DISEASE NOTE

FIRST REPORT OF CANDIDATUS PHYTOPLASMA MALI IN TURKEY

G. Sertkaya¹, M. Martini² and R. Osler²

¹Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, 31034 Antakya, Turkey
²Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy

In Turkey, 17 randomly selected apple orchards were surveyed from August to September 2004 in Adana (6 orchards) and Mersin (3 orchards) provinces of eastern Mediterranean region and Nigde (8 orchards) province of central Anatolia for the presence of apple proliferation disease. Since symptoms resembling those induced by this disease were observed, shoot and leaf samples from 10 different cultivars were collected from 28 symptomatic and 3 symptomless trees for laboratory testing. Total genomic DNA extracted from leaf mid-veins as described by Malisano et al. (1996) was used for nested-PCR with universal primers P1/P7 in direct PCR, followed by primer pair R16F2n/R16R2. RFLP analysis with TruI II gave the same pattern as AP15 and PD1 reference phytoplasma strains. Nested-PCR with 16SrX group-specific primer pair f01/r01 (Lorenz et al., 1995) confirmed the 16Sr phytoplasma group affiliation. RFLP patterns with BsuRI and SspI of f01/r01 PCR products were the same as that of AP15 reference strain, providing evidence that Candidatus Phytoplasma mali of subgroup 16SrX-A (Lee et al., 1998) occurred in infected Golden Delicious trees from Adana (1/3 positive sample) and Nigde (2/2) and in cvs Scarlet Spur (2/4) and Jonathan (1/3) from Adana. Thus, 6 of 28 apples showing typical proliferation symptoms such as witches’-broom and chlorotic leaves with enlarged stipules tested positive for C. Phytoplasma mali. Further surveys will investigate the presence of the same pathogen in other important apple-growing regions of Turkey and of its psyllid vectors. To our knowledge this is the first report of C. Phytoplasma mali in Turkey.


Corresponding author: M. Martini
Fax: +39.0432.558501
E-mail: marta.martini@uniud.it
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DISEASE NOTE

DETECTION OF A BEGOMOVIRUS ASSOCIATED WITH LEAF CURL DISEASE OF ORNAMENTAL CROTON (CODIEUM VARIEGATUM) IN SOUTHERN INDIA

Y.S. Mahesh¹, K.S. Shankarappa¹, K.T. Rangaswamy¹, H.A. Prameela¹, D.S. Aswathanarayana¹ and M.N. Maruthi²

¹Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bengaluru 560 065, India
²Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK

A leaf curl disease was observed on ornamental croton plants (Codieum variegatum, family Euphorbiaceae) in 15 home, office and botanical gardens in and around Bengaluru, South India. Diseased plants showed thickening of leaf veins, severe leaf curling, decrease in leaf size and stunting. The causal pathogen was transmitted to healthy croton plants by grafting with infected scions, and through whiteflies (Bemisia tabaci) given acquisition access and inoculation access periods of 24 h and 48 h, respectively. Association of a begomovirus with the disease was confirmed by amplification of viral DNA fragments of ca. 520 bp and 575 bp of the coat protein (CP) gene in begomoviral DNA A, with the aid of degenerate primers (Deng et al., 1994), from total DNA extracts of infected but not of healthy croton plants. An example of a 575 bp fragment, representing the core region of the CP gene (Brown et al., 2001) was cloned and sequenced. The resulting nucleotide sequence was most similar (94-96% identity) to the equivalent sequences of four begomoviruses from Asia [Ageratum yellow vein virus (AYVV, Pakistan), Crotalaria juncea leaf curl virus (CJLCV, Lucknow), Tobacco curly shoot virus (TbCSV, Yunnan 1) and Tomato leaf curl New Delhi virus (ToLCNDV)]. The extent of the relationship between the croton virus isolates from Bengaluru and that detected in C. variegatum in northern India (Raj et al., 1996) is not yet clear.


