Phytoprotection

Incidence of virus diseases in maize fields in the Trakya region of Turkey
Incidence des maladies virales dans les champs de maïs de la région de Trakya en Turquie

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See table of contents

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Article abstract

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Incidence of virus diseases in maize fields in the Trakya region of Turkey

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Keywords: ELISA, IC-RT-PCR, JGMV, maize, MDMV, SCMV.

[Incidence des maladies virales dans les champs de maïs de la région de Trakya en Turquie]

Une enquête sur les maladies virales du maïs a été menée dans la région de Trakya en Turquie avec l'examen, en 2004 et 2005, de respectivement 32 496 et 46 871 plantes. Selon l'endroit, le taux de plantes symptomatiques a varié entre 3,7 et 63,6 %. Les résultats de tests biologiques et sérologiques ont révélé la présence de la souche PAV du virus de la jaunisse nanisante de l'orge (BYDV-PAV), du virus de la mosaïque nanisante du maïs (MDMV), du virus de la mosaïque de la canne à sucre (SCMV) et du virus de la mosaïque du sorgho d'Alep (JGMV). En 2004, 142 échantillons foliaires ont été recueillis aléatoirement sur 6492 plantes présentant des symptômes. Soixante-douze des 142 échantillons étaient infectés par le MDMV, deux étaient infectés par le BYDV-PAV, 19 par le MDMV et le BYDV-PAV, deux par le MDMV, le BYDV-PAV et le SCMV, alors qu'un seul contenait les quatre virus. En 2005, 100 nouveaux échantillons foliaires ont été recueillis aléatoirement de 11 739 plants de maïs présentant des symptômes. Des tests sérologiques ont montré que 50 % des échantillons étaient infectés par le MDMV et le SCMV; cependant, cinq échantillons étaient infectés par des combinaisons de deux ou trois des virus testés. Des infections par un seul virus parmi le MDMV, le SCMV, le BYDV-PAV et le JGMV ont été respectivement détectées dans cinq, trois, deux et quatre échantillons. La présence du MDMV a été confirmée par buvardage Western et par immunocapture suivie de transcription inverse et réaction en chaîne de la polymérase (IC-RT-PCR). Le SCMV a aussi été détecté par IC-RT-PCR. La présente étude est la première à signaler, en Turquie, la présence du SCMV et du JGMV dans le maïs.

Mots clés : ELISA, IC-RT-PCR, JGMV, maïs, MDMV, SCMV.

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INTRODUCTION

Maize (*Zea mays* L.) production has been increasing steadily every year in Turkey since 2000. In 2005, the cultivated area reached 800 000 ha for grain maize and 29 500 ha for silage maize, with 3 500 000 tons of grain crops and 720 000 tons of silage harvested (FAO Statistics 2005). Virus diseases on maize, which cause reduction in yield, have occurred recently. Among them some important viruses belonging to the potyvirus group have been detected (Berger *et al.* 2005); these viruses can cause significant losses in agricultural, pastoral, horticultural and ornamental crops (Shukla *et al.* 1994). By evaluating their biological, serological and molecular properties, Shukla *et al.* (1992) reclassified the sugarcane mosaic subgroup of potyviruses and identified sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), sorghum mosaic virus (SrMV) and Johnson grass mosaic virus (JGMV) as distinct viruses. Recently, Zea mosaic virus (ZeMV), another potyvirus species infecting sorghum and maize in Israel, was identified by Seifers *et al.* (2000). Another virus infecting maize and sorghum, described by Fan *et al.* (2004), was proposed as another member of the SCMV subgroup of potyviruses and named Pennisetum mosaic virus.

