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Resistance of melon to *Cucumber Vein Yellowing Virus* (CVYV)

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Abstract

Cucumber vein yellowing virus (CVYV) belongs to the *Ipomovirus* genus, family *Potyviridae*. It is transmitted by the whitefly *Bemisia tabaci*, but can be transmitted mechanically in artificial inoculation. A collection of 1188 melon accessions has been inoculated with a CVYV strain isolated from melon in Spain. Five phenotypes have been observed: 46 % of the accessions are susceptible with mosaic and vein-banding symptoms like the control Védrantais; 50 % are highly susceptible with a severe mosaic, yellowing and stunting. Ouzbeque 2 is one of these accessions; few accessions exhibited necrotic symptoms with a rapid death of the plants. HSD 93-20-A from Sudan is a representative of this phenotype; only one accession (HSD 2458 from Sudan) was tolerant with very mild mottle but the virus can be detected by DAS-ELISA or RT-PCR in the plant apex; only an inbred line derived from the accession PI 164323 from India was resistant, exhibiting no symptom and the virus cannot be detected in non-inoculated leaves either by DAS-ELISA or RT-PCR. Inheritance of these behaviours was studied in F_1, F_2 and BC progenies between the above mentioned accessions. Three loci seem to be involved. At a first locus tentatively named *Cucumber vein yellowing* resistance (symbol *Cvy-1*), three alleles have been identified: *Cvy-1*$^+$ for susceptibility (present in Védrantais), *Cvy-1*^{1} controlling resistance in PI 164323 and *Cvy-1*^{2} controlling necrosis in HSD 93-20-A. At an independent second locus, the recessive allele *cvy-2* present in HSD 2458 controls the tolerance. And at a third locus the allele *Cvy-3*, present in Ouzbèque 2, controls the highly susceptible type of symptoms.

INTRODUCTION

*Cucumber vein yellowing virus (*CVYV) has been first described in Israel (Cohen and Nitzany 1960), then in other countries of the Middle-East and more recently in the western part of the Mediterranean area (Cuadrado et al. 2001;

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Lecoq et al. 2007; Lecoq and Desbiez 2012). It belongs to the *Ipomovirus* genus, family *Potyviridae* and is transmitted in the semi-persistent manner by the whitefly *Bemisia tabaci*, but can be transmitted mechanically in artificial inoculation. It induces mosaic and yellowing symptoms on the leaves and can severely reduce the yield and the fruit quality.

Resistance has been described in cucumber as controlled by a dominant gene (Picó et al. 2008). Wild *Cucumis* species (*C. prophetarum*, *C. africanus*, *C. dipsaceus*) have been described as resistant (Marco et al. 2003) but up to date no accession of *C. melo* has been observed to be resistant (Montoro et al. 2004).

In this paper, we describe the screening of melon germplasm and the inheritance of observed different phenotypes.

MATERIALS AND METHODS

Plant material

A total number of 1188 accessions of a melon collection maintained in France by a network associating INRA and private seed companies have been sown in trays with 10 seeds per accession. Some accessions with a typical phenotype were then used to study the inheritance of these traits, namely: 'Védrantais', 'Ouzbèque 2', PI 164323, HSD 2458 and HSD 93-20-A. The F_1, F_2 and back-cross progenies were produced. A recombinant inbred lines population (126 RILs) was developed between Védrantais and PI 164323.

Virus material and inoculation method

A CVYV strain isolated from melon in Spain in 2003 was used for mechanical inoculation on the cotyledons at the pointing first leaf-stage. Symptoms were visually rated. DAS-ELISA and RT-PCR tests were performed on plants with no symptoms or very weak symptoms to assess the presence or absence of the virus. DAS-ELISA was done using an antiserum produced against the CVYV coat protein expressed in bacteria and RT-PCR using primers previously described (Lecoq et al. 2007).