Corresponding author: K.T. Rangaswamy
Fax: +91.080.3330277
E-mail: ktr_uasb@rediffmail.com
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DISEASE NOTE

COMPLETE GENOME SEQUENCE OF AN INDIAN ISOLATE OF SUGARCANE YELLOW LEAF VIRUS

R.K. Gaur1, G.P. Rao2, M. Singh2 and A. Lehrer3

1 Department of Biotechnology, Faculty of Arts, Science and Commerce, MITS Laksbhanagar, Sikar, 332 311, Rajasthan, India
2 Sugarcane Research Station, Kunrhat, Gorakhpur, 273008, UP, India
3 Hawaii Biotech Inc., 99-193 Aiea Heights Drive, Aiea, Hawaii 96701, USA

Yellow leaf syndrome (YLS) has recently been recognized as new disease of sugarcane causing severe decreases in the sugar content of the sugarcane. Symptoms of the disease are yellowing leaves with a bright yellow midrib, often when the rest of the lamina is still green. In India, YLS has been linked to infection by Sugarcane yellow leaf virus (ScYLV; genus Polerovirus, family Luteoviridae) on the basis of particle morphology and serological relationships (Rao et al., 2000). In this study, we determined the complete nucleotide sequence of an Indian isolate of ScYLV by using reverse transcriptase-polymerase chain reaction (RT-PCR). Virions were purified from the leaves of sugarcane (cv. CoLk 8102) showing typical midrib yellowing using the enzyme-digestion method and RNA was extracted from them by phenol extraction, each as described by Moonan et al., (2000). For sequencing, the RNA was polyadenylated by using yeast polyA polymerase and oligo (dt)30 primer was used in RT-PCR procedures as described by Moonan et al. (2000). The 5899 nucleotide sequence of ScYLV-India (AY 236971) was found to be identical to that of isolate CP65-357 from Australia (AF491288) and 98% homologous to that of the Texas isolate (AF369928). The sequence differences resulted in 5 changes in amino acid sequence. Unrooted phylogenetic tree analysis revealed that ScYLV-India was most closely related to and in the same cluster as isolates CP92-1654, Florid 1999, LHo83-153, SP71-6163B, Q136 Argentina and CP65-357.


* Present Address: Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, SE 90187 Umeå, Sweden.

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DISEASE NOTE

FIRST REPORT OF FUSICOCCUM AESCULI CAUSING DIEBACK ON SWEET CHESTNUT (CASTANEA SATIVA Mill.) IN THE BLACK SEA COST OF TURKEY

S. Akilli1, Y.Z. Katircioglu2 and S. Maden2

1 Forestry Faculty of Karatekin University, Çankırı, Turkey
2 Department of Plant Protection, University of Ankara, 06110 Ankara, Turkey

During a survey for chestnut blight along the Black Sea coast of Turkey conducted in September 2006, cankers without fructifications were observed on sweet chestnut (Castanea sativa Mill.) trees in six provinces. The cankers differed from those of chestnut blight, which usually have reddish-yellow conidiomata of the causal agent, Cryphonectria parasitica. To determine the cause of the cankers, isolations were made from small pieces of inner-bark tissues on potato dextrose agar (PDA). Dark brown to almost black colonies were obtained that produced hyaline, thin-walled, aseptate, fusiform, straight and irregularly guttulate conidia with an obtuse apex and a distinctly truncate base, measuring 17.5-25×5-7.5 µm. The fungus was identified as Fusicoccum aesculi Dd apud Strum based on the description by Sutton (1980). Pathogenicity tests on four chestnut stems of 2-year-old saplings grown in the greenhouse were done by removing a bark disc 5 mm in diameter with a cork borer and placing mycelial plugs with conidia taken from a 10-day-old culture on PDA. Inocula were protected with Parafilm to prevent dehydration. Two months after inoculation, bark necrosis averaging 1.0×2.6 cm around the disc area was observed on chestnut shoots. The fungus was reisolated from the necrotic areas, while the control shoots inoculated with PDA plugs, remained healthy. This pathogen was isolated most frequently from Zonguldak, Ordu and Rize provinces. This is the first report of F. aesculi (teleomorph Botryosphaeria dothidea (Moug:Fr.) Ces. & De Not.) causing shoot blight on sweet chestnut along the Black Sea coast of Turkey.


