Neth. J. Pl. Path. 93 (1987) 153-158

Viruses of pepper in plastic houses in Crete

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Accepted 29 April 1987

Abstract

During a survey of virus diseases affecting pepper grown in plastic houses in Crete, during 1984-1986, tomato mosaic virus (ToMV) and tobacco mosaic virus (TMV) were detected. The most common virus was TMV, being present in samples of alle pepper cultivars carrying the L_1 resistance gene, while ToMV was isolated only from susceptible pepper cultivars. According to responses of *Capsicum* spp. the isolates from 640 samples checked were classified into three pathotypes: P_0, $P_{1.2}$ and $P_{1.2.3}$. Results of this study show that $P_{1.2}$ represents at present the major threat to the Cretan pepper industry.

Additional keywords: tomato mosaic virus, tobacco mosaic virus.

Introduction

Pepper virus diseases have not previously been surveyed in Crete and little has been published on the incidence of viruses in plastic house-grown peppers. In 1980 a severe disease caused by a pepper strain of tobacco mosaic virus (TMV) was noticed on pepper cultivars resistant to common isolates of TMV and tomato mosaic virus (ToMV) (Avgelis, 1986). Later the effect of this virus on yield and marketability of pepper grown in plastic houses was reported (Avgelis and Drossos, 1983) as well as the extensive presence of TMV in seeds of commercial pepper cultivars (Avgelis, 1982).

At present the acreage of peppers grown in plastic houses in Crete is rather limited (about 120) and it is nearly wholly concentrated in the Ierapetra area (southern coast of the island) where it represents an important commodity of the local vegetable industry. The purpose of this investigation was to determine which strains of TMV and ToMV occur in pepper grown in plastic houses and whether they have the potential to attack pepper plants possessing the resistant genes L_1, L_2 and L_3.

Materials and methods

Survey procedures. Thirty-two and thirty-five holdings with at least 0.25 acre plastic house-grown pepper were chosen at random in the area of Ierapetra during the growing seasons of 1984-85 and 1985-86. The plastic houses were inspected once during January, February or March and the following records were made: cultivars, total number of plants and number of plants with virus symptoms on leaves and/or fruits. Within each plastic house ten plants were selected at random from those with obvious symptoms. Each plant was labelled with a sample number and samples (leaves or

fruits) were frozen at −20 °C in plastic bags before testing for virus identification.

Virus identification. The collected samples were checked for the presence of virus using indicator plants and serology. All test plants were raised in steam-sterilized soil and kept in an insect-proof glasshouse at 18 to 26 °C. Routine inoculations were made with frozen pepper tissue ground in 0.1 M phosphate buffer (pH 7.2). Serological tests in agar gel using crude extracts of naturally or mechanically infected pepper plants were routinely carried out for ToMV and TMV and occasionally for tomato bushy stunt virus.

As only two viruses were consistently isolated – identified as isolates of ToMV and TMV – new tests were undertaken to better characterize these. So additional host plants were used, including *Capsicum* spp. possessing all known resistant genes to ToMV and TMV. As will be mentioned below, three pathotypes P_0, $P_{1.2}$, $P_{1.2.3}$ (Boukema et al., 1980) were identified and were maintained on tomato 'Dombo', *Capsicum frutescens* and *Capsicum chinense*, respectively. Plants of these hosts, infected by a randomly chosen isolate of each pathotype, were used to obtain purified virus preparations. Purification was carried out by the method of Steere (1965) followed by sucrose density centrifugation (90 min at 90 000 *g* through 10-40% sucrose density gradients in 0.02 M phosphate buffer pH 7.2). The density gradient tubes were scanned with an ISCO density gradient fractionator at 254 nm and collected virus zones were concentrated at 120 000 *g* for 90 min. Pellets were resuspended in distilled water. Antisera to purified virus, obtained by two cycles of density gradient fractionation, were prepared in rabbits by giving two intravenous injections five days apart followed by two intramuscular injections seven days apart in Freund's incomplete adjuvant. Antigen concentration was about 1 mg in each injection. Serological tests were carried out by the agar gel double diffusion technique using purified virus.

Results

During the growing season of 1984-85 and 1985-86, there were collected 260 and 380 samples, respectively, of virus-infected pepper plants which were checked. Identification was based on host range responses and serological tests. The predominant virus infecting pepper in plastic houses was TMV (in about 88% of the samples). The other virus was ToMV which was detected only in cultivars without resistance factors (in about 30% of the samples from susceptible cultivars). TMV was present in susceptible cultivars, in about 70% of the samples and in all samples of resistant cultivars possesing the L_1 gene (Table 1).

