Eur J Plant Pathol (2011) 129:579–590
DOI 10.1007/s10658-010-9722-x

Susceptibility of different plant species and tomato cultivars to two isolates of *Pepino mosaic virus*

Ahmad Fakhro · Susanne von Bargen · Martina Bandte · Carmen Büttner · Philipp Franken · Dietmar Schwarz

Accepted: 15 November 2010 / Published online: 4 January 2011

Abstract As *Pepino mosaic virus* has become a pathogen of major importance in worldwide tomato production, information is needed on possible differences between the sensitivity of cultivars towards infection. Furthermore, it is important what hosts other than *Solanaceae* may be virus reservoirs and are, therefore, threats for tomato cultivation. Two PepMV isolates (PepMV-Sav, E397, a European tomato isolate and PV-0554, a Peruvian pepino isolate) differing in their origin and virulence were used for several experiments to investigate these issues. The response to mechanical inoculation with PepMV was studied using 25 tomato cultivars, seven indicator plant species, and nine other possible horticultural host plants. Symptom development after infection with PepMV was monitored and the virus was detected by DAS-ELISA and IC-RT-PCR. Garlic and broad bean were shown to be additional hosts of PepMV depending on the virus isolate. *Nicotiana benthamiana* seems to be the most sensitive indicator among all tested indicator plants developing symptoms. Both PepMV isolates infected all tested tomato cultivars. Development of disease symptoms depended on the cultivar and the virus isolate but symptoms were not visible in all cases. None of the cultivars showed tolerance against the two isolates but two responded with a lower susceptibility at an absorbance level of 0.2 (healthy control 0.09). It was observed that some cultivars grown hydroponically showed also lower losses in biomass and yield. Data indicated a correlation between absorbance level in DAS-ELISA and reduction in total tomato growth.

Keywords DAS-ELISA · IC-RT-PCR · Indicator plants · PepMV · Potexvirus · Solanaceae · Symptoms

A. Fakhro · P. Franken · D. Schwarz (✉)
Plant Nutrition, Leibniz-Institute of Vegetable and Ornamental Crops, Großbeeren/ Erfurt e.V.,
Theodor-Echtermeyer-Weg 1,
14979 Großbeeren, Germany
e-mail: schwarz@igzev.de

A. Fakhro · S. von Bargen · M. Bandte · C. Büttner
Faculty of Agriculture and Horticulture, Department of Crop- and Animal Sciences, Division Phytomedicine,
Humboldt-Universität zu Berlin,
Lentzeallee 55/57,
14195 Berlin, Germany

Introduction

Among the important pathogens in soil, substrate, and soilless tomato cultivation systems several viral pathogens are associated with crop losses, among them *Pepino mosaic virus* (PepMV). This potexvirus (family Alpha-*flexiviridae*) has attracted much attention since 1999 when it was firstly detected in Europe. In the following years it was found in greenhouse tomatoes in many European countries, in Morocco, Syria, South and North America, and China (see references in Spence et al. 2006).

PepMV can infect different crops belonging to the *Solanaceae*, such as tomato, potato, tobacco, bell

pepper, petunia, and wild plants of *Lycopersion* spp., black nightshade (*Solanum nigrum*), and other *Solanaceae* (*S. aethiopicum, S. dulcamara, S. luteum*) (Kazinczi et al. 2005). Species of other families are susceptible as well: *Cucurbitaceae*, such as cucumber (*Cucumis sativus*, Villemson et al. 2003), *Amaranthaceae,* such as pigweed (*Amaranthus* sp.), *Asteraceae*, such as common sowthistle (*Sonchus oleraceus*), *Malvaceae,* such as cheeseweed mallow (*Malva parviflora*), and *Lamiaceae* such as basil (*Ocimum basilicum)* (Jordá et al. 2001; Kazinczi et al. 2005; Davino et al. 2009).

So far, four different genetic clusters of PepMV, referred to as EU-, LP-, CH2-, and US2-isolates found capable of infecting tomato and have been identified by determination of full length sequences of the genomic ssRNA (Cotillon et al. 2002; Verhoeven et al. 2003; Hanssen et al. 2008; Hasiow et al. 2008; Gomez et al. 2009; Hanssen and Thomma 2010). In Europe, PepMV isolates of the EU- as well as of the LP-genotype have predominated in virus infected tomato plants until 2007, but recently the CH2 genotype was found predominantly to be spreading in this crop particularly in the Western European countries (Pagan et al. 2006; Hanssen et al. 2008; Hasiow et al. 2008; Gomez et al. 2009; Hanssen and Thomma 2010). Authors mention also that these results are indicative and that EU genotypes are still present and persistent in mixed infections. It is not clear if this can be concluded for the whole of Europe, particular for the eastern countries (Pospieszny and Borodynko 2006). Contrary to Western Europe, the EU genotypes are still dominating in North America over US1, US2, and CH2 genotypes. Finally, it was reported that seed transmission—a major reason for dissemination of PepMV—profers an apparent advantage to the EU genotypes. There is no clear correlation between PepMV induced symptoms in tomato and sequence variability found among virus strains (Lopez et al. 2005; Pagan et al. 2006; Hanssen et al. 2008, 2009b).

The knowledge on yield responses of cultivars due to an infection with PepMV is very important because of the potential reduction in tomato production. Here, reports are conflicting. While Soler-Aleixandre et al. (2005) published high losses caused by the collapse of up to 90% of plants, others describe lower yield losses of up to 15% (Verhoeven et al. 2003), or even no quantitative yield losses but significant reduction in fruit quality, and thus marketable yield reductions up to 40% (Spence et al. 2006).