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Corresponding author: R.K. Gaur
Fax: +91.157.3225044
E-mail: gaurrajarshi@hotmail.com

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Corresponding author:
Fax: +90.312.3187029
E-mail: maden@agri.ankara.edu.tr

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In May 2005 and 2006, diseased watermelon plants (cv. Giromontiana) showing mosaic symptoms and collapse of the leaves were observed in northern China. The yield losses were 30% to 50% in most fields, and occasionally the disease resulted in a complete loss of the crop. Based on symptoms, infection with Watermelon mosaic virus (WMV) was suspected. A week after inoculating healthy plants with extracts from diseased plants, mosaic symptoms were observed in the top leaves and, after a further two days, the diseased plants appeared wrinkled and malformed. Young leaves were collected from 80 randomly selected hosts from 10 regions in Shaanxi province and tested for the presence of WMV by using RT-PCR of total RNA extracts with primers specific for the WMV coat protein gene (Desbiez and Lecoq, 2004). In all tests, this yielded the expected 843 bp DNA amplicon. Sequences of amplicons obtained for one isolate from each region were all the same (GenBank Accession No. DQ399708), which suggests that the disease in Shaanxi province had spread from a single source. Comparison tests showed that the Shaanxi isolates were 93.0% to 95.0% identical in coat protein gene sequence to isolates from other countries (WMV-isolates were 93.0% to 95.0% identical in coat protein gene sequence to isolates from other countries (WMV-Spain AJ579524). The Shaanxi isolate coat protein gene differed from that of WMV Heilongjiang (AY464948), which suggests that the disease in Shaanxi province had spread from a single source. Comparison tests showed that the Shaanxi isolates were 93.0% to 95.0% identical in coat protein gene sequence to isolates from other countries (WMV-France, AY437609; WMV-USA, D13913; WMV-Japan, AB001994; WMV-Pakistan, AB127934; WMV-Australia, D00535; WMV-Israel, AF322376; WMV-Tonga, L22907; WMV-Spain AJ379524). The Shaanxi isolate coat protein gene differed from that of WMV Heilongjiang (AY464948) in 10 nucleotide positions and thus represents a distinct gene differed from that of WMV Heilongjiang (AY464948).}

Desbiez C, Lecoq H. 2004. The nucleotide sequence of Watermelon mosaic virus (WMV, Potyvirus) reveals inter specific recombination between two related potyviruses in the 5’part of the genome Archives of Virology 149:1619-1632.
DISEASE NOTE

A NEW CHINESE ISOLATE OF APPLE STEM GROOVING VIRUS FROM APPLES

H.N. Yue, Y.F. Wu, T. Wei, W. Hou, Y.R. Li and K.K. Wu

College of Life Science and Plant Protection and Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwestern A&F University, Yangling, Shaanxi 712100, China

China is one of the largest apple producers in the world and Shaanxi province in northwestern China produced 27% of the Chinese crop in 2005. Low yields and decline of the trees are widespread problems in this area. In a survey from May to July 2006 in Yangling, Shaanxi province, we found that many apple trees of cultivar Red Fuji had long grooves on the woody stem and obvious symptoms of decline suggesting infection with Apple stem grooving virus (ASGV). Bark samples were collected from six symptomless and 30 symptomatic apple plants in 10 different regions in Yangling and tested for the presence of ASGV by reverse transcription RT-PCR assay of total RNA extracts (Vera et al., 1998). Primers specific for the ASGV coat protein gene were designed based on comparisons among sequences available in Genebank (accession Nos. D14995, AF465354, AY886760, AF438409, AB004083, AF522459). Primer sequences were 5'-ATG AGT TTG GAA GAC GTG C-3' (ASGV-cp1) and 5'-CTA ACC CTC CAG TTC CAG A-3' (ASGV-cp2). The tests yielded a 714 bp fragment only with samples from symptomatic apple plants. Sequences of amplicons obtained for one isolate from each region were all the same (GenBank accession No. EU236258) and were identical at 657, 656 and 653 nucleotide positions, respectively, to the sequences of isolates from Brazil (AF438409), Japan (AB004063) and a Chinese pear (AY886760). To our knowledge, this is the first report characterising a Chinese isolate of ASGV from apples.