RESULTS

Germplasm evaluation

Among the 1188 accessions, a first group (46.0% of the accessions) exhibited typical mosaic symptoms like the control 'Védrantais' and a second group (50.3%) exhibited very severe mosaic symptoms with yellowing and stunting. The accession 'Ouzbèque 2' was selected as a representative of this second group for inheritance studies. A majority of accessions belonging to the botanical varieties *cantalupensis*, *reticulatus* and *flexuosus* were in the first group with mosaic symptoms. A majority of accessions of the botanical varieties *agrestis*, *acidulus*, *ameri*, *chate*, *chinensis*,

dudaim, *makuwa*, *momordica*, and *tibish* were in the second group with severe symptoms. About 50% of the accessions of the botanical varieties *chito*, *conomon* and *inodorus* had mosaic symptoms and 50% severe symptoms. Twenty-three accessions exhibited a systemic necrosis a few days after inoculation; most of them originated from Sudan. HSD 93-20-A was used in the inheritance studies as a representative of this group. One accession from Sudan, HSD 2458, exhibited very mild symptoms of mottling but the virus can be detected by DAS-ELISA or by RT-PCR. Finally, an inbred line in an accession from India, PI 164323, was the only resistant accession with no symptoms and no detection of the virus in non-inoculated leaves, although very rarely few plants presented the necrotic phenotype. Interestingly, all plants from other inbred lines of PI 164323 developed a systemic necrosis few days after inoculation.

Genetic control

Inheritance of resistance

In F_1, F_2 and BC progenies from the cross 'Védrantais' × PI 164323, four phenotypes were observed: no symptom (like PI 164323), necrosis (like HSD 93-20-A), mosaic (like 'Védrantais') and severe mosaic, yellowing and stunting (like Ouzbèque 2) (Table 1a). The necrotic symptom, of the F_1 and a majority of plants in the F_2 and BC progenies, correspond to the heterozygous status. In the segregating progenies, the pooled numbers of plants with no symptom or necrosis *versus* mosaic or severe mosaic can be explained by one dominant gene. In the F_2, 267 *vs* 100 (χ^2 for 3:1 = 0.989, Prob = 33%); in the BC_s 85 *vs* 87 (χ^2 for 1:1 = 0.002, Prob = 88%) and no susceptible plant in the BC_r but one; in the RILs population, 44 RILs *vs* 38 RILs (χ^2 for 1:1 = 0.439, Prob = 51%). In the BCr, no susceptible plant (but one with severe mosaic) was observed. These segregations fitted a "one dominant gene for resistance" hypothesis with necrosis at the heterozygous status. We propose to name this gene *Cucumber vein yellowing virus* resistance (symbol *Cvy-1*).

This genetic control was confirmed in crosses between PI 164323 and HSD 2458 (Table 1c), when pooling the numbers of plants with no symptom or necrosis *versus* mild mottle, mosaic or severe mosaic. In the F_2, 393 *vs* 134 (χ^2 for 3:1 = 0.051, Prob = 82%); in the BC_s 45 *vs* 50 (χ^2 for 1:1 = 0.263, Prob = 61%) and no susceptible plant in the BC_r but one.

Inheritance of necrosis

The necrotic symptom observed in the accession HSD 93-20-A (Table 1b and 1e) was also observed occasionally in PI 164323 and frequently in the F_1, F_2 and BC progenies with PI 164323 as a parent (Table 1a and 1c). The segregation observed in the F_2 between Ouzbèque 2 and HSD 93-20-A (Table 1b) fitted a 3 (resistant or necrotic) *vs* 1 (mosaic) segregation corresponding to a monogenic dominant control

($\chi^2 = 2.881$, Prob = 9%).

In crosses between PI 164323 and HSD 93-20-A, the F_1 was resistant and in the F_2 no susceptible plant was observed (157 resistant and 38 necrotic). It can be concluded that the same locus *Cvy-1* is involved in the genetic control of necrosis in HSD 93-20-A but that another allele is present in HSD 90-20-A. We propose the symbol *Cvy-1*^{1} for the allele in PI 164323 and *Cvy-1*^{2} for the allele in HSD 93-20-A with the following symptoms: resistance for the homozygous *Cvy-1*^{1} / *Cvy-1*^{1} or the heterozygous *Cvy-1*^{1} / *Cvy-1*^{2} and necrosis for the homozygous *Cvy-1*^{2} / *Cvy-1*^{2} or the heterozygous (*Cvy-1*^{1} / *Cvy-1*$^+$ or *Cvy-1*^{2} / *Cvy-1*$^+$).

