Sources of Resistance to Cucurbit Aphid-borne Yellows Luteovirus in a Melon Germ Plasm Collection

Catherine Dogimont, Sofiane Slama, Jérôme Martin, Station d'Amélioration des Plantes Maraîchères, **Hervé Lecoq,** Station de Pathologie Végétale, and **Michel Pitrat,** Station d'Amélioration des Plantes Maraîchères, Institut National de la Recherche Agronomique (INRA), BP 94, 84143 Montfavet cedex, France

ABSTRACT

Dogimont, C., Slama, S., Martin, J., Lecoq, H., and Pitrat, M. 1996. Sources of resistance to cucurbit aphid-borne yellows luteovirus in a melon germ plasm collection. Plant Dis. 80:1379-1382.

Cucurbit aphid-borne yellows virus (CABYV) is a tentative member of the luteovirus group transmitted by *Myzus persicae* and *Aphis gossypii*. Five hundred twenty-three *Cucumis melo* L. accessions were screened in field conditions in the south of France under severe natural inoculum pressure. Double antibody sandwich–enzyme-linked immunosorbent assays were conducted to confirm the presence or absence of virus in the plants. Several potential sources of CABYV resistance were found, most originating from India, but some from Korea and Africa. The resistance of the most promising genotypes was confirmed in a field trial in five locations and under controlled conditions, with viruliferous *Myzus persicae* used to inoculate plants. Genotypes found to be resistant were Faizabadi Phoont, 90625, PI 124112, PI 124440, PI 255478, PI 282448, and PI 414723.

Cucurbit crops are commonly affected by many viruses that are nonpersistently transmitted by aphids, among which cucumber mosaic cucumovirus (CMV), watermelon mosaic potyvirus 2, (WMV-2), and zucchini yellow mosaic potyvirus (ZYMV) are the most prevalent (10). Recent outbreaks of a yellowing disease of cultivated cucurbits have been observed in France and the causal agent designated cucurbit aphid-borne yellows virus (CABYV) (11), a new member of the luteovirus group (20). In contrast with previously known cucurbit viruses, CABYV is a phloem-limited virus; it is not transmissible by mechanical inoculation, but is transmitted by *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) in a persistent manner.

CABYV is widespread throughout the Mediterranean basin and infects most cucurbit crops, including melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), squash (*Cucurbita* sp.), and watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) (11). It has also been detected in Asia and in Africa (12,13; H. Lecoq, *unpublished data*) and in California (15). CABYV has probably been present in cucurbit crops for a long time (11), but symptoms may have been incorrectly attributed to nutrient deficiencies, senescence, or other viruses, such as lettuce infectious yellows virus (LIYV) or cucumber yellows virus (CuYV), that induce yellowing symptoms and are transmitted by whiteflies (4,16, 19).

Symptoms include initial chlorotic patches followed by leaf thickening and overall bright yellowing of leaves. Yellowing symptoms are induced mainly on the older leaves of Charentais type melons, commonly grown in France, which usually develop less severe symptoms than American cantaloupe or Spanish tendral, amarillo, and rochet types. CABYV severely reduces yields of melon and cucumber by reducing the number of fruit per plant as a result of a high percentage of flower abortions (40 and 51%, respectively) but does not alter the fruit shape or quality. The virus does not affect fruit production of a zucchini squash cultivar (11). Little is known at present about the biological variability of CABYV.

Development of resistant cultivars is a promising approach to control the virus. An accession from India (PI 124112) has already been described as resistant (14). The objective of this research was to identify other sources of resistance to CABYV in a melon germ plasm collection. Numerous attempts to cross *C. melo* with other *Cucumis* species have failed (1); therefore, the genetic resources useful for melon breeding are restricted to the natural variability of *C. melo*.

MATERIALS AND METHODS

Plant material. Five hundred twenty-three melon accessions, including improved cultivars, breeding lines, primitive cultivars, land races, and wild melons, were evaluated for resistance to CABYV under field conditions in southern France in 1993 and 1994. The geographical origins of these accessions are given in Table 1. The complete list of the tested accessions can be obtained from the authors. All these accessions were increased by selfing.

Field trials. Field experiments were conducted by the experimental unit of the Vegetable Breeding Station in Montfavet, France. In 1993, 430 melon accessions (3 plants per accession) were tested. Sowings of about 20% of this collection were made every week for 5 weeks from 4 May to 9 June. Seedlings were transplanted 3 weeks after sowing from 26 May to 24 June. Plants were spaced 0.30 m in the row, with 2.0 m between rows. No plastic mulch was used and no aphicide treatments were applied.

