Evaluation of the status of capsicum viruses in the main growing regions of Turkey

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The incidence, severity and distribution of six viruses infecting capsicum were determined in the main growing areas of Turkey during the 2004 growing season. The surveys covered 50 randomly selected capsicum fields from four different areas in south-east Anatolia and the eastern Mediterranean region. 515 samples were individually collected and tested by DAS-ELISA for *Cucumber mosaic cucumovirus* (CMV), *Alfalfa mosaic alfamovirus* (AMV), *Potato X potexvirus* (PVX), *Potato Y potyvirus* (PVY), *Pepper mild mottle tobamovirus* (PMMoV) and *Tobacco etch potyvirus* (TEV). 64.8% of ELISA-tested capsicum samples (334 out of 515) were infected by one (41.7%) or more (23.1%) viruses. PVY was the most widespread (26.4%), followed by PVX (25.8%), AMV (25.2%), TEV (23%), PMMoV (9.1%) and CMV (8.3%). Surprisingly high AMV infection was found in three areas (Kahramanmaraş, Şanlıurfa and Gaziantep) where AMV is reported for the first time in this study. However, AMV was not detected in Hatay. PMMoV is another new virus, in all the tested areas.

Introduction

Production of capsicum is expanding in Turkey and currently exceeds 1750 000 t, making this country one of the most important producers in the Mediterranean Basin (Anonymous, 2002). Most of the production comes from Hatay, Şanlıurfa, Gaziantep and Kahramanmaraş provinces and their districts. Çukurova region is another very important growing area. Cultivation in Turkey is mostly done with local seed material, saved and reproduced by growers over the years. This practice has caused a serious loss of agricultural quality due to the spread and transmission of viral diseases from season to season. Recently, universities and regional research institutes have developed breeding programmes to release improved inbred lines and hybrids of the local cultivated types, with desired agronomic characters and virus-free status. Virus infections have a negative impact on the quality and quantity of capsicum in Turkey and efforts to define the sanitary status of capsicum have thus far been limited to one region (Mediterranean coast). *Tobacco mosaic tobamovirus* (TMV), *Cucumber mosaic cucumovirus* (CMV), *Tobacco etch potyvirus* (TEV) (Yilmaz & Davis, 1984) and *Potato Y potyvirus* (PVY) (Erkan, 1986) infections are frequently found and cause great economic losses along the Mediterranean coast. The presence of TEV, known to be confined to the USA and the Caribbean, was first reported in the coastal region of Turkey by Yilmaz *et al*. (1983), followed by reports in the Şanlıurfa district (Guldur & Unlu, 2004). Guldur *et al*. (1994) identified *Pepper mild mottle tobamovirus* (PMMoV) infections in Cukurova by biological, serological and agarose slab gel electrophoresis tests. An extensive study was carried out by Palloix *et al*. (1994) in glasshouses and plastic tunnels from Demre to Adana, and in traditional field plantings for dried capsicum powder (Kahramanmaraş) and for capsicum paste (Karaisali). The results demonstrated that the Karaisali district suffered heavy losses from virus complexes of CHV and PVY. Guldur & Unlu (2004) reported the presence of TEV for the first time in Şanlıurfa districts by means of sereological analysis. As a first step in a fuller evaluation of the impact of viruses on capsicum in Turkey, an extensive survey was undertaken on 50 plantings in four districts. The presence and incidence of six viruses was examined. Once the phytosanitary status of this important crop has been determined, efforts can be made to produce virus-free seeds and improve seed supplies through breeding.

