Reactions of some cucumber *(Cucumis sativus L.)* lines and hybrids to zucchini yellow mosaic virus (ZYMV) and selection of tolerant breeding lines

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Summary: In the past years zucchini yellow mosaic virus (ZYMV) has been appeared as a new pathogen of cucurbitaceous plants in Hungary. It caused severe disease outbreaks on the fields of pickling cucumber hybrids which are highly tolerant to cucumber mosaic virus (CMV). Preliminary inoculation tests have showed that all of the important pickling cucumber hybrids produced in Hungary were susceptible to ZYMV-P. However, a selection of Chinese Long and the breeding line I- KS10C proved to be highly tolerant. The F1 plants of the crosses between the tolerant lines and the susceptible genotype H1 were susceptible to ZYMV-P. F2 populations segregated for susceptible and tolerant individuals at a ratio of 3:1. The results strongly suggest that the resistance to ZYMV in our sources is controlled by a single recessive gene. Inoculations of the ZYMV and CMV susceptible cultivar Budai csemege and the CMV tolerant hybrid Perez F1 with the complex of ZYMV+CMV resulted extremely severe symptoms (strong mosaic and necrotic spotting of the leaves) on both cultivars. On the Chinese Long line, which is tolerant to ZYMV and CMV, respectively, the complex of the two viruses caused mild symptoms. The results show a synergistic pathological effect of ZYMV and CMV on differrent cucumber genotypes. More detailed studies on the interactions among the plant genotypes, viruses and virus strains are needed to develop cucumber hybrids that are highly resistant to the ZYMV+CMV complex.

Introduction

Cucumber (*Cucumis sativus L.*) is susceptible to a great number of plant viruses of which only the aphid borne *cucumber mosaic Cucumovirus* (CMV) caused economic losses in the past in Hungary. The aim of the breeding strategy of the Vegetable Crops Research Institute at Budatétény was to develop hybrids of pickling cucumber having high resistance to the most important pathogens including viruses. Resistance to CMV found in a line of *Chinese Long (Ruskó* and *Balogh,* 1994) was incorporated to the hybrids Perez F1 and Mohikán F1, which are also resistant to downy mildew (*Pseudoperonospora cubensis*) and powdery mildew (*Shpaerotheca fuliginea*) (*Balogh* et al. 1994; 1996).

Likely to many countries of the world, the epidemic appearance of *Zucchini Yellow mosaic Potyvirus* (ZYMV) has been demonstrated recently on the cucurbitaceous crops in Hungary (*Tóbiás* et. al., 1996; 1998; *Salamon* et al., 1997). The economic importance of ZYMV promted us to study the resistance of cucumber to ZYMV as well as to start a breeding program aimed to develop pickling hybrids resistant to this virus. Preliminary studies on the resistance to the complex of ZYMV+CMV have also been carried out.

Materials and methods

ZYMV-P was isolated from naturally infected cucumber showed severe necrotic mosaic disease. It was separated from CMV-P by transmission from a single chlorotic lesion of *Chenopodium quinoa Willd.*, then propagated and maintained on cucumber (cvs. Budai csemege and Perez F1). CMV-P was separated from ZYMV-P by inoculation of tobacco (*Nicotiana tabacum* cv. Xanthi-nc) which is immune to ZYMV. CMV-P was maintained and propagated on Xanthi-nc tobacco plants. The complex of ZYMV and CMV (ZYMV+CMV-PP) was maintained on cucumber.

For inoculation, cucumber seeds were sown in 10 cm pots and germinated in light boxes at 28–30 C°. Seedlings were grown at 24–26 C° under light using 16/8 h photoperiod. They were mechanically inoculated by rubbing the cotyledons with glass-spatula dipped in fresh leaf extracts of the propagative hosts of viruses. Carborundum was applied as an abrasive. Symptoms were evaulated weekly for 4–6

weeks. The presence of viruses in symptomless plants were demonstrated by re-isolation to test plants (*Chenopodium quinoa and/or Nicotiana tabacum*).