Symptom development in tomato is highly variable ranging from latent infections to mild mosaic up to severe leaf distortion and bubbling (Hanssen and Thomma 2010). In detail it is described as follows: filiform leaves, chlorosis and scattered necrotic spots, bubbles, yellow mosaics or leaf spots (van der Vlugt et al. 2000; Roggero et al. 2001), nettle like plant heads (Hanssen et al. 2009b), and occasionally yellow chlorotic angular spots on and irregular ripening of fruits (Jordá et al. 2001). Symptom development and intensity as well as biomass and yield reduction depends on many factors, such as strain (van der Vlugt et al. 2002), cultivar (Villemson et al. 2003), and climate (Spence et al. 2006; Schwarz et al. 2010). Other plant species show similar and also widely varying symptoms as described for tomato.

This study aimed to test i) the suitability of plant species of the *Solanaceae* and *Chenopodiaceae* family as indicator plants and propagation hosts, ii) the susceptibility of different crop species to the virus, iii) the susceptibility or tolerance of tomato cultivars to the virus, as well as iv) the effect of PepMV infection on tomato growth and yield.

Materials and methods

PepMV isolates, propagation, and detection

Two different PepMV isolates were used based on their different origin and aggressiveness.

- Pepino isolate PV-0554 (DSMZ, Braunschweig, Germany) was isolated from *Solanum muricatum* plants in Peru. Partial sequences of the viral replicase and the coat protein coding region of PV-0554 (EMBL accessions FN429032 and FN429033) showed 99% nucleotide identity to PepMV isolate SM74 (AM109896) and LP-2001 (AJ606361) of the LP-genotype (Hanssen et al. 2008; Hanssen and Thomma 2010). Henceforth in this paper it is denoted as a representative of the LP-genotypes. Typical induced symptoms are mild mosaic and leaf distortion in indicator plants but in tomato only symptomless infections have been reported (Lopez et al. 2005). Yield losses have not been determined.

– PepMV-Sav, E397 (Schwarz et al. 2009) was isolated from tomato fruits. Partial genomic sequences of the isolate (AM930243 and FN386458) showed 99% nucleotide identities to a genotype from France (AF340024, Cotillon et al. 2002) and 98% to other European genotypes (Hanssen et al. 2008). Hence, within this text it is denoted as a representative of the EU-genotypes. It induces typical but mild symptoms in indicator plants but rarely in tomato. Yield losses up to 40% were determined in tomato cultivars "Castle Rock" and "Hildares" (Schwarz et al. 2010).

Plant sap of PepMV infected tomato leaves (cv. Hildares) was prepared for inoculation as described by Schwarz et al. 2010. To prevent mechanical spread of the virus, plant handling in all experiments was done wearing disposable rubber gloves that were changed after each plant.

All plant samples were tested serologically by DAS-ELISA referring to Clark and Adams (1977) and modified by Schwarz et al. (2010). In all experiments the same commercially available polyclonal antibody was used according to the instructions provided and thus allowed comparison of virus titres between samples of the same isolate (AS-0554, German Collection of Microorganisms and Cell Cultures, DSMZ Braunschweig, Germany). Each ELISA test always included the buffer and non infected plant material of the species/cultivar tested as a negative control. A positive control was also included prepared from purified virus material of the applied isolate at a concentration of 582 ng/µl. Samples were rated positive if the absorbance measured at 405 nm was greater than twice the level obtained from healthy controls (Cordoba-Selles et al. 2007). Absorbance levels for the negative controls ranged between 0.09 and 0.10 in all experiments. Furthermore, this antibody was suitable to recognize LP- as well as EU-genotypes under standard conditions usually indicating high concentrations of the virus in plant material (Roggero et al. 2001; Salomone and Roggero 2002; van der Vlugt et al. 2002; van der Vlugt 2009).

Immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR, Schwarz et al. 2010) was applied for the detection of PepMV in leaf material of inoculated indicator and tomato plants. The method was used to confirm results of ELISA.

Plant species and tomato cultivars

Different plant species were tested for their susceptibility to PepMV. In a first experiment (E1) potential indicator plants from the *Nicotiana* spp. and *Chenopodiaceae* family were tested (Tables 1 and 2). The second experiment (E2) included *Solanaceae*, such as potato and bell pepper, *Cucurbitaceae,* such as cucumber, *Alliaceae*, such as garlic and onion, *Fabaceae*, such as runner bean, chickpea and cowpea (Tables 1 and 2). Tomato cultivars[1] were selected to evaluate their susceptibility or tolerance to PepMV based on previous tests against PepMV (unpublished) for a third experiment (E3; Table 3). A fourth experiment (E4) was carried out with selected tomato cultivars[1] based on available information on their susceptibility, tolerance, or resistance against other important tomato pathogens, such as *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Alternaria solani*, *Phytophthora infestans*, *Fusarium spp.*, *Verticillium spp*. (Table 4). Here, local cultivars from the Middle East, landraces as well as hybrids, were also tested since PepMV has not been detected in tomatoes in this region and their reaction to the virus are unknown.