Materials and methods

Field surveys

Field surveys were undertaken during the growing season of 2004 (July–September) in the main capsicum-growing districts of Hatay, Kahramanmaraş (eastern Mediterranean), Şanlıurfa and Gaziantep (south-east Anatolia). In total, 515 samples were collected from 50 field plantings from 28 towns of four districts. The number of samples collected from each was proportional to the area planted with capsicum in that region: Hatay, 59 samples from 12 plantings; Kahramanmaraş, 171 samples from 16 plantings; Şanlıurfa, 152 samples from 13 plantings; Gaziantep, 133 samples from 9 plantings. Fields were divided into two groups: A (< 0.5 ha), B (0.5–1 ha). The sampled fields were randomly selected from each region. Five to ten samples were collected from group A, 10–15 from group

B. The samples for laboratory virus testing consisted of leaves that were collected at random following the 'W' pattern as a sampling procedure. All samples were placed individually in plastic bags and stored at 4°C until testing, normally after 2–3 days.

Serological tests

The double antibody sandwich (DAS) ELISA protocol was used for virus detection (Clark & Adams, 1977). Antibodies for all viruses were commercially provided by Loewe Biochemica (DE) for PVY, PVX, CMV and AMV, by Bioreba (CH) for (PMMoV) and by BIO-RAD Plantest ELISA (INRA, FR) for TEV. ELISA plates were read with a Titertek Multiskan SIRIO apparatus. The test was considered positive when the mean absorbance value of a sample was over twice that of healthy controls.

Biological assays

Ten percent of the collected samples were used to inoculate a range of herbaceous indicator hosts to check the presence of mechanically transmissible viruses. Symptomatic and non-symptomatic leaves were ground in 0.03 M phosphate buffer (pH 7.2) with 0.2% DIECA and the extract was rubbed on celite-dusted leaves of *Chenopodium amaranticolor*, *Chenopodium quinoa*, *Nicotiana tabacum* cv. 'Samsun', *N. tabacum* cv. 'Xanthi', *N. tabacum* cv. 'Xanthi nc', *Nicotiana benthamiana*, *Nicotiana rustica*, *Capsicum annuum* 'Maras', *Lycopersicon esculentum* 'H-2274'. Inoculation with buffer alone was performed on the plants for each virus as negative controls. Inoculated plants were kept in a growth chamber at 22–24°C and inspected daily for the appearance of symptoms.

Results and discussion

Field surveys

The plants from the field plantings were not homogeneous in terms of cultivar and age. They were derived from field-germinated seeds originating from the previous season's fruits. The capsicum crops were densely sown along the row, which made observation of virus symptoms difficult. In the Şanlıurfa fields, the major problem was mixed cultivation with cucurbits (cucumber, watermelon, squash) or other solanaceous crops (aubergine).

Mosaic with some mottle, vein banding and bronze discoloration symptoms were very common at Hatay. Reduced growth and short internodes were also present in this area. Vein clearing in young leaves, leaf deformations and up-rolled leaves were widespread at Şanlıurfa and Gaziantep. Symptoms of virus infections at Kahramanmaraş were generally chlorotic spots with some necrosis, and yellow discoloration of leaves. Symptomatic plants mostly had overall lighter colour, with etiolation of at least some leaves and stunting and curling.

Serological tests

Infections by at least one virus were detected by ELISA in 334 out of 515 samples (64.8%) (Table 1). Virus incidence varied among regions and was lowest (52.6%) in Kahramanmaraş and highest (84.7%) in Hatay. PVY was the commonest, being detected in 26.4% of the samples. Its incidence was particularly was high at Şanlıurfa, where it was detected in 38.2% of the samples.

PVX was the second commonest virus. It was detected in 25.8% of the tested capsicum samples on average, with a high infection ratio in Hatay (71.2%) and Gaziantep (39.8%). CMV was less common (8.3%), followed by PMMoV (9.1%); however, CMV was commonly found at Hatay, being present in 23.7% of the total tested samples. This study found PMMoV in all four areas for the first time.