Following the initial description and identification of MDMV infections in the United States by William and Alexander (1965), Ellet *et al.* (1965) and Josephson *et al.* (1969) had reported MDMV epidemics and a reduction in grain yield in the United States, where maize is a major field crop. For the first time, Bremer and Raatikainen (1975) described a mosaic virus disease on maize transmissible by *Myzus persicae* (Sulz.) [Homoptera: Aphididae] in western Turkey, which was probably caused by MDMV. Baloglu *et al.* (1991) also reported MDMV infections in maize fields in the Çukurova region of Turkey. Besides individual infections of different viruses on maize, co-infections are also very common. For example, co-infections by MDMV, SCMV, barley yellow dwarf virus BYDV-MAV and BYDV-PAV were reported by Huth and Lesemann (1991) in Germany. Co-infection by SCMV-MDB and SCMV-BC was reported by Krstic and Tosic (1995) in Yugoslavia, and co-infections by MDMV and SCMV were detected in the Czech Republic by Pokorny and Porubova (2001). Finally, Fidan and Yılmaz (2004) detected individual infections and co-infections of MDMV, maize mosaic virus (MMV), maize stripe virus (MSpV) and BYDV on maize plants, on some weed species, in aphid vectors as well as in plant hopper species by employing reverse transcription-polymerase chain reaction (RT-PCR) in the Çukurova region of Turkey. However, SCMV in sugarcane plants was detected by Putra *et al.* (2003) using RT-PCR on infected plants obtained after artificial inoculation. They also found that this virus moved more slowly in the moderately resistant sugarcane cultivars than in the susceptible ones. Furthermore, by comparing antibody-based tests with genome-based techniques, Balamuralikrishnan *et al.* (2004) determined that the RT-PCR assay method was more sensitive for detecting SCMV in sugarcane plants with less predominant symptoms than ELISA tests.

Besides maize virus diseases, other cereal virus diseases have been occurring with high rates of incidence in the Trakya region of Turkey since 1999. Ilbagi *et al.* (2005) identified wheat streak mosaic virus (WSMV), oat necrotic mottle virus (ONMV) and BYDV-PAV on winter wheat, *Triticum aestivum* (L.); barley, *Hordeum vulgare* (L.); and oat, *Avena sativa* (L.), in this region of Turkey. Pocsai *et al.* (2003) and Ilbagi *et al.* (2003) also detected the presence of BYDV-PAV, BYDV-MAV, BYDV-RMV, BYDV-SGV, cereal yellow dwarf virus-RPV (CYDV-RPV) and wheat dwarf virus (WDV) in major cereal producing provinces of Turkey. These cereal viruses overwinter naturally in the winter sown cereal crops. However, their summer hosts are unknown and must be identified among annual and perennial plants from this region. Maize, one of the host plants, has been grown both as grain maize and silage maize in the Trakya region of Turkey, and their production has increased steadily.

In order to assess maize as a summer host of some cereal viruses and to identify major maize viruses, a two-year study was conducted using serological DAS-ELISA and PTA-ELISA tests as well as molecular techniques on infected maize leaf samples collected from the Trakya region of Turkey.

MATERIALS AND METHODS

Sampling

In June and July 2004, a survey was carried out for the detection of cereal viruses on maize in the Trakya region of Turkey. Samples were collected from field trial experiments established as randomized block design sown with 36 maize accessions in four different locations of the Tekirdag and Kırklareli provinces. A total of 142 samples were collected from maize plants showing infection symptoms, such as dwarfing, streaks and chlorosis along the leaves, which formed mosaic or mottled patterns and a general yellowing and reddening of the leaves. In order to determine the rate of infection, symptomatic plants were counted among the total number of maize plants in each location and the percentage of the symptomatic plants was calculated as a rate of infection. The rate of infection was determined in four locations. The survey was repeated again in July 2005 in the maize field trial experiments established as randomized block design with 21 accessions in three locations of the Trakya region. In order to confirm the 2004 results, 100 maize leaf samples were collected from plants showing characteristic virus symptoms. Samples were kept at -80ºC until use in biological and serological tests.