Experimental design (Table 1)

Indicator plants (E1) and crop species (E2) were grown in pots filled with potting mix (Gramoflor, Vechta, Germany) in a greenhouse, daily supplied with tap water following plant demands (Table 2). Seedlings were mechanically inoculated with either a PepMV LP- or EU-isolate, or with the inoculation buffer (Schwarz et al. 2010; Table 1). The distance between the pots did not allow contact between plants. Twenty one days after inoculation, the next to the youngest leaf on the top of each plant was sampled to test for PepMV infection.

Seeds of seven tomato cultivars selected (E3, Tables 1 and 3) were germinated in sterilized coarse sand and cultivated in 80 mm pots. At the 6–7 leaf stage, tomato seedlings were transferred into troughs ($8 \times 0.2 \times 0.1$ m) in a greenhouse. Each trough contained two plants each of seven cultivars randomly distributed, with two troughs per treatment. Treat-

[1] Information on origin and characteristics of all cultivars used can be requested from the authors

Table 1 Overview on experiments (E1-E4) performed: number of plants and replications, treatments, substrates used. Total duration (pre-inoculation/treatment time), method for PepMV detection (E397=European tomato isolate, EU; PV-0554=Peruvian pepino isolate, LP), and environmental conditions are given, such as relative humidity (RH), temperature (T), mean daily radiation (PAR)

Exp	Plants	no	Treatment	Replication no	Substrate	Total duration d	RH day/night %	T day/night °C	PAR $MJ \cdot m^{-2} d^{-1}$	Detection methods
E1	7 indicator	42			0.25 l pots	21/28	69	25/19	8.23	DAS-ELISA, IC-RT-PCR
	plant	21	EU	3	soil					
	species	21	mock	3						
E2	9 plant	81			0.25 l pots	21/31	68	24/18	7.69	DAS-ELISA
	species	27	EU	3	soil					
		27	LP	3						
		27	mock	3						
E3	7 tomato	84			8-m troughs	21/86	74.1/79.5	19.5/18.2	5.49	DAS-ELISA, IC-RT-PCR
	cultivars	28	EU	4	hydroponics					
		28	LP	4						
		28	mock	4						
E4	22 tomato	198			0.25 l pots	21/42	70	24/18	7.43	DAS-ELISA, IC-RT-PCR
	cultivars	66	EU	3	soil					
		66	LP	3						
		66	mock	3						

Table 2 Plant species and cultivars and their origin tested in experiment E1 and E2 against two PepMV isolates (E397=European tomato isolate, PV-0554= Peruvian pepino isolate)

Exp.		Plant species	Cultivar	Origin
E1	Garlic	*Allium sativum*	Namdo	China
	Onion	*A. cepa*	Golden bulb	China
	Cucumber	*Cucumis sativus*	Vorgebirgstrauben	Germany
	Runner bean	*Phaseolus vulgaris*	Saxa	Germany
	Broad bean	*Vicia faba*	Gisa	Egypt
	Chickpea	*Cicer arietinum*	Kabuli "Ghab"	Syria
	Cowpea	*Vigna unguiculata*	Idlib	Syria
	Bell pepper	*Capsicum annuum*	California Wonder	Germany
	Potato	*Solanum tuberosum*	Corona	Germany
E2	Quinoa	*Chenopodium quinoa*		
	Tobacco	*Nicotiana glutinosa*		
		N. clevelandii		
		N. tabacum	Samsun	
		N. benthamiana		
		N. rustica		
	Jimson weed	*Datura stramonium*		

ments were separated by a distance of at least 0.8 m. Troughs separating control from PepMV infected plants were separated by a distance of 2 m to avoid contact and thus transmission of the virus. There was also no contact between plants from different troughs. The distance within the row was 0.5 m. Each trough was irrigated from a separate tank containing a pump continuously recirculating about 100 l of nutrient solution at a flow rate of about 2 l min^{-1} (De Kreij et al. 1997). Electrical conductivity was adjusted to 2.0 dS m^{-1} and the pH to 5.6, and was controlled manually three times a week. Cultivation was performed following commercial practices (Table 1). Harvested plant parts, including roots, shoots, and fruits were weighed both fresh and dry. The dry weight was measured after drying sub-samples in an oven at 80°C for 72 h. Two young leaves (sixth or seventh from the top) were sampled and mixed for detection of PepMV at 21, 36, and 58 days after inoculation.

Further tomato cultivars (E4, Tables 1 and 3) grew in the same potting mix as in experiment E1 in a greenhouse, daily supplied with tap water depending on plant demands. Sampling was 21 days after inoculation using the same procedure as in experiment E3. All tomato cultivars were visually assessed for symptom development weekly.

Table 3 Reduction in fresh weight of seven tomato cultivars inoculated with two PepMV isolates (E397=European tomato isolate, EU; PV-0554= Peruvian pepino isolate, LP) compared with mock inoculated plants. All treatments depicted significant differences compared with the control (two-way-ANOVA) and showed also significant interactions between factor cultivar and isolate at $P=0.05$

Cultivars	Reduction compared with control, %							
	Shoot		Root		Fruit		Total	
	EU	LP	EU	LP	EU	LP	EU	LP
Balkonstar	32.7	9.03	18.6	51.3	1.97	65.3	17.8	41.9
Counter	2.77	13.2	6.71	22.2	17.8	29.1	9.09	21.5
Fruehzauber	8.09	1.79	67.5	8.35	62.2	60.5	45.9	23.5
Gnom	5.01	55.9	11.8	36.1	74.1	70.8	30.3	54.3
Goldene Koenigin	11.5	11.7	38.4	44.2	53.4	38.3	34.4	31.4
Hildares	2.11	40.1	23.1	15.3	42.3	3.13	22.5	19.5
Master	1.18	0.59	34.5	13.1	82.9	85.1	39.5	32.9

