An Austrian Cucumber Mosaic Virus Isolate is Causing Severe Symptoms on Resistant *Cucurbita pepo* Cultigens

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This article is being reprinted in its completion, from 2007.

Introduction: In 2005, we discovered some plants of a zucchini yellow mosaic virus (ZYMV)-tolerant oil-pumpkin breeding line (*Cucurbita pepo*), severely affected by a virus. Our first assumption was that ZYMV might have overcome the resistance, but ELISA tests revealed that most likely cucumber mosaic virus (CMV) caused the symptoms, although ZYMV was detected in very low concentrations as well. Using fruit flesh of such infected plants for inoculation of pumpkin seedlings lead to immediate death of all plants, independent of whether the plants were ZYMV-tolerant or susceptible. Therefore, we decided to isolate the CMV for further investigation.

Materials and methods: *Artificial inoculation.* An Austrian isolate of CMV was established as follows. Fruit flesh from oil-pumpkin with multiple virus infestation was collected in fall 2005 and used to inoculate tobacco plants. Tobacco, *Nicotiana tabacum,* is not susceptible to zucchini yellow fleck potyvirus (ZYFV) and squash mosaic comovirus (SqMV) (Plant Viruses Online: http://image.fs.uidaho.edu/vide/famly124.htm#Nicotiana%20tabacum). Tobacco is also not known to be susceptible to ZYMV, the most common virus in oil-pumpkin. Leaves of tobacco plants showing severe mosaic were tested with ELISA for CMV, ZYMV, WMV2 and SqMV. The presence of any virus other than CMV was excluded. Then CMV was increased on plants of the susceptible *C. pepo* variety Gleisdorfer Ölkürbis, because inoculations on *Cucurbita moschata*, using infected leaves of tobacco, failed. The Hungarian isolate (HI), provided to us by István Tóbiás (Plant Protection Institute, Hung. Acad. Sci., Budapest, Hungary), was purified and tested in the same way as the Austrian isolate (AUTI). The French isolate (FI), received from Muriel Archipiano (Clause Tézier, Domaine de Maninet, Route de Beaumont, Valence, France) was treated in the same way. The inoculum for the experiments was prepared from 1.0 g of infected leaves, ground in a mortar on ice, in 10ml inoculation buffer containing 1% K_2HPO_4. Finally, 1.0 g Celite® 545 was added. Seedlings were inoculated twice: first the two cotyledons, when the first true-leaf just appeared, and three days later the first true-leaf itself. Inoculation was carried out by gently rubbing the leaf surface with a finger in rubber gloves. The leaves were rinsed with water immediately after rubbing. Simultaneously the Hungarian and French isolates of CMV were tested.

Plant material: Nine *C. pepo* and nine *C. moschata* cultigens (Table 1) were grown in pots in the greenhouse at 23°-25°C day and 20°-22°C night temperatures at 50-70% RH. Natural illumination was supplemented with a combination of mercury and sodium vapor lamps (ca. 10,000 lux), maintaining a day-length of 14 hours during the whole experiment.

Evaluation: Plants were observed 14 and 24 days after the first inoculation. Leaf symptoms (LS) were rated from 0 (no symptoms) to 9 (severe mosaic). A rating of 10 was introduced for dead plants. Additionally, the approximate growth reduction (GR), in relation to normal growth, was scored in percent. For further evaluation a total rating (TR), using the formula TR=LS+GR*0.05, was calculated. Plants with a TR=0 to 5 were classified as tolerant, such with TR>5 as susceptible. TR-values greater than 10 were limited to 10. To verify the TR-value, we applied the 0 to 5 rating system described by Walkey and Pink (4), who combined leaf symptoms and stunting in one score. After the last evaluation the experiment was terminated and the plant material was autoclaved.

Results and Discussion: A comparison of results obtained by the infection experiment (Table 1), shows that AUTI is the most aggressive isolate. The symptoms caused by HI were half as severe as those caused by AUTI, those caused by FI were still somewhat milder. Comparisons of results obtained with *C. pepo* and *C. moschata*, revealed that, except against AUTI, most of the *C. moschata* cultigens showed a high level of CMV resistance. 'Nigerian Local', however, developed severe symptoms when inoculated with AUTI, although we had hoped that it could be the source of a high level of resis-