Sugar beet is a known natural host of Tobacco mosaic virus (TMV) (Krstic et al., 1997). In 2006, samples were collected from symptomless sugar beets in a field in the Inner Mongolia province of China. Viral RNA was extracted from virus partially purified from beet roots and tested by RT-PCR using a specific primer set [TMV-480F(+): 5'-CAT GAA GGC CAG AAA GAC AG-3' and TMV-1300R(-): 5'-GCC TGG TAT GTT CGA ATG TG-3']. The primer was designed to amplify a 821 bp fragment from the TMV RdRp gene (GenBank accession No. AJ011933). Eight of the eighteen sugar beet samples tested gave the amplicon of the expected size. An isolate recovered from Chenopodium quinoa leaves inoculated with the partially purified TMV preparation was positively recognized by immunosorbent electron microscopy and western blotting using a TMV-specific antiserum. In addition, sequence analysis of the cloned PCR amplicon showed that the virus isolated from Chinese sugar beets had an average identity of 92.3% (range 63.0-99.7%) at the nucleotide level and 95% (range 61.6-99.2%) at the amino acid level with other available TMV sequences. In particular, our TMV isolate had the highest nucleotide identity (99.7%) and amino acid identity (99.2%) with another Chinese TMV isolate (B935A) from Vicia faba (Zhou et al., 2000). In the course of the survey it was ascertained that TMV consistently coexisted in the same field and often in the same plant with Beet necrotic yellow vein virus (BNYVV) and Beet soil-borne virus (BSBV). To our knowledge, this is the first report of natural occurrence of TMV in sugar beet in China.


Corresponding author: Y.F. Wu
Fax: +86.29.87092559
E-mail: wuyf@nwu.edu.cn

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FIRST REPORT OF TOBACCO MOSAIC VIRUS ON SUGAR BEET IN CHINA

B. Wang, H. Pu, C-G. Han, D. Li and J. Yu

Department of Plant Pathology, and State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing 100094, China

Sugar beet is a known natural host of Tobacco mosaic virus (TMV) (Krstic et al., 1997). In 2006, samples were collected from symptomless sugar beets in a field in the Inner Mongolia province of China. Viral RNA was extracted from virus partially purified from beet roots and tested by RT-PCR using a specific primer set [TMV-480F(+): 5'-CAT GAA GGC CAG AAA GAC AG-3' and TMV-1300R(-): 5'-GCC TGG TAT GTT CGA ATG TG-3']. The primer was designed to amplify a 821 bp fragment from the TMV RdRp gene (GenBank accession No. AJ011933). Eight of the eighteen sugar beet samples tested gave the amplicon of the expected size. An isolate recovered from Chenopodium quinoa leaves inoculated with the partially purified TMV preparation was positively recognized by immunosorbent electron microscopy and western blotting using a TMV-specific antiserum. In addition, sequence analysis of the cloned PCR amplicon showed that the virus isolated from Chinese sugar beets had an average identity of 92.3% (range 63.0-99.7%) at the nucleotide level and 95% (range 61.6-99.2%) at the amino acid level with other available TMV sequences. In particular, our TMV isolate had the highest nucleotide identity (99.7%) and amino acid identity (99.2%) with another Chinese TMV isolate (B935A) from Vicia faba (Zhou et al., 2000). In the course of the survey it was ascertained that TMV consistently coexisted in the same field and often in the same plant with Beet necrotic yellow vein virus (BNYVV) and Beet soil-borne virus (BSBV). To our knowledge, this is the first report of natural occurrence of TMV in sugar beet in China.


Corresponding author: C-G. Han
Fax: +86-10-62813785
E-mail address: hanchenggui@cau.edu.cn

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FIRST REPORT OF LILY MOTTLE VIRUS INFECTING NARCISSUS PSEUDONARCISSUS IN CHINA

B. Liu1, J. Ming1, C. Liu1, D. Mu1, E.-X. Luo2 and X.-W. Wang1

1Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, 100094 Beijing, China
2College of Technology and Horticulture of Jinling, 210038 Nanjing, P.R. China