Crosses of Chinese Long x H1, I KS10C x H1 and Chinese Long x I KS10C were made in greenhouse. F1 and F2 plants were inoculated with ZYMV-P. Disease tolerance and susceptibility of the individuals were evaluated by analysis of symptoms. Preliminary experiments were made to check the tolerance of some F2 populations to ZYMV+CMV complex.

Results and Discussion

Symptomathological surveys carried out by the authors in 1995 and 1996 have showed that a disease, called *necrotic mosaic* (*Salamon* et al., 1997) appeared in the fields of pickling cucumber hybrids irrespective of their resistance background. So, the hybrids having high tolerance to CMV became affected by symptoms characterised by mosaic, necrotic spotting and deformation of the leaves *(Fig.1)* as well as by green-yellow mottling and severe distortions of the fruits.

Virological tests using differential test plants (*Capsicum, Chenopodium, Cucumis, Datura, Nicotiana spp.*) have showed that the plants affected by necrotic mosaic disease were infected always by at least two viruses. One of them was isolated by inoculation of tobacco (cv. Xanthi-nc) and identified as the pathotype C of CMV.

The second virus was detected by inoculation of *Chenopodium quinoa Willd.* with the extract of naturally infected cucumber. On the inoculated leaves of *C. quinoa* chlorotic lesions developed among the necrotic ones caused by CMV. The virus responsible for the chlorotic lesions did not infect tobacco and on cucumber (cv. Budai csemege and Perez F1) it caused systemic symptoms consisted of severe vein clearing, green puckered islands and severe malformation of the leaves *(Fig.2)*, characteristic to ZYMV (cf. Lisa and Lecoq, 1984). In DAS-ELISA tests the virus reacted strongly with an antiserum prepared to ZYMV, but did not react with an antiserum of watermelon mosaic virus (WMV).

A Hungarian isolate of ZYMV, marked ZYMV-10 was characterized as pathotype 2 of ZYMV by *Tóbiás* et.al (1998). The pathotype of our isolate, marked ZYMV-P has not yet been determined, but in respect of the symptoms caused on cucumber it is very similar to those of ZYMV-10.

Inoculation of a wide range of cucumber cvs. by ZYMV-P have showed that some old cultivars (e.g. Budai csemege, Delicatess) and all of the current hybrids (e.g. Accordia, Duett, Claudia, Firmate, Harmonia, Mohikán, Oricat, Ouverture, Passavia, Pazano, Perez, Profi, Ringo) produced in Hungary were highly susceptible to the virus. However, a line of Chinese Long and a breeding line marked I-KS 10C (both are tolerant to CMV) proved to have resistance (high tolerance) to ZYMV-P. On these lines, ZYMV did not cause any symptoms, but the virus could be re-isolated from the top leaves.