Bioassays on tests plants

In October 2004, young seedlings were inoculated with sap obtained from infected maize just before tiller stage. Corn seedlings at the four-leaf stage were used as part of a series of six indicator plant species. For this purpose, two pots of seedlings of wheat, *Triticum aestivum* cv. Alcedo; barley, *Hordeum vulgare* cv. Erfa; rye, *Secale cereale* (L.) cv. Nikita; corn, *Zea mays* cv. Helix; bird seed, *Phalaris canariensis* (L.) cv. Sp.P1.(1753)54; and millet, *Panicum miliaceum* (L.) cv. Local German, were dusted with celite and

mechanically inoculated with each inoculum. Inocula were prepared with 1:10 dilution (wt/vol.) by grinding leaves of infected maize leaf samples in potassium phosphate buffer (0.05 M, pH 7.0). Indicator plants were inoculated with sap from 142 infected maize leaf samples, washed under tap water, maintained at greenhouse conditions and examined for characteristic symptom expression. Three weeks after inoculation, positive and negative reactions were recorded.

Transmission electron microscopy (TEM)
Extracts obtained from the different symptomatic maize plants of the field trials were placed on grids individually. They were negatively stained with 2% uranyl acetate (Milne 1984) and examined with a transmission electron microscope (Zeiss Opton 902, Germany), and electron micrographs were taken from the observed virions.

Double antibody sandwich-ELISA (DAS-ELISA)
A double antibody sandwich-immunosorbent assay test (DAS-ELISA) was used as described by Clark and Adams (1977). Eleven antisera prepared against MDMV, SCMV, JGMV, BYDV-PAV, CYDV-RPV, WDV, WSMV, ONMV, cocksfoot mottle virus (CofMV), wheat spindle streak mosaic virus (WSSMV), and barley stripe mosaic virus (BSMV) were used to determine the presence of these viruses in maize leaf samples. Antisera were produced from rabbits at the Institute of Resistance Research and Pathogen Diagnostic (Aschersleben, Germany). The absorbance of the samples at 405 nm was measured using a microplate reader (Tecan Spectra, Salzburg, Austria) after incubation for 1 h at room temperature. Readings of samples were considered positive when their absorbance values were greater than three times the mean value of the negative control.

Plate-trapped antigen-ELISA (PTA-ELISA)
In order to detect the occurrence of potyviruses in the samples, an indirect plate-trapped antigen-ELISA (PTA-ELISA) was performed with Nunc polysorb ELISA plates using broad reacting antibodies according to Richter *et al.* (1995). The plant extracts were diluted with 1:50 coating buffer solution and plates were coated overnight at 4°C with 100 µL of the extracts. The plates were emptied without washing. Blocking solution containing 1% non-fat dry milk powder in phosphate buffer solution (PBS, pH 7.4) was added and incubated for 1 h at 37°C and was then washed three times with phosphate buffer solution+ tween 20 (PBST, pH 7.4). Two microlitres of antiserum number PAS-314 to turnip mosaic virus (TuMV) showing broad cross-reactions to members of the Potyviridae (Richter *et al.* 1994) diluted with 10 mL of PBS was added and incubated for 2 h at 37°C and washed four times with PBST. Then, 100 µL of goat anti-rabbit IgG labelled with alkaline phosphatase at a dilution of 1:2000 in Tris HCl-buffer (pH 8.0) was added and incubated for 1 h at 37°C. After washing four times with PBST, the conjugate was detected by addition of p-nitrophenyl phosphate (1 mg 1 mL^{-1}) in 0.1 M diethanolamine buffer (pH 9.8). The absorbance of the samples at 405 nm was measured using a microplate reader (Tecan Spectra, Salzburg, Austria) after incubation for 1 h at room temperature. Similarly to DAS-ELISA, readings of the samples were considered positive when their absorbance value at 405 nm was greater than three times the mean value of the negative control.