In 1994, 93 accessions (3 plants per accession) were evaluated. Sowing of about 50% of the collection was made on 2 June and the remaining accessions were sown

Table 1. Geographical origin of *Cucumis melo* accessions tested for cucurbit aphid-borne yellows virus resistance

Region/country	Accessions (no.)
Europe	227
France	64
Spain	97
Other southern Europe	13
Central Europe	40
Others or undetermined	13
Far East and India	103
Japan	29
Korea	4
China	9
Other Far East	10
India	51
Middle East	56
Turkey	11
Iran	28
Central Asia	9
Israel	6
Saudi Arabia	2
Africa	30
North Africa	14
Sudan	11
Others	5
America	84
U.S.	73
South America	3
West Indies	8
Unknown origin	23

Corresponding author: C. Dogimont
E-mail: dogimont@avignon.inra.fr

Accepted for publication 30 August 1996.

Publication no. D-1996-0923-04R

on 14 June. Seedlings were transplanted respectively on 30 June and 15 July. Plants were spaced 0.50 m in the row, with 2.0 m between rows. A plastic mulch was used for weed control and no aphicide treatments were applied.

In both trials, fungicide treatments were applied to control powdery mildew. Plants of the CABYV-susceptible cultivars Védrantais (a Charentais type, Vilmorin, France) and Ouzbèque-2, an accession from Uzbekistan, were regularly planted in each row as indicators of CABYV infection.

The 11 most promising accessions in the 1993 trial were re-evaluated in a trial conducted in five additional locations in 1994. About 10 plants of each accession were planted in five different sites among nine experimental fields located in southeastern France within 30 km of Montfavet, except for the accession PI 124112, which was planted in all nine fields. All the accessions were planted in Montfavet. In 1995, four other accessions were re-evaluated in four sites including Montfavet. Ten plants of both susceptible cultivars, Ouzbèque-2 and Védrantais, were planted as indicators of CABYV infection in each experimental site. The similarity of the sites for genotype behavior toward CABYV infection was tested by monofactorial analysis of variance without interactions, with SYSTAT Windows 5 (SYSTAT, Inc., Evanston, IL).

Controlled inoculation. The 11 accessions selected after the 1993 field observation and the susceptible checks, Védrantais and Ouzbèque-2, were inoculated by viruliferous aphids in 1994. The CABYV isolate used (CABYV-N) was originally obtained from a melon plant collected at Nérac, southwest France, in September 1989 (11), and was maintained by serial transfer in *Capsella bursa-pastoris* (L.) Medik. with *M. persicae* as vector, in a growth room (24°C day/18°C night, 14-h day length). Melon seeds were sown on 11 May. All plants were inoculated twice: a first inoculation was made on 31 May at the two-fully-expanded-leaf stage and another one was made on 9 June. Plants were inoculated by placing a small piece of infected *C. bursa-pastoris* leaf carrying viruliferous aphids (10 to 30 larvae and adults) on the youngest-expanded-leaf. Viruliferous aphids were left to feed for 48 h on each plant and then were killed by an aphicide spray (0.75 g l^{-1} of Pirimor G [pirimicarb] Sopra, SOPRA, Vélizy-Villacoublay, France). Inoculated plants were subsequently grown in an insect-free greenhouse. On 17 June, plants were transplanted in a plastic tunnel and trained to a single vertical branch. Two blocks containing randomized plots of five plants of each accession were planted (except in some cases of poor seed germination in which fewer plants were available). Aphicide treatments were regularly applied to prevent infection of plants with other viruses or with another strain of CABYV naturally occurring in the area. Serological assays were conducted on the ninth or tenth leaf from the apex of each plant, 7 weeks and 9 weeks after the first inoculation.

Serological assays. Screening for CABYV resistance with foliar symptoms used as the sole selection criteria is not possible because susceptible plants are often asymptomatic and CABYV-induced yellowing symptoms are difficult to distinguish from other yellowing due to senescence, environmental factors, or other viruses. The absence of virus in the plants was revealed by double antibody sandwich–enzyme-linked immunosorbent assay (DAS-ELISA) (3,11) and used as a basis for assessing resistance to CABYV. Sap was extracted from 1-g samples of leaves of individual plants with a roller press and was diluted with 4 ml of extraction buffer (sodium phosphate 0.1 M, pH 7, containing 0.2% DIECA [sodium diethyldithiocarbamate] and 2% skimmed milk). Two hundred microliters of each sample was loaded into duplicate wells of a microtiter plate (Nunc-Immuno Plate F96 MaxiSorp, Poly Labo, Strasbourg, France) previously coated with immunoglobulin and was incubated overnight at 4°C. Coating globulin was applied at 0.5 μg ml^{-1} and alkaline phophatase-conjugate at a 1/4,000 dilution, and incubated 3 h at 37°C. Absorbance values (A_{405}) were measured with a Titertek Multiskan spectrophotometer (EFLAB, Helsinki, Finland). Test samples were considered positive if their A_{405} values exceeded twice that of healthy control samples.