Mosaic on the young leaves, puckering or/and necrosis of the fruits and stunting were the main symptoms observed on pepper plants infected by TMV. ToMV-symptoms, however, were characterized by mild mosaic or masked symptoms on the leaves, necrosis on fruits and stems and premature leaf dropping. The mean percentage of plants with evident symptoms was very low (0.5 to 2%). A significant increase in the incidence of TMV was noticed from one year to the next (51% and 66% of the plastic houses surveyed growing pepper L_1 cultivars were infected by TMV in the 1984-85 and 1985-86 growing season, respectively), and the mean percentage of plants with symptoms in these plastic houses was 17 in 1984-85 and 25 in 1985-86. The host plant reactions to the isolates of ToMV and TMV are shown in Table 2. All isolates of ToMV

Table 1. Distribution of tomato mosaic virus (P_0) and tobacco mosaic virus ($P_{1.2}$ and $P_{1.2.3}$) pathotypes in plastic houses-grown pepper in Crete based on test plants reactions.

Pepper type source of pathotype	Total number of samples	Number of infected samples with			Number of plastic houses infected/inspected
		P_0	$P_{1.2}$	$P_{1.2.3}$	
1984-85 growing season					
Bell pepper L_1L_1	130	—	121	9	13/25
Sweet long L_+L_+	60	4	56	—	6/6
Hot long L_+L_+	70	40	30	—	7/10
Total	260	44	207	9	26/41
1985-86 growing season					
Bell pepper L_1L_1	250	—	250	—	25/31
Sweet long L_+L_+	70	11	59	—	7/10
Hot long L_+L_+	60	22	38	—	6/12
Total	380	33	347	—	38/53

Table 2. Response of test plants to mechanical inoculation with tomato mosaic virus (P_0) and tobacco mosaic virus ($P_{1.2}$ and $P_{1.2.3}$) pathotypes isolated from pepper grown in plastic houses.

Test plant	P_0		$P_{1.2}$		$P_{1.2.3}$	
Capsicum annuum 'No 14' L_+L_+	LLa	SNaM		SM		SM
Capsicum annuum 'Early Calwonder' L_+L_+	LLa	SM		SM		SM
Capsicum annuum 'Verbeterde Glas' L_1L_1	LLa			SM		SM
Capsicum annuum 'Lamuyo' L_1L_1	LLa			SM		SM
Capsicum frutescens 'Tabasco' L_2L_2	LLa			SM		SM
Capsicum annuum 'Bruinsma 903/80' L_+L_3	LLa		LLa	SN		SM
Capsicum annuum 'Bruinsma 916/80' L_+L_3	LLa		LLa	SN		SM
Capsicum chinense 'Bruinsma 940/79' L_3L_3	LLa		LLa			SM
Capsicum chinense 'Miscucho' L_3L_3	LLa		LLa			SM
Capsicum chinense 'P.I.159236' L_3L_3	LLa		LLa			SM
Lycopersicon esculentum 'Dombo' +/+		SM		—		—
Lycopersicon esculentum 'Dombito' Tm-2^2Tm-1/+ +	LL			—		—
Nicotiana benthamiana	LL	SN		SM		SM
Nicotiana glutinosa	LL		LL		LL	
Nicotiana rustica	LL		LL		LL	
Nicotiana tabacum 'Samsun'		SM		—		SL

LL = Local lesions, a = abscission, SM = systemic mosaic, SN = systemic necrosis, – = absence of symptoms (no infection detected by back inoculation to *Nicotiana glutinosa*), SL = latent systemic infection.

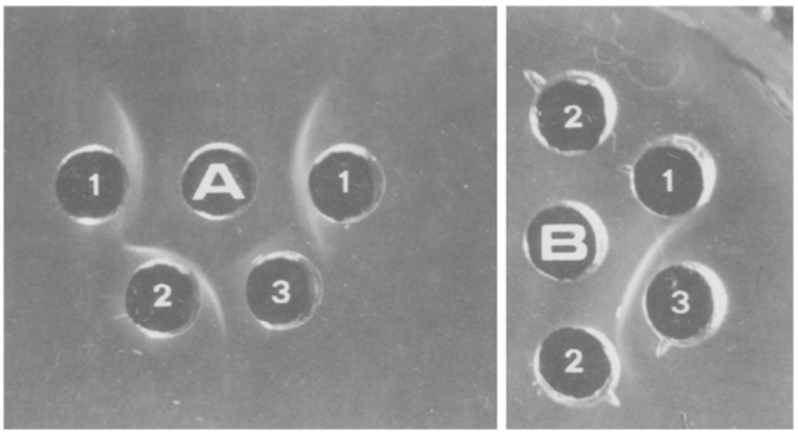

Fig. 1 Serological reactions between P_0, $P_{1.2}$ and $P_{1.2.3}$ pathotypes of tomato mosaic virus and tobacco mosaic virus. A = anti-$P_{1.2.3}$, B = anti-P_0, 1 = $P_{1.2.3}$, 2 = $P_{1.2}$ and 3 = P_0.