In Şanlıurfa, Kahramanmaraş and Gaziantep, all the viruses were detected. The most prevalent were PVY (38.2% and 33.9% of the samples) in Şanlıurfa and Hatay, respectively; AMV (35.7 of the samples) in Kahramanmaraş; and PVX (39.8% of the samples) in Gaziantep followed by AMV in Şanlıurfa (28.3%), and TEV (27.8% and 21.6%) in Gaziantep and Kahramanmaraş, respectively. AMV infection was not detected in Hatay capsicum fields. In the other three areas, this is the first report of its presence.

Of the 334 ELISA-positive samples, 215 (41.7%) were infected with one virus. Samples with single, double and more virus infections were also found at varying incidence. The greatest number of double infections were found to be caused by AMV/PVY (9.9%), AMV/PVX (7.4%) and PVX/PVY (6.9%). CMV/PMMoV (0.4%) caused the fewest. The most double and multiple infections were recorded from the Kahramanmaraş district. Four viruses (AMV/CMV/PVX/PVY) were found in only one sample, five and/or six of them were not detected in any of the tested samples.

Biological assay

Different types of symptoms were observed in capsicum cultivars and indicator hosts by the viruses detected above. AMV symptoms were bright yellow blotches with some mottle, bronze discoloration on some leaves and chlorotic or necrotic spots in *L. esculentum* H2274. CMV induced chlorotic local lesions in *C. amaranticolor* and *C. quinoa*, and vein clearing followed by white mosaic in capsicum cv. 'Maras'. *L. esculentum* H2274 plants appeared lighter in color and were stunted. Close-up symptoms included mosaic, especially on the younger leaves. Yellow colour along with mosaic pattern was induced by CMV in *N. rustica*, *N. tabacum* cv. Samsun. Stunting and systemic mosaic were caused by PVX in *L. esculentum* H2274. Veinal necrosis and mild mosaic symptoms were typically induced in most *N. tabacum* plants by PVY. PMMoV caused chlorotic ringspots in *C. quinoa*, *C. amaranticolor*, necrotic local lesions in *N. rustica*, chlorotic lesions accompanied by mosaic in necrotic lesions in *Nicotiana benthamiana*, necrotic local lesions in capsicum cv. 'Maras',

Table 1 Incidence of viral infections in the main capsicum-growing areas in south-east Anatolia (Şanlıurfa and Gaziantep) and eastern Mediterranean (Hatay and Kahramanmaraş) regions, as determined by ELISA

	Samples infected			Viruses (%)					
Provinces	Tested	Infected	%	AMV	CMV	PVX	PVY	PMMoV	TEV
HATAY	59	50	84.7	–	23.7	71.2	33.9	3.4	3.4
Oymakli	25	20	80	–	20	72	54	8	5
Babatorun	12	11	91.6	–	33.3	83.3	66.7	–	–
Serinyol	11	10	91.6	–	45.4	81.8	63.6	–	–
Yalankoz	11	9	81.8	–	–	45.5	36.4	–	9.1
ŞANLIURFA	152	86	56.6	28.3	11.2	5.3	38.2	12.5	16.4
Gurcutepe	29	13	44.8	31	–	3.4	48.3	10.3	24.1
Konuklu	22	16	72.7	100	72.7	31.8	100	50	31.8
Boydere	10	8	80	40	–	–	50	20	30
Kisas	91	49	53.8	8.8	1	–	8.8	16.5	8.8
KAHRAMANMARAŞ	171	90	52.6	35.7	4.6	17.5	21.1	8.2	21.6
Beyoglu	11	10	90.9	45.5	–	27.2	18.2	–	72.7
Sekeroba	9	5	55.5	22.2	–	22.2	11.1	11.1	22.2
Turkoglu	10	6	60	60	10	–	30	10	50
Cerkezler	15	11	73.3	53.3	6.7	13.3	20	–	6.7
Doganli	6	3	50	–	–	–	–	–	50
Orta Dehliz	5	2	40	20	–	–	20	20	40
Dehlizburnu	10	9	90	50	–	–	50	–	50
Cinarli	5	2	40	20	–	–	40	–	20
Merkez Cigili	10	7	70	–	–	–	30	10	70
Kilili	10	9	90	80	–	70	50	20	10
C. Hasanaga	80	26	32.5	31.3	6.3	20	13.8	10	12.5
GAZIANTEP	133	83	62.4	18.8	3.8	39.8	16.5	8.3	27.8
Golbasi	11	6	54.5	–	–	45.5	18.2	18.2	–
Gulluhuyuk	11	1	9	9.1	–	9.1	9.1	9.1	–
Yelliburun	20	13	65	10	–	5	–	–	55
Ortulu	17	16	94.1	76.5	–	53	64.7	29.4	29.4
Elbistan	12	7	58.3	16.7	–	41.7	–	–	50
Zincirli	7	2	28.6	14.3	–	–	–	–	28.6
Gozluhuyuk	22	11	50	27.3	–	27.3	13.6	–	4.5
Yesemek	24	24	100	–	12.5	87.5	12.5	8.3	12.5
Akinyolu	9	3	33.3	–	22.2	55.6	22.2	11.1	–
Total									
%			64.8	25.2	8.3	25.8	26.4	9.1	23
No.	515	334		130	43	133	136	47	118