For the field-grown plants, preliminary tests were conducted on the susceptible cultivars 3 weeks after transplanting to assess natural CABYV infection in the field. About 6 weeks after transplanting, ELISAs were carried out on all the plants. For the screening of the collection, each sample for ELISA consisted of equal parts of leaf from each plant of each accession. When the A_{405} value was low ($A_{405} < 0.3$), a second test was conducted 15 days later and leaves of each plant were tested individually. For the trial conducted at multiple locations, each plant was tested individually.

RESULTS

Screening of a *C. melo* collection for CABYV resistance under field conditions. In both trials, 100% of plants of the two susceptible cultivars, Ouzbèque-2 and Védrantais, were infected 3 weeks after transplanting. Most accessions tested were susceptible to CABYV but a total of 16 accessions were observed with some level of resistance (Table 2). In 1993, three accessions were found resistant to CABYV (all three plants tested): Faizabadi Phoont, PI 124112, and PI 282448. Four accessions segregated for CABYV resistance (one or two virus-free plants out of three tested plants): PI 164487, PI 164797, PI 255478, and 90625. Four other accessions showed a very low A_{405} value 7 weeks after transplanting but could not be tested again because of the death of the plants due to attack by other pathogens: PI 123501, PI 124440, PI 164723, and PI 183307.

In 1994, two new accessions were found to be uniformly resistant (PI 313970 and PI 414723) and three others showed segregation (Inde-5, PI 140471, and T-EK 92-2).

Assessment of the resistance of selected accessions in a multilocal field trial. In all the experimental sites, the susceptible checks, Védrantais and Ouzbèque-

Table 2. Cucurbit aphid-borne yellows virus detection by enzyme-linked immunosorbent assay (ELISA) in some *Cucumis melo* accessions in 1993 and 1994 field trials

		Positive plants (no.)/tested plants (no.)[a]	
Accession	Geographical origin	First evaluation in 1993[b]	Trial conducted in 10 locations in 1994[c]
Védrantais	France	44/44	76/78
Ouzbèque-2	Uzbekistan	3/3	60/61
Faizabadi Phoont	India	0/3	1/39
90625	India	1/3	1/42
PI 123501	India	0/3[d]	8/21
PI 124112	India	0/3	2/77
PI 124440	India	0/3[d]	0/40
PI 164487	India	2/3	18/33
PI 164723	India	0/3[d]	2/33
PI 164797	India	1/3	4/37
PI 183307	India	0/3[d]	3/17
PI 255478	Korea	1/2[e]	1/39
PI 282448	South Africa	0/3	0/40

[a] A sample was considered positive if its A_{405} value exceeded two times that of the healthy control.
[b] Each sample for ELISA consisted of equal parts of leaf from each of three plants of each accession. When the A_{405} value was low ($A_{405} < 0.3$), a second test was conducted 15 days later and leaves of each plant were tested individually.
[c] Each plant was tested individually.
[d] Plants considered to be resistant 6 weeks after transplanting but were not tested later because of the death of plants.
[e] Only two plants were tested because the third one died.

2, were, respectively, infected at 97 and 98% in 1994 and at 88 and 97% in 1995 (Table 3). During both years, the incidence of CABYV was satisfactory to evaluate the resistance of the other accessions. Seven accessions showed a high level of resistance and consistent reactions toward CABYV in the different sites, with less than 3% of plants positive in ELISA: Faizabadi Phoont, 90625, PI 124112, PI 124440, PI 255478, PI 282448, and PI 414723. Eight others were found to segregate for CABYV resistance. In 1994 and 1995, respectively, the 10 and four experimental sites were similar ($F = 0.73$, $P = 0.68$; $F = 0.39$, $P = 0.76$).

Behavior of some accessions toward CABYV in controlled inoculation. The CABYV aphid transmission procedure resulted in 100% infection of the inoculated susceptible check plants of Védrantais and Ouzbèque-2. Three weeks after inoculation, the plants of Ouzbèque-2 showed intense yellowing symptoms on all leaves; Védrantais presented only mild symptoms of yellow spots on the oldest leaves. Nevertheless, the A_{405} values of Védrantais were not lower than those of Ouzbèque-2 (respectively, $A_{405} = 1.979$ and $A_{405} = 1.962$; A_{405} (healthy control) = 0.098). Eight accessions were found uniformly resistant: Faizabadi Phoont, 90625, PI 124112, PI 124440, PI 164723, PI 164797, PI 255478, and PI 282448. One plant of PI 255478 was observed slightly positive 7 weeks after inoculation ($A_{405} = 0.258$) but negative 2 weeks later ($A_{405} = 0.066$). PI 123501 and PI 164487 were found to segregate for CABYV resistance (respectively, 2 and 3 positive plants out 10). The 9 plants of PI 183307 were found infected.