induced similar symptoms, indistinguishable from those of the common strain of ToMV (Holdings and Huttinga, 1976). *Capsicum* responses are in agreement with those of pathotype P_0 (Boukema et al., 1980). The response of some hosts to inoculation of TMV isolates appeared to be different from that of the common tobacco strain (Zaitlin and Israel, 1975) but similar to other TMV pepper strains previously described (Feldman and Oremianer, 1972; Greenleaf et al., 1964; Rast, 1977; Selassie et al., 1981; Tobias et al., 1982; Avgelis, 1986). Taking into account the reactions of some hosts the local TMV pepper isolates were differentiated in two main groups: those of one group did not infect *Nicotiana tabacum* 'Samsun', while *Capsicum chinense* 'Miscucho' and 'PI. 159236' were only infected locally; those of the other group infected tobacco 'Samsun' but latently and caused mosaic symptoms in als *Capsicum* species tested. According to Boukema et al. (1980) these two groups could belong to pathotypes $P_{1.2}$ and $P_{1.2.3}$, respectively.

Pathotype $P_{1.2}$ was found in about 80 and 91% of the total number of samples during 1984-85 and 1985-86 growing season, respectively. Pepper cultivars with L_1 resistance were more affected (58.5% and 72% of the $P_{1.2}$ infected samples) than susceptible cultivars (41.5 and 28% of the $P_{1.2}$ infected samples). Pathotype $P_{1.2.3}$ was isolated only from nine samples during 1984-1985 growing season in three plastic houses growing L_1 resistant pepper cultivars. P_0, $P_{1.2}$ and $P_{1.2.3}$ pathotypes were easily purified according to Steere's procedure and in sucrose density centrifugation no differences were noticed, all sedimented as a single band. The homologous titres of antisera were: 1 : 128, 1 : 128 and 1 : 256 for P_0, $P_{1.2}$ and $P_{1.2.3}$, respectively. During the serological comparison antisera to $P_{1.2}$ and $P_{1.2.3}$ reacted with both homologous and heterologous antigens without spurs, while antiserum to P_0 reacted only with the homologous antigen. When antigens of P_0, $P_{1.2}$ and $P_{1.2.3}$ were tested in adjacent wells against antisera to $P_{1.2}$ or $P_{1.2.3}$ clear spurs were formed (Fig. 1).

Discussion

The combination of host reactions and serological tests allowed successful identification of two viruses, ToMV and TMV, on pepper grown in plastic houses on the island of Crete. Moreover the use of a selected host range revealed three pathotypes: P_0, $P_{1.2}$ and $P_{1.2.3}$ (Boukema et al., 1980). Although the serological tests among these pathotypes need further word to clarify their serological relationships, it is evident that $P_{1.2}$ and $P_{1.2.3}$ are related while P_0 seems to be serologically different.

The surveys in the two growing seasons indicate that $P_{1.2}$ is the predominant pathotype in resistant (L_1L_1) bell peper cultivars and is also increasingly found in susceptible (L_+L_+) sweet long and hot long pepper cultivars. The apparent decrease in occurence of $P_{1.2.3}$ pathotype, which a few years ago appeared to be more widely spread (Avgelis, 1986) is rather difficult to explain. It may be worth pointing out that all seeds of bell pepper cultivars are imported from Europe and USA, while sweet long and hot long pepper seeds are mainly produced locally. In any event the actual wide spread of $P_{1.2}$ pathotype, which represents the major threat to the local pepper industry, suggests that the use of phytosanitary uncontrolled seed (Avgelis, unpublished data) favours its perpetuation and tends to explain its prevalence. Taking into consideration that there is at present no effective management practice to control this virus desease, it has become urgent to select more suitable cultivars from among the newly resistant ($L_{3/+}$) bell pepper cultivars (L.G. van den Berkmortel, personal communication) and to continue surveys in order to monitor the pathogenicity of these isolated virus.

Acknowledgments

I wish to thank Dr A.Th.B. Rast, Glasshouse Crops Research and Experiment Station, and Dr L.G. van den Berkmortel, Bruinsma B.V., Naaldwijk, Holland, for providing *Capsicum* seeds.

Samenvatting

Virussen van paprika in plastic-foliekassen op Kreta

Bij een in de jaren 1984-1986 gehouden inventarisatie van virusziekten in paprika in plastic-foliekassen op Kreta werd zowel het tabaksmozaïekvirus (TMV) als het tomatemozaïekvirus (ToMV) aangetoond. TMV kwam zeer algemeen voor: het werd aangetroffen in alle monsters van de paprikarassen met het resistentiegen L_1. ToMV werd alleen geïsoleerd uit planten van vatbare paprikarassen. Volgens de waargenomen symptomen die de isolaten van 640 monsters op *Capsicum* spp. vertoonden, konden de isolaten in drie pathotypen, nl. P_1, $P_{1,2}$ en $P_{1,2,3}$, worden geklassificeerd. Uit het onderzoek is gebleken dat $P_{1,2}$ de belangrijkste bedreiging vormt voor de teelt van paprika op Kreta.

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