SDS-PAGE and Western blot analysis
Maize leaf samples for polyacrylamide gel electrophoresis were prepared by grinding leaf tissue in water (1:3, w/v) and, after a brief centrifugation (10,000 rpm for 3 min) the supernatant was mixed with an equal volume of SDS buffer. The plant samples were boiled for 3 min and proteins separated by electrophoresis on SDS-10% polyracrylamide gels. After electrophoresis, the viral coat proteins were electrophoretically transferred to a nitrocellullose membrane (Hybond-ECL, Göttingen, Germany) in a Trans-Blot Semi Dry system (Biometra, Göttingen, Germany) in a transfer buffer (39 mM glycine, 48 mM tris base, 0.037% SDS, 20% methanol). The nitrocellullose membrane was incubated in blocking solution (1% non-fat dry milk powder in Tris buffer (pH 8.0)) for 30 min, and 300 µL IgG (1 mg 1 mL^{-1}) was diluted with 30 mL Tris buffer (pH 8.0) and shaken for 1 h. It was then washed with Tris buffer (pH 8.0). After that, 15 mL of goat anti-rabbit IgGs conjugated with alkaline phosphatase (Dianova, Hamburg, Germany) were added to 30 mL Tris buffer (pH 8.0) and shaken for 1 h, washed four times in Tris buffer (pH 8.0), and another four times in Tris buffer (pH 9.0). Finally, the membranes were exposed to a substrate solution of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and p-nitroblue tetrazoliumchloride (NBT).

Immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR)
In order to detect MDMV and SCMV at the molecular level, IC-RT-PCR was carried out. Polyclonal IgG prepared against MDMV was diluted at 1:50 in coating buffer and added to PCR tubes. The tubes were incubated for 4 h at 37°C and were then washed three times with PBS and once with distilled water. Twelve maize leaf samples, freshly obtained from mechanical inoculations of the sap obtained from naturally infected maize samples, were homogenized and their extracts were diluted at 1:10 in PBS buffer, and 100 µL of the extracts were put into PCR tubes. After overnight incubation at 4°C, the tubes were washed three times with PBS and once with distilled water. Universal primers that transcribed and replicated a 1.6 to 2.1 kbp fragment from the 3´ end of the genome of potyvirids were used according to Gibbs and Mackenzie (1997) and as verified by Chen *et al.* (2001). In order to obtain RT of 12 samples in 12 different tubes, a reaction mixture was prepared by adding of 1 pmol primer 3´ Potyg (dT)3 (CAC GGA TCC TTT TTT TTT TTT TTT TTV, V = C or G, T) as initial primer, 8 µL 5 mM dNTP, 8 µL 5x buffer, 0.25 µL RNase inhibitor, and 23 µL water. For the cleaving of the virus particles, 35 µL reaction mixture was added for each tube and RT-1 started with the following temperature program: 5 min at 70°C, 10 min at 42°C, and the reaction ended at 4°C. In order to start c-DNA synthesis, 5 µL reaction mixture, which contained 0.25 µL M-MLV reverse transcriptase enzymes, was added to each sample and the following timing program was used: 1 h at 42°C, 5 min at 96°C, and the reaction ended at 4°C. That is how c-DNA was obtained. In the next step, the following reaction

mixture was used for PCR reaction: 3 µL 10x buffer (Biogene), 3 µL 1 mM dNTP, 0.3 µL Primer 3´ Potyg(dT)3 (CAC GGA TCC TTT TTT TTT TTT TTT TTV, V = C or G, T) (20 pmol), 0.3 µl Primer 5´ Potyg (CCA CGG ATC CGG BAA YAA YAG YGG DCA RCC, B = A or G, Y = C or T, D = A, G or T, R = A or G) (20 pmol), 0.2 µL Taq DNA polymerase, and 23.5 µL water for each tube. Finally, it was mixed and 5 µL c-DNA product and 30 µL PCR reaction mixture were added into a separate tube. The conditions for degenerate PCR amplifications to amplify fragments of the 3´ end of the potyvirus genome were as previously described by Chen *et al.* (2002). Products of IC-RT-PCR fragments were isolated electrophoretically on 1.6% agarose-gel.

RESULTS

A survey conducted in 2004 in maize field trials sown with 36 maize accessions revealed that characteristic virus symptoms such as yellow stripes, dwarfing and redness on leaves were observed in four different locations of the Tekirdag and Kırklareli provinces. The average rates of symptomatic plants were in the range of 3.7 to 63.6% in the different locations (Table 1). A 20% average rate of symptomatic plants in maize fields was recorded in the Trakya region of Turkey in 2004. Results of the survey conducted in July 2005 are shown in Table 1. The highest rate of symptomatic plants recorded was 52.7% and the lowest rate was 8.0%. The average rate of symptomatic plants was 25.1% in 2005.