Lily mottle virus (LMoV) (family Potyviridae) has been reported in lily crops in the USA, Asia, Europe, Australia (Asjes, 1972) and Taiwan as well as Fujian and Zhejiang provinces of China. Plants are frequently stunted and flowers may be malformed with color break, brown rings and necrotic spots on the bulb scales (Lawson et al., 1996). In August 2007, total RNA was extracted from bulb tissue of symptomatic plants of two varieties of Narcissus pseudonarcissus and tested for LMoV by using a reverse transcription-polymerase chain reaction (RT-PCR) with primers L1 (5’-TGCGCACCCTTGTGAATTACA-3’) and L2 (5’-CACGGGAGGCATACAGCA-3’). Primer sequences were based on published sequences of LMoV coat protein gene (GenBank accession Nos. AJ748256; AJ748257). The predicted 553 bp amplicon was obtained from a sample of cv. Pink-charm that had brown spots on the bulbs and grew poorly in tissue culture. The nucleotide sequence of the amplicon (GenBank accession No. EU167936) showed more than 98% identity with sequences of other isolates of LMoV (AJ748256, AJ564636, AB053256, AF531458, AJ564637, AJ748257) and encoded an amino acid sequence (ABW16938) identical to that in the database for LMoV (NP-945145). This is the first report of infection of Narcissus pseudonarcissus by LMoV.


NEW HOSTS FOR SCLEROTINIA STEM ROT OF CANOLA

M.A. Aghajani1 and N. Safaei2

1Plant Protection Research Department, Agricultural and Natural Resources Research Center of Golestan Province, Gorgan, Iran
2Department of Plant Pathology, College of Agriculture, Tarbit Modarres University, Tehran, Iran

Sclerotinia sclerotiorum is an important plant pathogen that causes stem rot of different plants (Anonymous, 2005). During spring 2006 and 2007, in an epidemiological study of sclerotinia stem rot of canola (*Brassica napus*) in the Golestan province (northern Iran), symptoms of leaf, pod and stem rotting were observed on the following weeds growing in canola fields: sweet sageswort (*Artemisia annua*), wild oat (*Avena sterilis* ssp. *ludoviciana*), shepherd’s purse (*Capsella bursa-pastoris*), peacock poppy (*Papaver pavoninum*), annual bluegrass (*Poa annua*), clustered dock (*Rumex conglomeratus*), blessed milkthistle (*Silybum marianum*), charlock mustard (*Sinapis arvensis*), stinging nettle (*Urtica dioica*) and volunteer wheat (*Triticum aestivum*). From all symptomatic plants a fungus identified as *S. sclerotiorum* was consistently isolated on potato dextrose agar (PDA). Pathogenicity of representative isolates was assessed by placing 8-mm disks taken from the margins of an actively growing colony on the stems and leaves of potted host plants which were then placed in a greenhouse at 25°C and more than 90% relative humidity for 3-7 days (Hollowell et al., 2003). Symptoms like those observed in the field developed on all inoculated plants and the fungus was reisolated from rotten tissues. According to literature records (Anonymous, 2007), all ten species are new hosts of *S. sclerotiorum* for Iran and six of them (*A. annua*, *A. sterilis ludoviciana*, *P. pavoninum*, *P. annua*, *R. conglomeratus* and *U. dioica*) are new in the world.


OCCURRENCE OF CHARCOAL ROT ON KIWI FRUIT IN IRAN

M.A. Aghajani

In July 2006 wilted kiwi fruit trees (*Actinidia chinensis* Planchon) showed severe root rotting and dry rotting of the trunks that expanded up to 30 cm above the crown in the Kordkuy area of the Golestan province (northern Iran). Numerous microsclerotia were present on rotted roots and, sometimes, the surface of rotted stems was covered with small pycnidia. Microsclerotia were black, irregular and very small (74.02×75 µm), whereas pycnidia were larger (231.6×299.2 µm), black and semi-globous. Pycnidiospores were extruded from the pycnidial pore as orange cirrhi. Conidia were hyaline, single-celled, elliptical to oval in shape and measured 5.5×13.8 µm. Culturing microsclerotia and pycnidia on potato dextrose agar (PDA) yielded colonies of a fungus identified as *Macrophomina phaseolina* (Tassi) Goid. based on morphological characteristics (Singleton et al., 1993). Fungal colonies were nearly colorless at first, turning to light white during growth. Abundant microsclerotia were produced withn 2-3 days of culture, which changed the colony color to black. Hyphae were mostly branched at right angle and had obvious constrictions. The toothpick method (Singleton et al., 1993) was used for pathogenicity tests, following which symptoms developed which consisted of chlorosis, necrosis and shedding of the leaves, along with rotting of the stem at the inoculation points. An extensive field survey showed that most of the recently established orchards in the Kordkuy area were infected, the same as another stand in the Noshahr area of Mazandaran province. This is the first report of *M. phaseolina*-induced charcoal rot disease of kiwi fruit in Iran.