systemic necrosis in *N. tabacum* cv. Samsun. Foliar mottling in *N. tabacum* cv. Samsun, mild mottling in cv. Xanthi, vein clearing and systemic mosaic in capsicum cv. 'Maras', light colouring, mosaic appearance with light and dark green areas on some leaves, leaf curling in *L. esculentum* H2274, were the symptoms caused by TEV. Symptoms were not observed in the control plants.

Conclusion

The present study provides an important contribution to the knowledge of the phytosanitary status of capsicum in south-east Anatolia and the eastern Mediterranean region, for which only scant information, based on a limited number of samples and viruses, was previously available. This is the first detailed work for the phytosanitary status of capsicum crops in the main capsicum-growing areas of Turkey. Previous work, on the best known viruses, such as CMV and TMV, was carried out in very limited areas, in single regions. We have obtained very significant results for the capsicum-growing areas in general. Despite the relatively small number of crops surveyed by comparison with the total growing area of capsicum in Turkey, distinct situations for the six viruses were encountered in the different provinces.

Infection rates detected ranged between 84.7% in Hatay and 52.6% in Kahramanmaraş. Despite the fact that Kahramanmaraş had a low virus infection rate (52.6%) on average, there was significant variation among sampled localities (32.5–90.9%). Individual viruses showed heavy virus infection in Kahramanmaraş by comparison with other districts, especially AMV (35.7%) followed by TEV (21.6%). It is interesting to note a significant incidence of AMV, now reported for the first time in Kahramanmaraş (35.7%), Şanlıurfa (28.3%) and Gaziantep (18.8%), but not in Hatay. TEV infection ranged

between 31.6% in Kahramanmaraş and 3.4% of Hatay, but had not been reported in Kahramanmaraş by Palloix *et al.* (1994).

CMV infection of capsicum was the first time reported in some provinces of Şanlıurfa by Unlu & Guldur (2004). They found infection rates of 47% and 76% at seedling and harvesting stages, respectively. This could be due to heavy infestations of aphid vectors at the harvesting period and also to the proximity of cucurbit crops (cucumber, watermelon, courgette) which are potential reservoirs of the virus (Martelli & Quacquarelli, 1988; Kaper & Waterworth, 1981; Guldur, 1998). In our study, CMV infection was only 11.2% in Şanlıurfa, which is very low with respect to the results of Unlu & Guldur (2004).