Visible symptoms of virus infections were observed on maize seedlings as early as June for both years. Similar symptoms were also observed on some perennial weeds such as *Phragmites communis* (L.) in the vicinity of these fields. Survey results obtained from the Tekirdag field trials revealed a higher rate of symptomatic plants than in the Kırklareli field trials.

The results of mechanical inoculations made using the sap of 142 samples revealed that only 12 samples caused stripe mosaic symptoms on the maize cultivar (Helix) used as an indicator test plant set. There were no symptoms on the other species.

TEM studies revealed the presence of flexuous rod-shaped virus particles of about 727 nm in length in negatively stained grids prepared from infected leaf extracts of 10 maize plants as indicated in Figs. 1 and 2.

As a results of the DAS-ELISA and PTA-ELISA tests, the mechanically transmissible MDMV, SCMV and JGMV viruses and the non-mechanically transmissible BYDV-PAV viruses were identified as shown in Table 2. A total of 96 samples out of the 142 collected in 2004 revealed the presence of at least one of these four viruses. The results of the PTA-ELISA tests that were carried out using antiserum prepared against TuMV, which shows broad cross-reactions with potyviruses, confirmed the results of the DAS-ELISA tests. With this confirmation, there was no need to repeat PTA-ELISA tests for the samples collected in 2005. Both serological tests revealed that MDMV was the most widespread virus, affecting 94 out of 142 tested maize samples, followed by BYDV-PAV, detected on 24 samples in individual and mixed infections. Two samples contained SCMV and only one sample had JGMV as part of a mixed infection. DAS-ELISA tests performed on the samples collected in 2005 revealed that 63 out of 100 samples were infected with at least one of the four viruses in individual or mixed infections. Fifty-three out of 100 maize leaf samples contained MDMV, and 52 out of 100 samples contained SCMV. JGMV was detected in seven samples and BYDV-PAV was detected in five samples, as shown in Table 2.

MDMV coat proteins of eight Turkish isolates from the Tekirdag and Kırklareli provinces were analyzed

Table 1. Average rates of virus symptomatic plants in maize field trials in the Trakya region of Turkey in 2004 and 2005

Year	Location	Maize plants (total number)	Symptomatic plants (number)	Symptomatic plants (%)
2004	T.V.R.I.[a]	7 832	4 010	51.2
	K.A.R.I.R.A.[b]	17 323	804	4.6
	K.L.S.S.F.[c]	4 991	184	3.7
	T.F.A.R.F.[d]	2 350	1 494	63.6
Total		32 496	6 492	20.0
2005	T.V.R.I.[a]	14 472	7 631	52.7
	K.A.R.I.R.A[b]	15 955	1 275	8.0
	K.L.S.S.F.[c]	16 444	2 833	17.23
Total		46 871	11 739	25.05

[a] Tekirdag – Viticulture Research Institute.
[b] Kırklareli – Atatürk Research Institute for Rural Affairs.
[c] Kırklareli – Lüleburgaz Sarmısaklı State Farm.
[d] Tekirdag – Faculty of Agriculture Research Field.

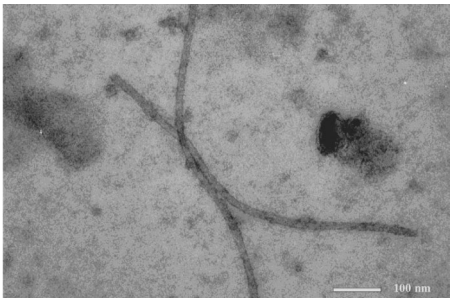

Figure 1. Electron micrograph of the maize dwarf mosaic virus (MDMV).

