CP alone inhibits systemic movement of RNA-CP. Three additional mutants were analyzed that were engineered to express either CP-RT only (mutant 5), CP only (mutant 6) or no protein at all (mutant 7). The results showed that the clone expressing CP did not move to the upper leaves but the other two clones could be detected in upper non-inoculated leaves (Fig. 1B lanes 5 and 7). As before, the other two RNAs were detected in upper leaves of plants infected with all mutants.

Extracts from leaves that had been inoculated with the seven different mutants were examined in the electron microscope and virus particles were found in all samples except for two clones, namely, mutant 5 which only expressed CP-RT and mutant 7 where protein expression was prevented by the insertion of two stop codons at the beginning of the CP open reading frame (data not shown). These results indicate that inhibition of movement to the upper leaves was not due to inability to form particles.

Yeast two-hybrid analysis reveals interactions between virus CP-RT and movement protein TGB1. We had previously shown that CP-RT was present at one extremity of virions and it is possible that CP-RT may function in systemic movement by interaction with other proteins. An obvious candidate is the TGB1 movement protein. We tested interactions between CP, CP-RT and TGB1. The results showed that TGB1 interacts with CP-RT but not CP. In addition, CP-RT interacts with CP presumably through the CP domain (Fig. 2). Similar analysis of the truncated CP-RT indicated that those RNA-CP mutants defective in long-distance movement encoded CP-RT that failed to interact with TGB1 (data not shown).

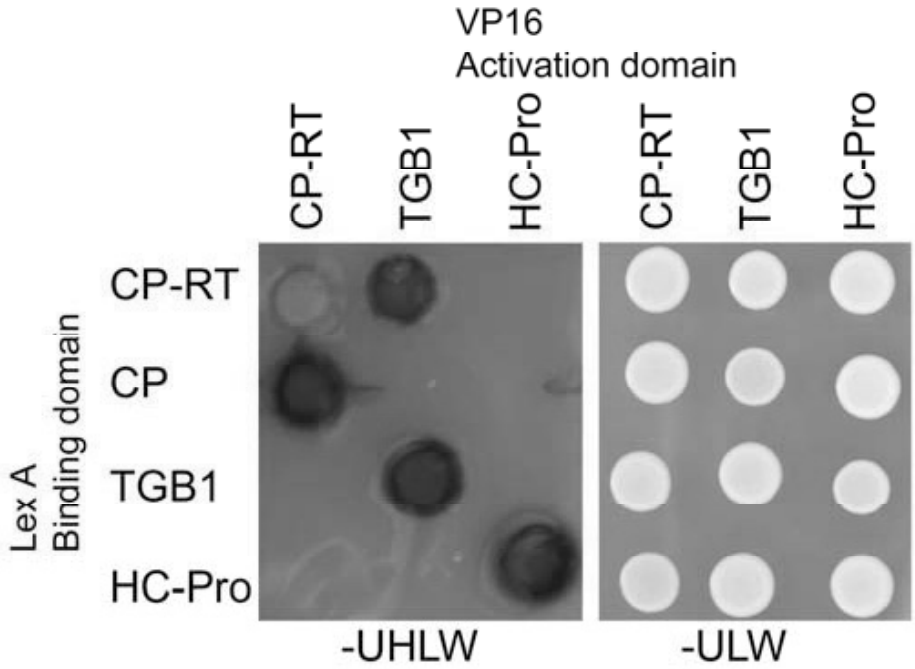

Fig 2. Interactions of PMTV proteins in the yeast two-hybrid system. Right panel: transformants were selected on medium lacking uracil, leucine and tryptophan (-ULW) to indicate growth of input plasmids. Left panel: transformants selected on medium lacking ULW and histidine (-UHLW) to indicate protein interactions. The left panel was also stained with ß-galactosidase to indicate the relative strength of the interactions. HC-Pro was used as a self-interacting control.

Discussion

Mutant RNA-CP clones carrying various deletions in the CP-RT coding sequence were used to investigate the long-distance movement of PMTV RNAs. The results show that systemic movement of RNA-CP was inhibited when truncated CP-RT was expressed together with CP but the movement of the other two genomic RNAs was unaffected. In contrast, RNA-CP mutants that expressed only the CP-RT polypeptide or no protein at all were able to move long-distance, whereas mutants that expressed only CP were inhibited in systemic movement. PMTV RNA1 and RNA-TGB have been shown to move long-distance in the absence of RNA-CP and must move as viral ribonucleoprotein (vRNP) complexes. The composition of PMTV vRNP is unknown but by analogy with *Barley stripe mosaic virus* most likely contains TGB1 (Lim et al., 2008). CP-RT expression does not enable virion formation and so presumably in this mutant (or mutant 7 where no protein is expressed), the RNA-CP can move as a vRNP complex similar to the other two RNAs. It is possible that when CP is expressed alone virus particles are formed but they are defective in long-distance movement. Movement defective CP-RT mutants also formed virus particles and protein interaction studies using the yeast two-hybrid system revealed that CP-RT and the movement protein TGB1 interact but TGB1 does not interact with CP. TGB1 also failed to interact with truncated CP-RT. The results suggest a novel hypothesis that virions competent in long-distance movement may require presence of CP-RT and TGB1 at one end and this is the subject of further study.

Acknowledgements

Research at SCRI is supported by the Scottish Government Rural Environment and Research Analysis Directorate; E. I. S received a sabbatical stipend from the Wenner-Gren Foundations, Stockholm.

References

Cowan, G.H., Lioliopoulou, F., Ziegler, A., and Torrance, L. 2002. Subcellular localisation, protein interactions and RNA binding of *Potato mop top virus* triple gene block proteins. Virology 298:106-115.

Cowan, G.H., Torrance, L., and Reavy, B. 1997. Detection of *Potato mop top virus* capsid readthrough protein in virus particles. J. Gen. Virol. 78:1779-1783.

Koenig, R., and Lesemann, D.E. 2005. Genus pomovirus. *In* van M.H.V. Regenmortel, C. Fauquet and D.H.L. Bishop (ed.), Virus Taxonomy, eighth report of the international committee on taxonomy of viruses, Harcourt International.

Lim, H.-S., Bragg, J.N., Ganesan, U., Lawrence, D.M., Yu, J., Isogai, M., Hammond, J. and Jackson, A.O. 2008. Triple gene block protein interactions involved in movement of *Barley stripe mosaic virus* J. Virol. 82: 4991-5006.

Reavy, B., Arif, M., Cowan, G.H., and Torrance, L. 1998. Association of sequences in the coat protein/readthrough domain of *Potato mop top virus* with transmission by Spongospora subterranea. J. Gen. Virol. 79:2343-2347.

Sandgren, M., Savenkov, E.I., and Valkonen, J.P.T. 2001. The readthrough region of *Potato mop top virus* (PMTV) coat protein encoding RNA, the second largest RNA of PMTV genome, undergoes structural changes in naturally infected and experimentally inoculated plants. Arch. Virol. 146:467-477.

Savenkov, E.I., Germundsson, A., Zamyatnin, A.A.Jr., Sandgren, M., and Valkonen, J.P.T. 2003. *Potato mop top virus*: the coat protein-encoding RNA and the gene for cysteine-rich protein are dispensable for systemic virus movement in Nicotiana benthamiana. J. Gen. Virol. 84:1001-1005.

Yelina, N.E., Savenkov, E.I., Solovyev, A.G., Morozov, S.Yu., and Valkonen, J.P.T. 2002. Long-distance movement, virulence, and RNA silencing suppression controlled by a single protein in Hordei- and Potyviruses: complementary functions between virus families. J. Virol. 76:12981-12991.