Table 4 Relative virus levels in tomato cultivars from different origins infected with two different PepMV isolates (E397= European tomato isolate, EU; PV-0554=Peruvian pepino isolate, LP) scored by mean absorbance in DAS-ELISA. Virus level is defined as low (+ absorbance level is 2–3 times higher compared with mock inoculated control plants), medium (++ 3–5 times), or high (+++ >5 times) absorbance. Control is the mean of three samples from each of all non infected cultivars (22)

Cultivars	EU-isolate	LP-isolate
Cal-Ace	++ 0.349±0.06	++ 0.416±0.16
Castle Rock	++ 0.366±0.02	++ 0.419±0.03
Counter	++ 0.405±0.06	++ 0.578±0.09
Diamond	++ 0.355±0.08	++ 0.394±0.07
Egypt local variety	+ 0.196±0.02	++ 0.248±0.03
Goldene Koenigin	+++ 0.498±0.03	++ 0.316±0.04
Hellfrucht	++ 0.286±0.02	++ 0.298±0.05
Hildares	++ 0.343±0.06	++ 0.458±0.07
Master	++ 0.404±0.06	++ 0.598±0.14
Oula	++ 0.314±0.03	++ 0.384±0-06
Pakmor	++ 0.282±0.02	++ 0.268±0.03
Peto 98	++ 0.319±0.04	+ 0.219±0.02
Petomech VF	++ 0.305±0.07	++ 0.366±0.09
Rawaj	++ 0.273±0.05	++ 0.283±0.01
Royesta	++ 0.298±0.05	++ 0.388±0.01
Super Red	++ 0.335±0.09	++ 0.389±0.05
Syrian local variety 1	++ 0.281±0.02	+++ 0.644±0.03
Syrian local variety 2	++ 0.290±0.01	++ 0.400±0.05
T-03	++ 0.286±0.03	++ 0.418±0.02
T-27	++ 0.292±0.01	++ 0.474±0.12
T-09	++ 0.276±0.05	++ 0.603±0.05
Tenshet Star	++ 0.274±0.03	++ 0.321±0.04
Healthy controls	0.095±0.03	0.082±0.06

Statistics

In experiment 1 (indicator plants) one-way ANOVA was done. Means were separated by Tukey's test procedure at $p=0.05$. In experiment 2–4 data were subjected to two-way ANOVA with species/cultivars and PepMV isolate as treatment factors. For all tests Statistica software (StatSoft Inc. 2004 Tulsa, OK, USA) was used. Significant differences are presented by different letters or asterisks and standard errors are given.

Results

Sensitivity of indicator plants (E1) and plant species (E2) to PepMV

Among the indicator plants infected and tested against the EU-isolate, *C. quinoa* and *N. tabacum* cv. Samsun neither showed symptoms nor reacted positively in PepMV-specific DAS-ELISA (Fig. 1). All other species developed distinct symptoms, such as leaf mottle (*N. benthamiana* and *N. clevelandii*, Fig. 2) or chlorotic leaf spots (*D. stramonium*), and reacted positively. *N. rustica* showed chlorotic or necrotic lesions and *N. glutinosa* a light green colour sometimes combined with chlorotic mottle. However, the most sensitive indicator was *N. benthamiana* with clear symptoms and the significantly highest virus concentrations (Fig. 2). All positive results of ELISA were confirmed by IC-RT-PCR.

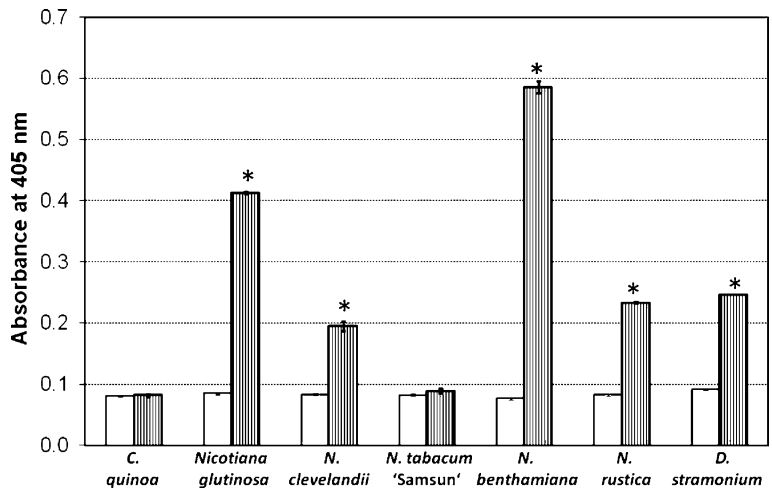

Fig. 1 Absorbance levels in DAS-ELISA measured from leaves of seven indicator plants sampled 21 days after inoculation with PepMV European tomato isolate (E397, vertically striped columns). *Bars* depict standard errors and *asterisks* positive reaction compared with healthy leaves (open columns)