tance, as was reported by Brown et al. (1). Nigerian local was found to be resistant against a number of viruses and was therefore used in many breeding programs (1). We obtained a similar result with 'Menina 15' (received from Michael Pitrat, INRA, Montfavet, France), which is, analog to Nigerian Local, highly resistant against ZYMV (2). Only 'Zhou', a Chinese, hull-less *C. moschata* cultivar named by us according to its discoverer Zhou Xianglin (5) and Soler, (kindly provided by L. Wessel-Beaver, USDA-ARS, Puerto Rico), seemed to have resistance against AUTI (Fig. 1). All *C. pepo* cultigens, including 'Linda', an American zucchini F1 variety from Harris Moran Seed Company (Modesto, California) described as CMV-resistant, showed high susceptibility to AUTI. The zucchini variety True French (kindly provided by Harry Paris, Newe Ya`ar Res. Center, Ramat Yishay, Israel), developed clearly less leaf symptoms than most of the other *C. pepo* cultigens. 1997, for the first time, a ZYMV-epidemic destroyed half of the oil-pumpkin harvest in Austria (3). We are alarmed by the fact that, in our first experiment, CMV in combination with ZYMV killed all our test plants. We are wondering, why CMV in the field so far occurs only on single plants. One possibility could be that AUTI lost its aphid transmissibility. A sequencing of the virus genome is in progress. Further investigations will have to be carried out to determine the potential danger posed by this isolate.

Literature Cited

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Table 1: Tested cultigens, their geographic origin, resistance behaviour, average rating for leaf symptoms (LS), growth reduction in % to control (GR), calculated total rating (TR) and Walkey and Pink (4) rating (WP) for comparison.

Cultigens/Origin	Resistance	CMV-AUTI[y]				CMV-HI[x]				CMV-FI[w]			
		LS	GR	TR	WP	LS	GR	TR	WP	LS	GR	TR	WP
						C. pepo							
True French Zucchini/GB	no	3.0	50	5.5	4.0	2.0	25	3.3	3.0	2.8	40	4.8	3.6
Gleisdorfer Ölkürbis/AUT	no	8.7	96	9.3	4.8	4.3	33	6.0	3.3	3.3	29	4.8	3.2
Linda Zucchini F1/USA	CMV	5.0	53	7.6	4.0	3.5	21	4.5	2.7	2.3	17	3.2	2.5
Linda selfing	CMV[z]	5.3	54	7.9	4.0	3.7	58	6.6	3.8	2.8	50	5.3	3.7
Linda x Gleisdorfer Ölkürbis		5.5	63	7.8	4.0	4.5	42	6.6	3.7	3.0	25	4.3	3.0
Tigress Zucchini F1/USA	ZYMV	5.2	50	7.7	4.0	4.5	25	5.8	3.0	3.8	40	5.8	3.6
Tigress selfing	ZYMV[z]	4.7	58	7.6	3.8	4.8	33	6.5	3.3	4.0	63	7.1	3.8
44/15 oil-pumpkin breeding line/AUT	ZYMV	5.7	67	7.3	4.2	4.3	42	6.4	3.7	3.0	29	4.5	3.2
True French resistant against ZYMV/Israel	ZYMV	9.2	79	10.0	5.0	2.0	75	5.8	4.0	3.2	50	5.7	4.0
Mean		6.0	65	8.0	4.2	3.7	39	5.7	3.4	3.1	38	5.0	3.4
						C. moschata							
Waltham Butternut/USA	no	4.2	75	7.3	4.2	4.0	45	6.3	3.8	0.5	0	0.5	0.5
Zhou x Watlham Butternut.		3.0	25	4.3	3.0	0.0	8	0.4	0.7	0.2	4	0.4	0.5
Zhou hull-less/China		1.4	25	2.7	3.0	0.8	0	0.8	0.5	0.0	0	0.0	0.0
Soler/Puerto Rico	ZYMV	2.2	25	3.4	3.0	0.2	4	0.4	0.5	0.0	0	0.0	0.0
Waltham Butternut. x Soler		3.0	50	5.5	4.0	1.3	0	1.3	1.3	0.3	0	0.3	0.3
Menina 15/France	ZYMV	5.0	50	7.5	4.0	0.0	0	0.0	0.0	0.0	0	0.0	0.0
Nicklow´s Delight selfing/USA		4.2	79	7.3	4.2	3.0	25	4.3	3.0	2.0	4	2.2	2.2
Nigerian Local/Nigeria	ZYMV, CMV, WMV2, PRSV-W	4.0	75	7.8	4.0	0.0	0	0.0	0.0	0.0	0	0.0	0.0
Waltham Butternut x NigerianLocal		5.0	50	7.5	4.0	0.5	4	0.7	0.5	0.0	0	0.0	0.0
Mean		3.7	52	6.0	3.7	1.1	10	1.6	1.1	0.3	1	0.4	0.4