Inheritance of tolerance

The accession HSD 2458 exhibited very weak symptoms (mild mottle) but the virus can be detected in DAS-ELISA or RT-PCR. In the F_2 between HSD 2458 and 'Védrantais' (Table 1d), a 1 tolerant: 3 mosaic segregation was observed ($\chi^2 = 0.114$, Prob = 74%) and in the F_2 with Ouzbèque 2, a 1 tolerant: 3 mosaic or severe mosaic segregation was observed ($\chi^2 = 0.346$, Prob = 56%). We propose the symbol *cvy-2* for this recessive gene controlling tolerance in HSD 2458. This genetic control was confirmed in the F_2 between HSD 2458 and PI 164323 (Table 1c) with a 1 tolerant: 15 resistant or necrotic or mosaic or severe mosaic segregation ($\chi^2 = 0.790$, Prob = 37%). The same 1:15 segregation was also observed in the F_2 between HSD 2458 and HSD 93-20-A (Table 1e): 11 tolerant plant *vs* 166 plants with necrosis, mosaic or severe mosaic ($\chi^2 = 0.0004$, Prob = 98%).

Inheritance of severe mosaic, yellowing and stunting

In the F_2, BC_s and RILs between 'Védrantais' and PI 164323 (Table 1a), and similarly in the F_2 between 'Védrantais' and HSD 93-20-A (data not shown), plants with mosaic or severe mosaic were observed. The "severe mosaic" symptom was dominant over the mosaic symptom. The segregation observed (Table 1a) in the F_2 ($\chi^2 = 0.754$, Prob = 39%), in the BC ($\chi^2 = 5.069$, Prob = 2%) and the RILs ($\chi^2 = 1.684$, Prob = 19%) fitted one dominant gene hypothesis. We propose the symbol *Cvy-3* for this gene with the allele *Cvy-3*$^+$ for mosaic symptom.

The allele *Cvy-3* is present in PI 164323, HSD 93-20-A and Ouzbèque 2 and the allele *Cvy-3*$^+$ is present in Védrantais and HSD 2458 as shown (Table 1d) by the absence of plants with severe mosaic in the F_2 between 'Védrantais' and HSD 2458 and the presence of plants with severe mosaic or mosaic in the F_2 between Ouzbèque 2 and HSD 2458.

Linkage and epistasis between the three loci

The locus *Cvy-1* is independent from *Cvy-3* as shown for instance by the 1:1 segregation for mosaic *vs* severe mosaic among the susceptible RILs (Table 1a).

The alleles *Cvy-1*^{1} and *Cvy-1*^{2} are epistatic and dominant on *Cvy-3* according to the higher number of resistant or necrotic plants over the susceptible (mosaic or severe mosaic) or tolerant plants. Moreover the two lines PI 164323 and HSD 93-20-A have respectively the genotypes [*Cvy-1*^{1} *Cvy-3*] and [*Cvy-1*^{2} *Cvy-3*] confirming the epistatic effect over *Cvy-3*. However the allele *Cvy-3* is not necessary for the resistance as it can be concluded from the observation of F_2 progenies between 'Védrantais' and RILs which were resistant: some of these F_2 were segregating with plants with no symptom and plants with mosaic but no plants with severe mosaic indicating that these RILs had the genotype [*Cvy-1*^{1} *Cvy-3*$^+$]

The F_1 between HSD 2458 and Ouzbèque 2, heterozygous for both *cvy-2* and *Cvy*-3, exhibited mosaic symptoms (Table 1d). In the F_2 progeny, we observed about 25% of tolerant plants corresponding to the homozygous [*cvy-2 cvy-2*] (see above inheritance of tolerance). Plants heterozygous for *cvy-2* or without the *cvy-2* allele [*cvy-2 cvy-2*$^+$] or [*cvy-2*$^+$ *cvy-2*$^+$] exhibited mosaic or severe mosaic symptoms. The observed 7:5 segregation (mosaic *vs* severe mosaic) ($\chi^2 = 2.489$, Prob = 11%), could be explained by the following interactions: plants heterozygous for both genes [*cvy-2 cvy-2*$^+$ *Cvy-3 Cvy-3*$^+$] like the F_1 or homozygous for *Cvy-3*$^+$ exhibited mosaic symptoms and plants homozygous *cvy-2*$^+$ and homo- or heterozygous *Cvy-3* [*cvy-2*$^+$ *cvy-2*$^+$ *Cvy-3* -] exhibited severe mosaic symptoms.