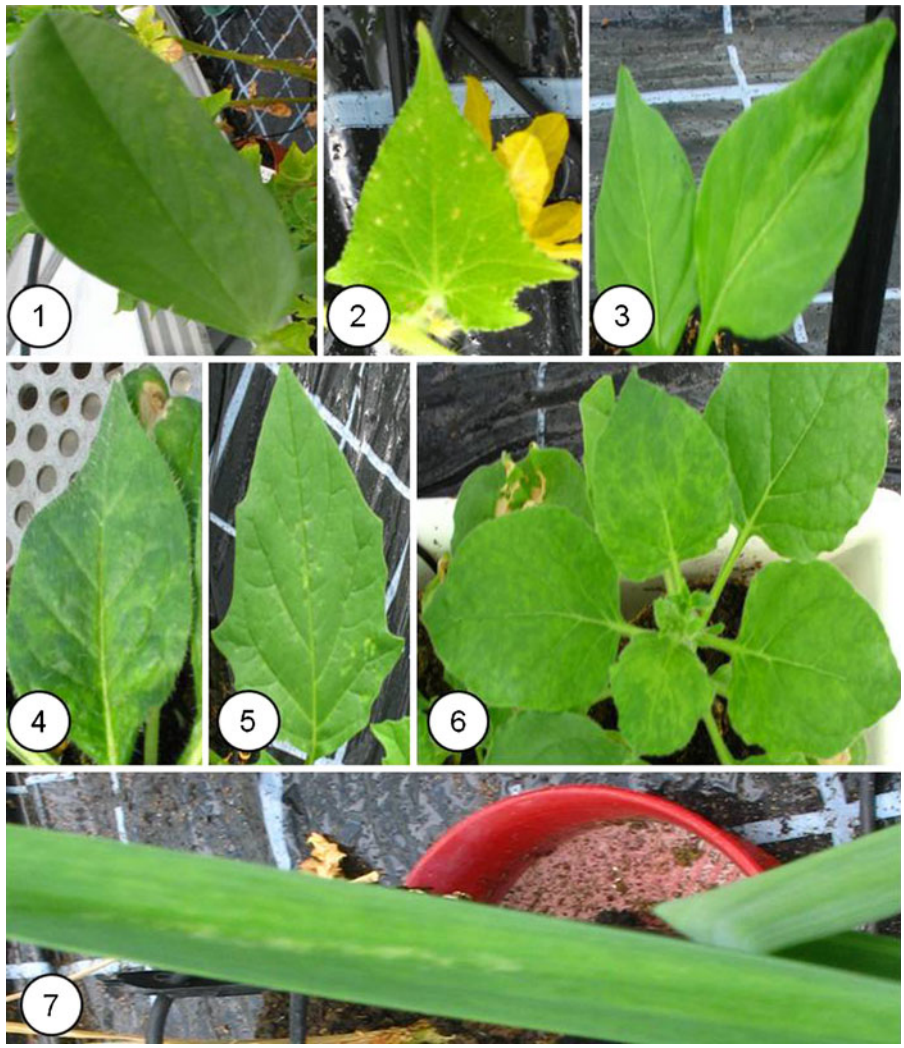

Fig. 2 Symptoms caused by infection with PepMV European tomato isolate E397: yellow spots and colour change on (*1*) broad bean, (*2*) cucumber, (*3*) bell pepper, (*5*) jimson weed, (*7*) garlic; (*4*) leaf mosaic on *Nicotiana clevelandii*; (*6*) change in colour and mosaic on leaves of *Nicotiana benthamiana*

Most plant species inoculated with either the EU- or LP-isolate did not develop any symptoms. However, on bell pepper, garlic, and cucumber leaves yellow spots or stripes and chlorotic mottle appeared at the earliest 7 days after inoculation (Fig. 2). EU and LP infection was confirmed by DAS-ELISA in inoculated potato, broad bean, and bell pepper, while cucumber and garlic plants were only susceptible to the LP-isolate (Fig. 3). The absorbance was significantly higher in samples from bell pepper, cucumber, and garlic when inoculated with LP- compared with the EU-isolate. On the other hand, potato and broad bean infected by the EU-isolate had higher absorbance values than the LP-isolate inoculated samples. Runner bean, chickpea, cowpea, and onion were not infected by either isolate.

Sensitivity of tomato cultivars in hydroponics to PepMV (E3)

Seven days after inoculation, young tomato leaves reacted with interveinal chlorosis (cv. 'Gnom') or mottle, yellow spots and bubbles (cv. 'Hildares'). Even at the end of the experiment, 86 days after inoculation, yellow spots were still visible also on old leaves. While all inoculated and infected 'Hildares' plants showed these symptoms only individual plants among the other cultivars developed symptoms.

Results of IC-RT-PCR as well as DAS-ELISA (Fig. 4) revealed that all seven cultivars tested in E3 (Table 3) were infected by the respective PepMV isolates. All tomato cultivars produced significantly higher absorbance in DAS-ELISA when infected by

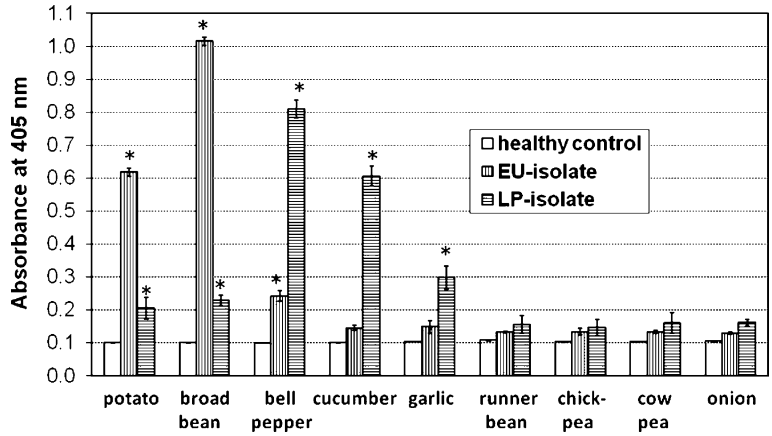

Fig. 3 Absorbance levels in DAS-ELISA measured from leaves of nine plants species sampled 21 days after inoculation with two PepMV isolates (E397=European tomato isolate, EU; PV-0554=Peruvian pepino isolate, LP). *Bars* depict standard errors and *asterisks* positive reaction compared with healthy leaves