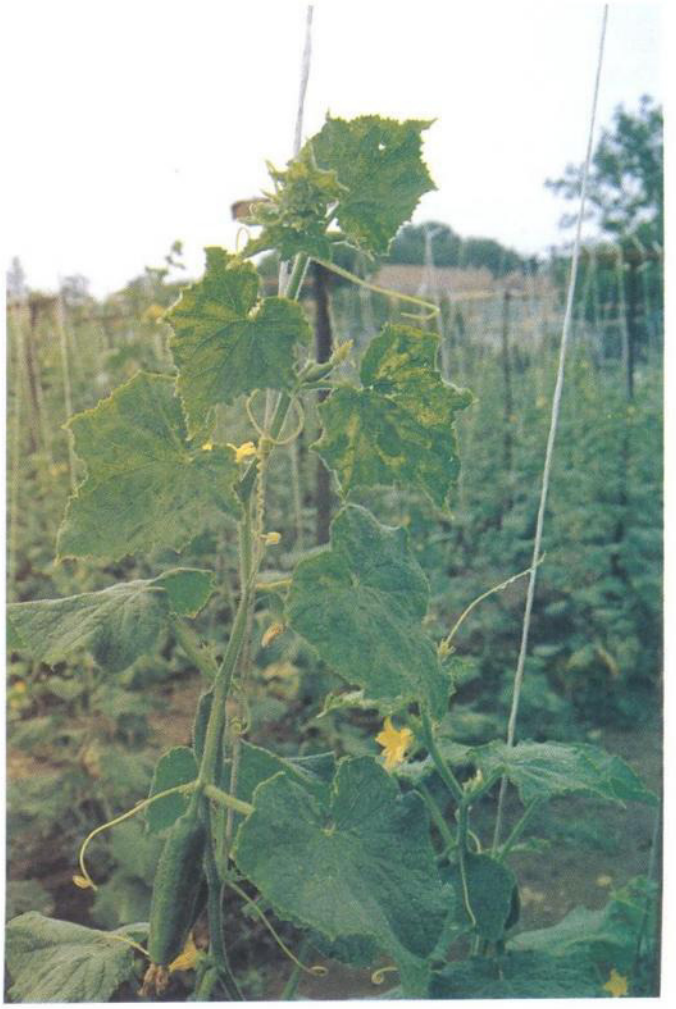

Figure 1 Symptoms of necrotic mosaic disease caused by ZYMV + CMV infection on naturally infected cucumber plants

Figure 2 Symptoms (green puckered mosaic, deformations) caused by ZYMV on susceptible cucumber

Inheritance of tolerance to ZYMV in Chinese Long and I-KS 10C was studied. Crosses were made between the two tolerant lines and the susuceptible parent H1 and the F1 and F2 generations were analyzed for segregation of susceptible and tolerant individuals. All of the F1 plants were susceptible

to ZYMV. In accordance to the literature data, the segregation ratio of the F2 populations for susceptible and tolerant plants *(Table 1.)* strongly suggest that the tolerance in our sources is controlled by a single recessive allele, most probably the recessive allele marked zym *(Provvidenti*, 1987). All of the F1 plants of the crosses Chinese Long x I KS10 C remained symptomless showing that the parents posses probably the same allele of the resistance to ZYMV.

ZYMV, CMV and their artificial mixture were back inoculated to cucumber cvs. either susceptible or tolerant to CMV. *(Table 2.)* The results clearly showed, that the CMV tolerant hybrids were susceptible to ZYMV. Mixed infections of ZYMV and CMV caused necrotic mosaic symptoms on both the CMV susceptible and CMV tolerant cultivars showing a pathological synergism between the two viruses. The synergic interference of the two viruses in cucumber was described by Poolpol and *Inouye* (1986) and *Palukaitis* and *Kaplan* (1998).

Because Chinese Long showed mild symptoms to the inoculation with CMV+ZYMV, plants of the F2 population of the cross Chinese Long x H1 were inoculated by the complex, too. The symptoms appeared on the individuals varied greatly and were not suitable to drawn any conclusions on the inheritance of tolerance to the complex infection of CMV and ZYMV. More detailed pathological and genetic studies are needed to analyse the interactions of the two viruses on differrent genotypes of cucumber.

Table 1 Segregations in F2 populations from crosses of ZYMV tolerant and susceptible cultivars

Crosses	Total plants number of	No. of susceptible plants		Ch2	P
		Observed	3:1		
I KS10C x H1	61	42	46	1,229	0,2-0,3
Chinese Long x H1	60	51	45	3,2	0,05-0,1
Crosses combined	121	93	91	0,223	0,7-0,9

Table 2 Symptoms caused by cucumber mosaic virus, zucchini yellow mosaic virus and their mixture on susceptible and tolerant cucumber genotypes

PLant Genotype	Symptoms		
	CMV	ZYMV	CMV + ZYMV
CMV susceptible (cv. Budai csemege)	yellow-green vein clearing, green mosaic	vein clearing, green puckered mosaic, severe deformation of leaves	severe mosaic, necrotic spots, deformations
CMV tolerant (cv. Perez F1)	mild mosaic, recovery	vein clearing, green puckered mosaic, severe deformation of leaves	severe mosaic, necrotic spots, deformations
CMV tolerant ZYMV tolerant (Chinese Long)	symtomless	symptomless	mild mottle, e yellow spots

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