[z] Segregating; [y] Austrian isolate; [x] Hungarian isolate; [w] French isolate

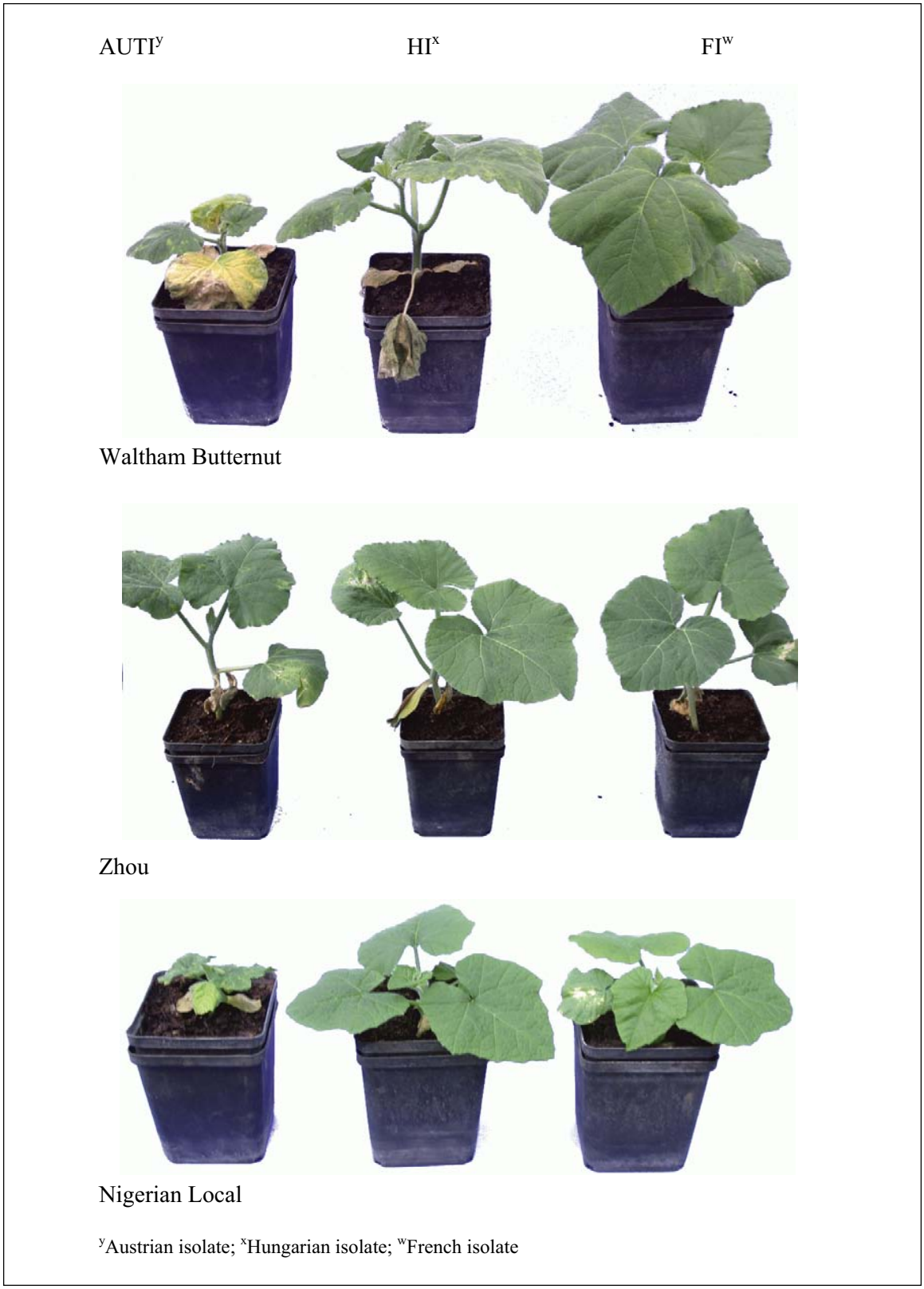

Fig. 1 Symptom development 24 days after inoculation with three isolates of CMV on the C. *moschata* cultigens 'Waltham Butternut', 'Zhou', and 'Nigerian Local'