the LP-isolate than plants infected with the EU-isolate. The mean absorbance of plant samples inoculated with the EU-isolate was three times higher and with the LP-isolate five times higher compared with the negative control at 0.089. The absorbance values in infected leaf material of both isolates increased over time (Fig. 5). No differences between responses of cultivars after inoculation with the EU-isolate were tested. When inoculated with the LP-isolate 'Gnom' samples gave a significant higher absorbance level compared with 'Hildares' and 'Balkonstar'. Absorbance levels for all other cultivars ranged in between these two groups (Fig. 4).

The growth rate of the cultivars differed significantly and that of 'Hildares' was the highest with 1,876 g plant^{-1} fresh weight followed by 'Counter' with 1,576 g. Total growth of all cultivars was significantly diminished by both PepMV isolates compared with mock inoculated plants, most for 'Gnom', 'Master', and 'Fruehzauber' (Table 3). 'Balkonstar', 'Gnom', and 'Counter' were more negatively affected by the LP- than by the EU-isolate while 'Master' and 'Fruehzauber' more by the EU- than by the LP-isolate. However, both PepMV isolates reduced growth similarly for the cultivars Goldene Koenigin and Hildares.

Growth of single plant parts, such as shoots, roots, and fruits was differently reduced and depended on the virus isolate present. For example, yield of 'Master' was diminished by >80% after infection with both isolates but shoot growth was not significantly affected. In contrast, the yields of 'Balkonstar' when inoculated with the EU- and of Hildares with the LP-isolate were not affected at all while their shoot growth was reduced by more than 30%. Root growth was also not diminished uniformly. Only 'Goldene Koenigin' and the LP-infected 'Balkonstar' showed the same strong reduction of root growth and

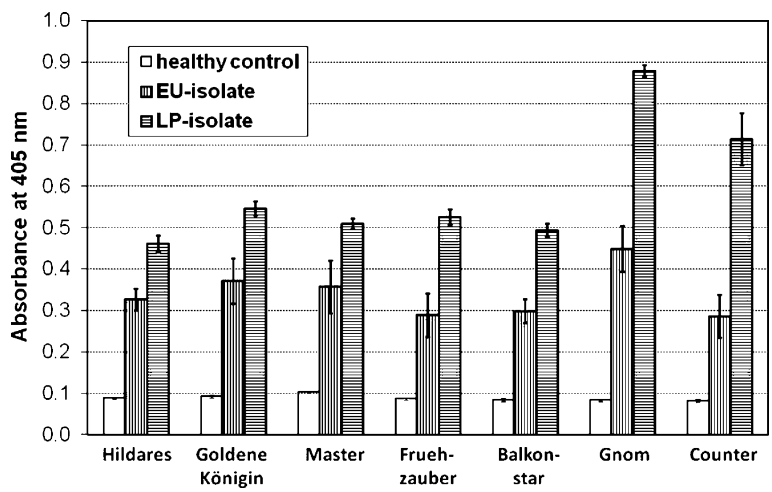

Fig. 4 Absorbance levels in DAS-ELISA measured on leaves of seven tomato cultivars 21 days after inoculation with two PepMV isolates (E397=European tomato isolate, EU; PV-0554=Peruvian pepino isolate, LP). *Bars* depict standard errors. All cultivars showed a positive reaction compared with the healthy control. When inoculated with the LP isolate 'Gnom' had a significantly higher absorbance level compared with 'Hildares' and 'Balkonstar'

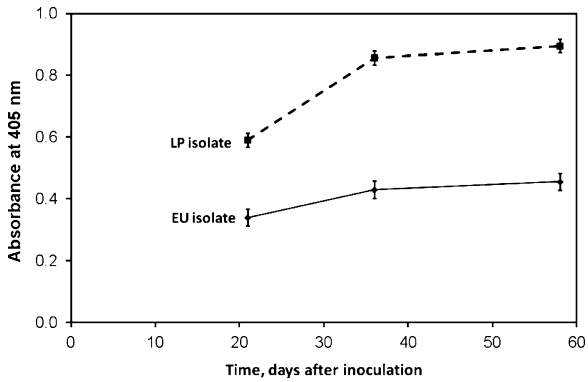

Fig. 5 Absorbance levels in DAS-ELISA measured from leaves of seven tomato cultivars and depicted as means 21, 36, and 58 days after inoculation with two PepMV isolates (E397=European tomato isolate, EU; PV-0554=Peruvian pepino isolate, LP). *Bars* depict standard errors

yield. Shoot growth of 'Gnom' and Hildares inoculated with the LP-isolate was considerably reduced >40% in comparison to healthy controls. Similarly, 'Balkonstar' infected by the EU was significantly shorter (about 30%) than the control plants.