From the segregation observed in the F_2 and BC between PI 164323 and HSD 2458 (Table 1c), we can conclude that *Cvy-1* is epistatic dominant over *cvy-2*: the plants homozygous *Cvy-1*^{1} *Cvy-1*^{1} were resistant whatever the allele at the *cvy-2* locus and the plants heterozygous at the locus *Cvy-1* were necrotic whatever the allele at the *cvy-2* locus.

DISCUSSION AND CONCLUSIONS

After the evaluation of the melon genetic resources with one isolate of CVYV, five phenotypes have been observed. Resistance has been found in only one accession as was the case for other viruses, *Zucchini yellow mosaic virus* (ZYMV) or *Papaya ringspot virus-watermelon strain* (PRSV-W), where a low number of resistant accessions have been identified. This resistance is controlled by one dominant allele but the heterozygous plants exhibited a necrotic reaction. In commercial F_1 cultivars resulting from breeding programs, both parents must be resistant. This necrotic reaction was also observed in some accessions which were homozygous for the allele *Cvy-1*^{2}. This type of symptom has been observed in melon in interaction with other viruses. Two pathotypes of ZYMV have been described: the pathotype NF induces mosaic symptoms on 'Védrantais' and 'Doublon' while the pathotype F induces mosaic symptoms on 'Védrantais' and wilting and necrosis on 'Doublon' (Lecoq and Pitrat 1984). One dominant gene (*Flaccida necrosis*, symbol *Fn*) present in 'Doublon' controls this phenotype (Risser et al. 1981). A similar

situation has been described with *Moroccan watermelon mosaic virus* (MWMV) and the gene *Nm* (Quiot-Douine et al. 1988). The interaction with PRSV-W is more similar to what is observed with CVYV. At the locus *Prv*, the allele Prv^1 controls resistance to all strains of PRSV and the allele Prv^2 controls resistance to some strains and systemic necrosis with other strains (Pitrat and Lecoq 1983).

The phenotype of partial resistance with very light symptoms has also been identified in only one accession and is controlled by one recessive allele *cvy-2*. Up to now only a few recessive genes have been described in melon for virus resistance: *cab-1* and *cab-2* for *Cucurbit aphid borne yellows virus* (CABYV), *nsv* for *Melon necrotic spot virus* (MNSV) and QTLs for *Cucumber mosaic virus* (CMV). The practical interest of the gene *cvy-2* in association with $Cvy\text{-}1^1$ or alone for the level and/or the durability of resistance has to be evaluated.

About half of the accessions presented severe yellowing and stunting symptoms. This type of symptom cannot be related to geographical origin or botanical types. It is controlled by one dominant allele *Cvy-3*. Surprisingly, this allele is present in the resistant accession PI 164323 with $Cvy\text{-}1^1$ or in the necrotic accession HSD 93-20-A with $Cvy\text{-}1^2$. But the allele is not necessary for the phenotype of resistance as plant with the [$Cvy\text{-}1^1$ $Cvy\text{-}3^+$] genotype are resistant. Nevertheless its interaction with the locus *Cvy-1* for the durability of resistance should be investigated. Indeed, in preliminary tests, CVYV isolates from other geographical origins could induce necrotic or stunting symptoms on the PI 164323 inbred line described in the paper.

The genotypes of the five lines studied in this paper are the following: 'Védrantais' [$Cvy\text{-}1^+$ $cvy\text{-}2^+$ $Cvy\text{-}3^+$], 'Ouzbèque 2' [$Cvy\text{-}1^+$ $cvy\text{-}2^+$ *Cvy-3*], PI 164323 [$Cvy\text{-}1^1$ $cvy\text{-}2^+$ *Cvy-3*], HSD 93-20-A [$Cvy\text{-}1^2$ $cvy\text{-}2^+$ *Cvy-3*] and HSD 2458 [$Cvy\text{-}1^+$ *cvy-2* $Cvy\text{-}3^+$].