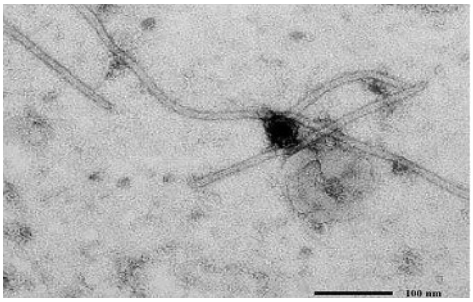

Figure 2. Electron micrograph of the sugarcane mosaic virus (SCMV).

from infected maize samples using Western blot analysis. The MDMV antibodies clearly reacted with 32 kDa coat protein molecules as shown in Fig. 3. Samples Nos. 1, 2, 3, 5, 6 and 8 reacted with higher molecular bands. Thus, MDMV proteins were readily detected in the infected maize leaf samples collected from the Trakya region even though sample No. 5 exhibited two bands. However, sample No. 7 had a very weak reaction and sample No. 4 did not exhibit any band at all in Western blot analysis in spite of their positive reaction in DAS-ELISA and PTA-ELISA tests.

Immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) was performed using potyvirus specific primers reacting also with MDMV and SCMV. For this purpose, 10 infected maize samples obtained by inoculating maize cultivar Helix with sap and that showed positive reactions to MDMV along with one sample that had a positive reaction to SCMV in biological and serological tests were used. The results revealed that PCR products of approximately 1800 bp, 800 bp and 344 bp in size were identified in the infected samples. According to the results shown in Fig. 4, ten maize leaf samples showed fragments of 1800 bp and 800 bp indicative of MDMV. Sample No. 11, from Kırklareli, gave two different bands, one of which was slightly higher than 1800 bp and 344 bp, indicative of SCMV. Samples Nos. 2 and 6 revealed the presence of all three fragments due to the presence of another strain of MDMV.

This confirmed the presence of two different strains of Turkish isolates of Turkish MDMV and one isolate of SCMV. A summary of the test results obtained in 2004 on selected maize samples is presented in Table 3.

In light of these results, it appears that MDMV was present in all leaf samples and that samples Nos. 1 and 8 contained BYDV-PAV, while sample Nos. 4 and 11 contained SCMV. However, JGMV was only identified in sample No. 4.

Table 2. Virus identification results of serological tests in symptom expressing maize samples collected in 2004 and 2005 in the Trakya region of Turkey

			Number of samples infected by different viruses and virus combinations									
Year	**Location**	**Samples (number)**	MDMV	SCMV	JGMV	BYDV-PAV	MDMV + SCMV	MDMV+ BYDV-PAV	SCMV+ JGMV	MDMV+ SCMV+ BYDV-PAV	MDMV + SCMV+ JGMV	MDMV+ SCMV+ JGMV+ BYDV-PAV
2004	T.V.R.I.[a]	58	30	-	-	1	-	11	-	1	-	1
	K.A.R.I.R.A.[b]	19	11	-	-	-	-	1	-	-	-	-
	K.L.S.S.F.[c]	18	6	-	-	1	-	-	-	1	-	-
	T.F.A.R.F.[d]	47	25	-	-	-	-	7	-	-	-	-
Total		142	72	-	-	2	-	19	-	2	-	1
2005	T.V.R.I.[a]	43	1	1	3	1	26	-	-	1	-	-
	K.A.R.I.R.A.[b]	30	1	2	-	-	6	-	-	-	2	-
	K.L.S.S.F.[c]	27	3	-	1	1	11	-	1	2	-	-
Total		100	5	3	4	2	43	-	1	3	2	-

[a] Tekirdag – Viticulture Research Institute.
[b] Kırklareli – Atatürk Research Institute for Rural Affairs.
[c] Kırklareli – Lüleburgaz Sarmısaklı State Farm.
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DISCUSSION

Virus infections on maize cultivars have been recorded in maize field trials located in the Trakya region of Turkey in 2004 and 2005. The average rates of symptomatic plants were 20% in 2004 and 25.1% in 2005. Similar rates of MDMV infection had been reported by Williams and Alexander (1965). Also, Josephson *et al.* (1969) in the United States and Huth and Lesemann (1991) in Germany had reported mixed MDMV and SCMV infections. The results presented in Table 1 reveal rates of symptomatic plants similar to those reported by Tosic and Mijavec (1991) in Yugoslovia. In contrast to their high rate of SCMV infection, we recorded a higher rate of MDMV infection than of SCMV on maize in 2004, and almost similar infection rates for both viruses in 2005.