A relationship calculated between absorbance levels of virus concentration in systemically infected leaves in DAS-ELISA and growth responses in terms of total fresh weight confirmed a correlation between both characteristics when the Peruvian isolate was used (R^2=0.5, significant; Fig. 6). The correlation was not significant when the EU-isolate was inoculated (R^2=0.015).

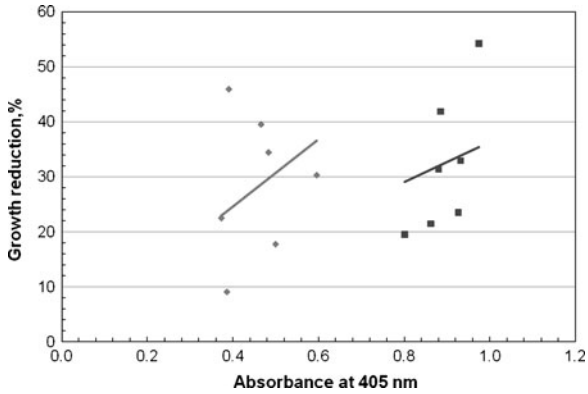

Fig. 6 Correlation between absorbance levels (DAS-ELISA) and reduction in growth (total plant fresh weight) depicted for seven tomato cultivars and two PepMV isolates (E397=PepMV European tomato isolate, rhomb, R^2=0.015 n.s.; PV-0554=Peruvian pepino isolate, square, R^2=0.5 sign.)

Sensitivity of tomato cultivars in a pot experiment to PepMV (E4)

Leaves of PepMV inoculated tomato developed similar symptoms after 1 week as described for PepMV infected cultivars in E3. At the beginning of week three all virus inoculated plants were shorter compared with mock inoculated plants.

All 22 tomato cultivars inoculated with either of the isolates were tested positive for PepMV by DAS-ELISA, which was confirmed by IC-RT-PCR. Based on the measured absorbances the cultivars could be separated into three groups according to virus concentration in the leaf samples, low, medium, and high (Table 4). Most of the cultivars belong in the medium group. The cultivar with the highest levels of the EU-isolate in systemically infected leaves was 'Goldene Koenigin' while LP absorbance was highest in the Syrian landrace 1. Cultivars with lowest virus concentrations were a local variety from Egypt infected by the EU- and 'Peto 98' affected by the LP-isolate.

Discussion

In this study we used two different PepMV isolates, representing the EU- and LP-genotypes. Isolates belonging to both groups were regularly found in PepMV infected tomato crops in Europe, particularly in mixed infections (Pagan et al. 2006; Hanssen et al. 2008; Hanssen and Thomma 2010). Comparisons of available full length genomes of PepMV isolates revealed that the LP- and the EU-isolates are closely related and shared approximately 95% overall nucleotide sequence identity (van der Vlugt 2009; Hanssen and Thomma 2010). Within the EU-genotype sequence identities over 99% were determined (Aguilar et al. 2002; Cotillon et al. 2002; Lopez et al. 2005), while they were less closely related with other genotypes exhibiting sequence identities of 86% or less to US1 and CH2 isolates.

In our study we were able to determine significant differences between the closely related LP- and EU-isolates. They could be distinguished by their host range and we observed variable reactions of affected tomato cultivars concerning symptom expression, growth reduction, and yield loss.

Indicator plants exhibited different susceptibility to the EU-isolate. With the exception of *N. tabacum* cv.

Samsun, all species investigated of the *Solanaceae* family became systemically infected by this isolate originating from tomato, while *C. quinoa* was not susceptible. Non-susceptibility of *C. quinoa* to PepMV isolates originating from 15 different tomato accessions has already been reported by Verhoeven et al. (2003) and recently also confirmed for a Polish isolate PepMV-PK from tomato belonging to the CH2 genotype (Pospieszny and Borodynko 2006; Hasiow et al. 2008). Salomone and Roggero (2002) described that *C. quinoa* was only locally infected in inoculated leaves by a PepMV isolate originating from a Sardinian tomato. This is in accordance with findings that the natural host range of PepMV is mainly restricted to species of the family *Solanaceae* (van der Vlugt 2009). In our experiment *N. benthamiana* was the best host for propagation as these plants developed clear symptoms after infection and accumulated higher virus titres in the leaves than *D. stramonium* and all other tested *Nicotiana* species. Corresponding to our results, *N. tabacum* was not susceptible to most of the EU-isolates investigated in the studies of van der Vlugt et al. (2002) and Verhoeven et al. (2003) although they used another cultivar (White Burley). Results presented and previous observations reveal that PepMV is easily mechanically transmissible especially to species in the *Solanaceae*. Symptom development considerably differs in indicator species, as well as concentration of virus particles in leaf tissue of susceptible host plants. Therefore, it is advisable to determine for each important PepMV isolate the species best suited for propagation.

The representative of the LP-genotypes exhibited a broader host range within the nine crop species investigated than those of the EU-genotypes. The LP-isolate was not only able to infect the investigated crop species belonging to the *Solanaceae* (potato, bell pepper) but also broad bean, cucumber, and garlic. In contrast the EU-isolate was more restricted to the *Solanaceae* and induced systemic infection of bell pepper and potato. Additionally, one member of the *Fabaceae,* i.e. broad bean, was highly susceptible to this isolate, as indicated by high virus concentrations in systemically infected leaves, but did not develop symptoms. Susceptibility of *S. tuberosum* to isolates belonging to both genotypes (EU and LP) is in accordance with reports of Jones et al. (1980) and Mumford and Jones (2005). However, bell pepper was only reported to be susceptible to isolates belonging to the LP-genotype but was never infected by any tomato isolates belonging either to EU- or CH2-genotypes used in studies by Salomone and Roggero (2002), van der Vlugt et al. (2002), and Verhoeven et al. (2003) or two PepMV isolates from Poland (Pospieszny and Borodynko 2006; Pospieszny et al. 2008). In our study systemic infection of this crop was also found with the tested EU-isolate, although virus concentrations were considerably lower in leaves systemically infected by the EU- than the LP-isolate. Likewise, virus concentrations differed considerably in infected tissue of other crop species depending on the isolate applied.