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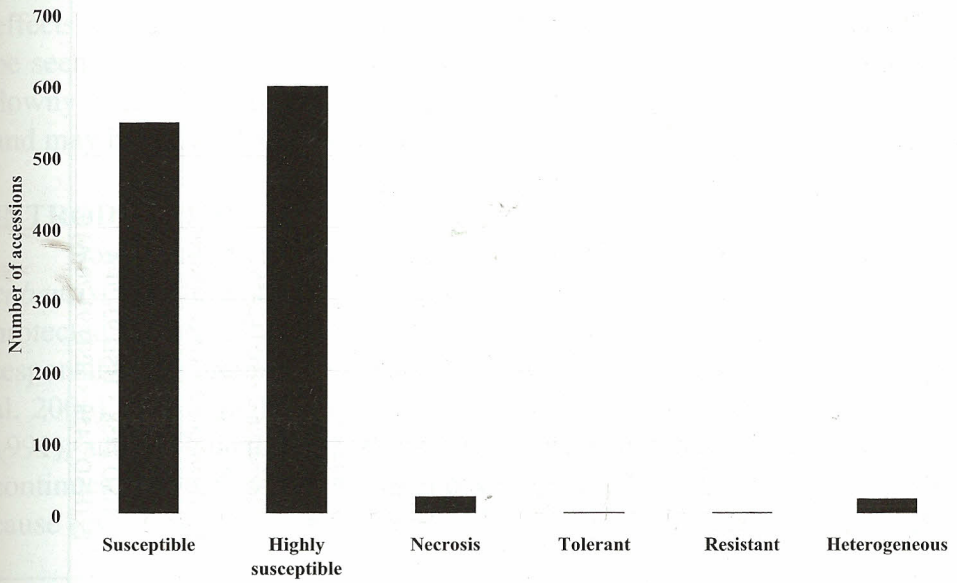

Fig. 1. Number of melon accessions according to the symptoms observed after inoculation with a strain of CVYV.

Table 1. Number of plants observed with different types of symptoms in progenies between different lines of melon (HSD 2458, HSD 93-20-A, Ouzbèque 2, PI 164323 and Védrantais) following inoculation with CVYV.

	Progeny	Symptoms				
		No symptom	Necrosis	Mild mottle	Mosaic	Severe mosaic
a	Védrantais				50	
	PI 164323	43	2			
	F_1 Védrantais × PI 164323		34			
	F_2 (Védrantais × PI 164323) ⊕	30	237		23	77
	BC_s (Védrantais × PI 164323) × Védrantais	1	85		33	54
	BC_r (Védrantais × PI 164323) × PI 164323	53	116			1
	RILs Védrantais × PI 164323	44			15	23
b	Ouzbèque 2					7
	HSD 93-20-A	4	12			
	F_1 Ouzbèque 2 × HSD 93-20-A		12			
	F_2 (Ouzbèque 2 × HSD 93-20-A) ⊕	10	147		4	63
c	HSD 2458	1		50		
	PI 164323	65	2			
	F_1 HSD 2458 × PI 164323	4	51			
	F_2 (HSD 2458 × PI 164323) ⊕	106	287	28	44	62
	BC_s (HSD 2458 × PI 164323) × HSD 2458	1	44	37	11	2
	BC_r (HSD 2458 × PI 164323) × PI 164323	29	70		1	
d	HSD 2458			20		
	Védrantais	1			19	
	Ouzbèque 2					25
	F_1 Védrantais × HSD 2458			13	7	
	F_1 Ouzbèque 2 × HSD 2458			1	34	
	F_2 (Védrantais × HSD 2458) ⊕	2		57	180	
	F_2 (Ouzbèque 2 × HSD 2458) ⊕	3		101	175	149
e	HSD 2458			10		
	HSD 93-20-A		20			
	F_1 HSD 2458 × HSD 93-20-A		5			
	F_2 (HSD 2458 × HSD 93-20-A) ⊕		110	11	22	34