High virus incidence is probably due to heavy infestations of aphid vectors and to the use of uncertified seeds. Aphid infestations were observed in Şanlıurfa and Hatay, less in Kahramanmaraş and Gaziantep. The tested viruses have been reported to be either aphid- and/or seed-transmitted and they have also been reported to infect a broad range of hosts (Pirone & Harris, 1970; Francki *et al.*, 1979; Pirone & Thombury, 1983; Palukaitis *et al.*, 1992; OEPP/EPPO, 2004). Virus diseases are difficult to control and can result in substantial crop losses. As there are no curative treatments for virus-infected plants in the field, all efforts should be directed at preventing infection. Therefore, the use of certified seeds is necessary to avoid early infections in the crop. It is also of great importance to control winged aphid vectors coming from surrounding crops. However, growers have little of knowledge to the proper timing and dosage of insecticide treatments. Spraying against aphids is mostly ineffective because of the density of planting along the rows.

The current status of virus infection in Turkish capsicum crops, and the use of uncertified seeds, put severe stress on the quality and quantity of capsicum production, resulting in dramatic losses in many places. The implementation of breeding and controlled seed production programmes in Turkey should help growers to improve their production capacity, by reducing the transmission of virus diseases and the consequent economic losses.

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Evaluation de la situation des virus des poivrons dans les principales régions productrices de Turquie

L'incidence, la sévérité et la distribution de six virus infectant les poivrons ont été déterminées dans les principales zones de culture de Turquie au cours de la saison 2004. Les prospections couvraient 50 champs de poivrons sélectionnés au hasard dans quatre zones différentes du Sud-Est de l'Anatolie et dans l'Est de la région méditerranéenne. 515 échantillons ont été collectés individuellement et analysés par DAS-ELISA pour le *Cucumber mosaic cucumovirus* (CMV), l'*Alfalfa mosaic alfamovirus* (AMV), le *Potato X potexvirus* (PVX), le *Potato Y potyvirus* (PVY), le *Pepper mild mottle tobamovirus* (PMMoV) et le *Tobacco etch potyvirus* (TEV). 64.8% des échantillons de poivrons testés par ELISA (334 sur 515) étaient infectés par un virus (41.7%) ou plusieurs (23.1%). Le PVY était le plus largement répandu (26.4%), suivi par le PVX (25.8%), l'AMV (25.2%), le TEV (23%), le PMMoV (9.1%) et le CMV (8.3%). Une infection étonnament élevée par l'AMV a été trouvée dans trois zones (Kahramanmaraş, Şanlıurfa et Gaziantep) où l'AMV est signalé pour la première fois dans cette étude. Cependant, l'AMV n'a pas été détecté en Hatay. Le PMMoV est un autre virus nouveau, dans toutes les zones testées.

Оценка статуса вирусов стручкового перца в основных производящих областях Турции

В течение вегетационного сезона 2004 г. в основных производящих областях Турции были определены встречаемость, вредоносность и распространение шести вирусов, заражающих стручковый перец. Эти обследования охватывали 50 произвольно отобранных полей стручкового перца в четырех различных областях в юго-восточной Анатолии и восточной части Средиземноморского региона. 515 образцов были индивидуально собраны и проверены с помощью системы DAS-ELISA на *Cucumber mosaic cucumovirus* (CMV), *Alfalfa mosaic alfamovirus* (AMV), *Potato X potexvirus* (PVX), *Potato Y potyvirus* (PVY), *Pepper mild mottle tobamovirus* (PMMoV), а также на *Tobacco etch potyvirus* (TEV). 64,8% проверенных с помощью ELISA образцов стручкового перца (334 из 515) были заражены одним (41,7%) или несколькими вирусами (23,1%). PVY был наиболее широко распространенным (26,4%), затем шли в порядке убывания: PVX (25,8%), AMV (25,2%), TEV (23%), PMMoV (9,1%) и CMV (8,3%). Удивительно высокое заражение AMV было найдено в трех областях (Рараманмараш, Шанлурфа и Газианатеп), причем AMV впервые отмечается в этом исследовании. Однако, AMV не был обнаружен в Гатае. PMMoV представляет собой еще один новый вирус для всех проверявшихся областей.

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