In our study neither the LP-isolate nor the EU-isolate was able to infect runner beans. This is in accordance with findings reported by van der Vlugt et al. (2002). However, tomato and pepino isolates used by this group were also not able to infect cucumber or broad beans, whereas; using different cultivars, the LP-isolate infected both species and the EU-isolate infected cucumber but not broad bean. It has been previously reported that cultivars of tomato, cucumber, and potato differ in their susceptibility to PepMV (Martin and Mousserion 2002; Villemson et al. 2003).

The susceptibility of garlic and broad bean to PepMV has not been reported previously. For growers it is important to know that these crops can be infected by certain PepMV isolates also affecting tomatoes and to avoid them in the rotation as a putative inoculum source for a new tomato crop. Additionally, weed species may serve as PepMV reservoirs in the surroundings of tomato production areas as suggested for instance by Cordoba et al. (2004), who detected the virus in several naturally infected weed species such as *Convolvulus* spp. and *Rumex* spp.. PepMV infected crops and weeds may be a threat to cultivated tomatoes. Also, if the virus isolates are causing a symptomless infection, which was the case with potato and broad bean in our study, they may escape attention.

Since 2005 new virus variants, more isolates from the US and CH2 cluster, and mixed infections of PepMV have started to appear and spread in tomato producing areas in Europe (van der Vlugt 2009; Hanssen and Thomma 2010). In our study tomato cultivars investigated showed no difference in general susceptibility to the two PepMV isolates representing the different genotypes LP and EU. All inoculated tomato plants became systemically infected by both virus isolates, most of them showing little differences

in susceptibility. However, the LP-isolate accumulated to higher levels in tomato leaves and produced more severe symptoms. This is noteworthy because it has been reported by van der Vlugt et al. (2002) and Lopez et al. (2005) that tomato is only latently infected by PepMV isolates belonging to the LP-genotype. Furthermore, both virus isolates used here induced significant differences in symptoms in tomato cultivars as well as in host range. Tomato cultivars with resistance against fungal diseases such as *Alternaria solani* and *Phytophthora infestans* or tolerance to tobamoviruses (ToMV, TMV) did not show resistance against the PepMV isolates tested.

Determined fruit losses from tomato cultivars grown in hydroponics and infected by either the LP- or the EU-isolate ranged between 2% and 85%, covering the whole spectrum of previously estimated losses (Verhoeven et al. 2003; Soler-Aleixandre et al. 2005; Spence et al. 2006; Schwarz et al. 2010). However, in our experiments symptom development and yield losses in tomato cultivars could not be connected to infection with PepMV genotype applied. This is also observed in increasing numbers of studies related to PepMV infection of tomatoes. For instance it has been reported that some EU-and CH2-isolates may induce mild mosaic symptoms but other isolates belonging to the same genotypes induce chlorosis or necrosis in tomato (Hanssen et al. 2009a, b; Hasiow-Jaroszewska et al. 2009). Therefore, it was concluded that symptom induction in tomato and subsequent crop losses depend greatly on environmental conditions such as light and temperature (Jordá et al. 2001; Martinez-Culebras et al. 2002; van der Vlugt 2009; Schwarz et al. 2010; Fakhro et al. 2009). However, in our study virus concentrations in LP-inoculated tomato cultivars were correlated with total growth reduction. This may support the speculation that virus titre in infected tomatoes additionally contributes to symptom development and severity. This was also postulated by Soler-Aleixandre et al. (2005) who found that elevated PepMV concentrations in the basal stem was associated with severe symptoms such as vascular necrosis and collapse of infected tomato plants. On the other hand this does not apply for the symptomless infections we observed in the other crops such as potato or *V. faba* infected by the EU-isolate which showed high accumulation of virus in leaf tissues. Additionally, the tomato cultivars infected by this isolate did not show a connection between virus concentration and growth reduction. A correlation may only exist if the isolate or mix of isolates exceeds a certain threshold of aggressiveness. However, in tomato a correlation between the aggressiveness of isolates to reduce plant growth and yield and symptom development could not be shown. Our results support the suggestions from Hanssen et al. (2009a, b) that the capacity of the virus to induce different symptoms in tomato is a property of the individual isolate and is not linked to the virus genotypes established to date. Additionally, this seems to apply to the capability of virus isolates to infect various plant species, their characteristics in mixed infections with different PepMV isolates and impact of environmental conditions on virus induced yield losses in tomato.

Acknowledgements The study was part of Ahmad Fakhro's PhD program and granted by the University of Al-Furat, Syria. The Ministries of Food, Agriculture, and Consumer Protection of the Federal Republic of Germany and of the states Brandenburg and Thüringen supported the experimental work. We appreciated the technical assistance of Gabriele Buddruss, Gundula Aust and we thank Dr. Winter (DSMZ Braunschweig, Germany) for providing the antibody.

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