DISEASE NOTE

FIRST REPORT OF RAMULARIA COLLO-CYGNI IN SLOVAKIA

J. Gubiš, M. Hudcovcová and L. Klčová

Slovak Agricultural Research Centre - Research Institute of Plant Production, Bratislavská cesta 122, 921 68 Priešt’any, Slovakia

In 1987, the fungus Ophiocladum borei Cav. was identified in Austria as the causal agent of a new barley disease (Huss et al., 1987). Sutton and Waller (1988) isolated the same fungus from triticale (Triticum x Secale) and other gramineous plants, revised its taxonomic position, and renamed it Ramularia collo-cygni. More recently this fungus has been identified in a number of countries where is gaining importance (Frei et al., 2007). During summer 2006 and 2007 ringspots of unknown nature were observed on the leaves of spring barley growing in central Slovakia (Viglas-Pstrusa area). Twenty symptomatic leaf samples were tested by polymerase chain reaction (PCR) for the presence of R. collo-cygni. Total genomic DNA from naturally infected barley leaves, was extracted using Dneasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according the manufacturer's protocol. Specific detection of the pathogen was done using specific primers and the amplification conditions described by Frei et al. (2007). Purified fungal DNA form a culture provided by Dr. P. Frei (Agroscope, RAC Changing, Nyon, Switzerland) was used as positive control. From fourteen samples of diseased barley leaves and the control, the expected product of 348 bp was amplified by PCR, confirming the presence of R. collo-cygni in symptomatic plants. To our knowledge, this is the first report of this pathogen in Slovakia.


Corresponding author: J. Gubiš
Fax: +421 33 7726306
E-mail: gubis@vurv.sk
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FIRST REPORT IN ITALY OF A TOMATO RESISTANCE-BREAKING STRAIN OF TOMATO SPOTTED WILT VIRUS FROM CHRYSANTHEMUM

G. Bubici1, M.M. Finetti-Sialer2, T. Mascia2, M. Amenduni1, M. Girilli1 and D. Gallitelli2

1 Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via Amendola 163/A, 70126 Bari, Italy
2 Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 163/A, 70126 Bari, Italy

In 2007, samples were received from near Bari (Apulia, Southern Italy) of greenhouse-grown chrysanthemums (Dendranthema grandiflora cvs. Boris Becker, Delianne, Eleonora, Euro, Il Mondo, Paso Doble Pink, Reagan, Taormina, Zebra and Snowdon) all of which showed distinct chlorotic spots on the top leaves, followed by wilting and necrosis of the leaves and stems. In cvs Delianne and Snowdon, petals and flowerheads were also necrotic. Tomato spotted wilt virus (TSWV) but not Impatiens necrotic spot virus was detected in all samples tested by dot blot hybridization with digoxigenin-labelled riboprobes (Finetti-Sialer and Gallitelli, 2000). No amplicons were obtained in RT-PCR tests for the presence of Chrysanthemum stem necrosis virus using primers corresponding to positions 69-90 and 827-849 of RNA-M (accession No. AF213675). Three isolates obtained from cv. Snowdon and the wild-type strain p105 of TSWV were mechanically inoculated onto 4 plants (at 2-3 true leaf stage) of the hybrid tomato cv. Diaz, carrying the Sw5 resistance gene to TSWV, and the susceptible cv. UC82. The three Snowdon isolates, but not strain p105, overcame the resistance and induced systemic necrosis. UC82 plants became systemically infected by all 4 isolates. The results show that the Snowdon isolates are resistance-breaking. Moreover a 670 bp DNA copy of part of the NS5 gene of each Snowdon isolate yielded a MacI restriction pattern like that of resistance-breaking strains of TSWV A-type (Finetti-Sialer et al., 2002; Ciuffo et al., 2005). This is the first report of RB strains of TSWV in chrysanthemum in Italy.


Corresponding author: D. Gallitelli
Fax +39 080 544 2911
E-mail: gallitel@agr.uniba.it
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