Mechanical sap transmission made from infected maize samples to six indicator plants revealed stripe mosaic symptoms on maize cultivar Helix in contrast to other bio-indicator species that did not exhibit any symptoms at all. This was confirmed by detecting MDMV on 10 samples and SCMV on only one sample using the IC-RT-PCR test. Conversely, Garrido *et al.* (1996) transmitted MDMV to a number of differential hosts by mechanical inoculations. In our TEM studies, flexuous rod-shaped virus particles of about 727 nm in length were observed in negatively stained infected leaf extracts of MDMV and SCMV, as shown in Figs. 1 and 2. Virion measurements of both viruses were in the range of potyviruses dimensions. However, using immunoelectron microscopical staining techniques, Huth and Lesemann (1991) measured longer particles for MDMV and SCMV, and Aftab *et al.* (1990) identified flexuous rods measuring about 750-800 nm in length from naturally infected maize hybrids. These measurements were therefore longer than the virion measurements in this study.

Identification of MDMV on maize with a high incidence rate, as shown in Table 2, is in agreement with the results of Bremer and Raatikainen (1975), Baloglu *et al.* (1991) and Fidan and Yılmaz (2004). Serological DAS-ELISA and PTA-ELISA test results revealed that MDMV was found in 72 of the 142 samples in 2004, and 53 out of 100 samples in 2005, as individual and mixed infection with SCMV, JGMV and BYDV-PAV from tested maize plants. MDMV was shown to be the most widespread virus in the Trakya region of Turkey.

BYDV-PAV was detected in 21 out of the 142 tested maize plants as individual and mixed infections. Fidan and Yılmaz (2004) had previously reported BYDV on maize in the Çukurova region of Turkey, but could not identify which BYDV species was present. According to our results, BYDV-PAV was identified as the virus spending summer on maize plants in the Trakya region, which had already been reported by Ivanovic *et al.* (1992) in Yugoslavia. Besides maize, Ilbagi (2006) identified *P. communis* as a summer host of BYDV-PAV in this region. BYDV-PAV has become the most important virus in cereals in Turkey as reported by Ilbagi *et al.* (2003, 2005) and Pocsai *et al.* (2003).

SCMV was found in 3 out of 142 tested samples as a mixed infection using both serological methods in 2004, and was identified in 52 out of 100 samples in 2005 in individual or mixed infections. The Turkish isolate of SCMV was propagated in maize plants, purified by ultracentrifugation and used for antiserum production in rabbits (F. Rabenstein, unpublished). This is the first reported identification of SCMV in Turkey using ELISA and IC-RT-PCR tests. SCMV was identified as one of the major viruses on maize in European countries (Huth and Lesemann 1991; Krstic and Tosic 1995; Pokorny and Porubova 2001).

As a result of the serological tests, JGMV was identified in only one sample along with three other viruses (MDMV, SCMV and BYDV-PAV) in 2004, and in 7 out of 100 samples in 2005. Being a distinct potyvirus as reclassified by Shukla *et al.* (1992) and differentiated by Marie-Jeanne *et al.* (2000) from other Poaceae viruses with a PCR test, JGMV was identified on maize samples in Turkey. This is the first report of the presence of JGMV in the Trakya region of Turkey.

In addition to serological test, MDMV was unequivocally identified using Western blot analysis as the same molecular weight of the capsid protein of MDMV was detected by Signoret and Alliot (1995). Qertel *et al.* (1997) identified three bands of the viral capsid proteins about 34 kDa, 36 kDa and 37 kDa in different SCMV isolates using Western blot analysis, and an unusual isolate of MDMV was found in Hungary by

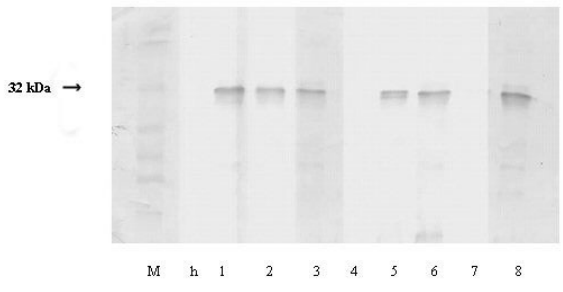

Figure 3. Estimation of molecular weights of the coat proteins of MDMV isolates using Western blot analysis (1-8: Tekirdag and Kırklareli isolates of MDMV; h: healthy control; M: marker, molecular weight markers in kDa).

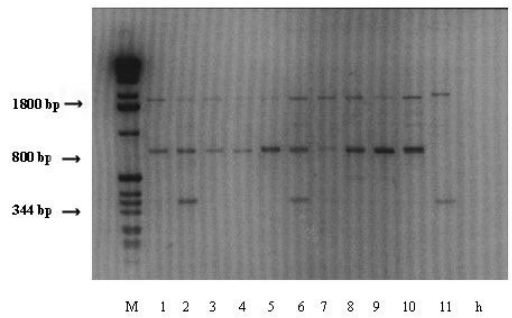

Figure 4. Molecular bands obtained as a result of IC-RT-PCR tests conducted on MDMV and SCMV infected maize samples (1-11 samples; h: healthy control; M: marker, molecular weight markers in bp).

Table 3. Summary of test results of selected maize samples collected in 2004

Sample (No.)	Designation of sample	DAS-ELISA*	PTA-ELISA*	Western blot analyses	IC-RT-PCR
1	Tekirdag (T.V.R.I.)	2.862	1.803	1 band	2 bands
2	Tekirdag (T.F.A.R.F.)	3.209	2.681	1 band	3 bands
3	Kırklareli (K.L.S.S.F.)	2.121	2.020	1 band	2 bands
4	Tekirdag (T.V.R.I.)	2.682	2.123	no band	2 bands
5	Kırklareli (K.L.S.S.F.)	3.316	2.929	2 bands	2 bands
6	Tekirdag (T.V.R.I.)	3.387	3.103	1 band	3 bands
7	Tekirdag (T.V.R.I.)	1.989	1.678	1 band	2 bands
8	Tekirdag (T.F.A.R.F.)	2.883	1.375	1 band	2 bands
9	Tekirdag (T.F.A.R.F.)	2.837	1.232	not tested	2 bands
10	Tekirdag (T.F.A.R.F.)	2.212	1.645	not tested	2 bands
11	Kırklareli (K.L.S.S.F.)	1.101	1.023	not tested	2 bands
Healthy controls: 0.200-0.215					
Positive controls: 1.930-1.922					

* $E_{405\ nm}$ after 1 h substrate incubation.

Tobias and Palkovics (2004) that contained a stretch of 13 additional amino acids in its coat protein. In addition to Western blot analysis, the results of IC-RT-PCR revealed three molecular bands of MDMV and two molecular bands of SCMV. These results of IC-RT-PCR confirmed that samples Nos. 1 to 10 are MDMV, as already shown using DAS-ELISA tests and Western blot analysis. Although Ricaud *et al.* (1989) pointed out the presence of SCMV in Turkey according to observations made in 1956, this study revealed natural infections of this virus on maize by employing reliable serological and molecular tests in Turkey.

Overall, in the maize samples collected from two provinces of the Trakya region, four viruses were identified: MDMV, SCMV, JGMV and BYDV-PAV. Among them, SCMV and JGMV were detected for the first time in Turkey. Therefore, this is the first report revealing the presence of SCMV and JGMV on maize in Turkey. Three molecular bands of MDMV and two molecular bands of SCMV were detected in the IC-RT-PCR tests. Thus, these results show that there are presumably two different strains of MDMV and one strain of SCMV. The use of specific primer combinations and subsequent sequencing (Tobias and Palkovics 2004; Yang and Mirkov 1997) of the genomes of these Turkish isolates would be desirable to analyze their phylogenetic relationships with other maize infecting potyviruses.

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