DISCUSSION

CABYV was characterized only recently (11) but its early and frequent outbreaks and extended distribution justified the search for genetic resistance. In this paper, we screened 523 accessions of *C. melo* for CABYV resistance. The tested collection was fairly representative of the natural biodiversity in *C. melo* even though some geographic origins such as South America are underrepresented. Different susceptible behaviors were observed; some accessions, such as those originating from the Middle East or of Canaria type, became bright yellow after CABYV infection. Some other accessions (such as Charentais type) expressed less severe symptoms even though CABYV multiplied actively in the infected plants. Among these 523 accessions, 16 accessions have been identified in which the virus could not be detected in at least some plants. Probably, some accessions found to be susceptible may also have genes for resistance that were not detected due to the small sample size tested (3 plants) and the high degree of heterozygosity that can be assumed for most of the accessions tested. Nevertheless, this screening revealed that sources of resistance to CABYV are relatively common in melon, compared with other virus resistances, e.g., ZYMV resistance, which has been found so far in a single Indian accession, PI 414723 (18). The 11 accessions first observed as resistant in 1993 were most intensively studied in 1994. The five accessions observed as resistant in 1994 are under study. Resistant accessions originate mostly from India. However, most of the Indian accessions tested (39 out of 51) were susceptible to CABYV. India is known to be an important center of diversification of *C. melo,* and sources of resistance to several pathogens have been identified in accessions coming from this area. Particularly, some genitors cumulate several resistances. For instance, PI 124112 is also resistant to powdery mildew (*Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollaci and *Erysiphe cichoracearum* DC. (7) and to downy mildew (*Pseudoperonospora cubensis* (Berk. & M. A. Curtis) Rostovzev) (5,6). PI 414723 is resistant to ZYMV and was reported to be partially resistant to some strains of WMV-2 (8); it is also resistant to *A. gossypii* (9) and powdery mildew (17), and partially resistant to downy mildew (5,6). Sources of CABYV resistance were also identified in accessions from other geographical origins. PI 255478 from Korea is also resistant to CMV and *A. gossypii*. Two resistant accessions from Africa were identified: PI 282448 from South Africa, and T-EK 92-2, a *C. melo agrestis* accession collected in Sudan.

The procedure of testing in natural conditions resulted in uniform infection, as verified on the susceptible checks, and was efficient for a first screening of resistance. Re-evaluation of most promising accessions in five locations did not reveal differences between the sites, so it seems that pathogenicity of CABYV strains on melon is uniform in southeast France. In artificial inoculation conditions, using viruliferous *M. persicae*, the 11 cultivars responded in the same way as observed in the field. The virus could not be detected in resistant plants. Absorbance values of susceptible artificially inoculated plants were generally lower than those in naturally infected plants, probably because of mixed infection with other viruses that may enhance CABYV multiplication (2); moreover, the virus was found irregularly distributed in all the infected plants.

Further research is underway to study the inheritance of CABYV resistances, to determine to what extent sources of resistance share the same genetic factors, and finally to evaluate the diversity of useful genes. Additional work is now needed to introduce resistance genes in adapted types and release varieties with good horticultural quality and resistance to CABYV.

Table 3. Cucurbit aphid-borne yellows virus detection by enzyme-linked immunosorbent assay (ELISA) in some *Cucumis melo* accessions in 1994 and 1995 field trials

		Positive plants (no.)/tested plants (no.)[a]	
Accession	Geographical origin	First evaluation in 1994[b]	Trial conducted in four locations in 1995[c]
Védrantais	France	17/18	43/49
Ouzbèque-2	Uzbekistan	NA[d]	33/34
Inde-5	India	1/2[e]	20/39
PI 140471	Unknown	2/3	33/40
PI 313970	India	0/3	11/30
PI 414723	India	0/3	0/30
T-EK 92-2	Sudan	1/3	NA

[a] A sample was considered positive if its A_{405} value exceeded two times that of the healthy control.
[b] Each sample for ELISA consisted of equal parts of leaf from each of three plants of each accession. When the A_{405} value was low ($A_{405} < 0.3$), a second test was conducted 15 days later and leaves of each plant were tested individually.
[c] Each plant was tested individually.
[d] Not available
[e] Only two plants were tested because the third one died.

ACKNOWLEDGMENTS

We thank the melon breeders and pathologists of the Agro 5 Recherche, Asgrow, ASL, Clause, Gautier, PetoSluis, Rijk Zwaan, Sluis & Groot, and Tézier seed companies for their participation in the multilocal trial of this research and for partially funding it. We also wish to thank Chantal Olivier, Monique Ricard, and Catherine Wipf-Scheibel for their technical assistance, and Patrick Rousselle for his critical review of the manuscript.

LITERATURE CITED

1. Beharav, A., and Cohen, Y. 1995. Attempts to overcome the barrier of interspecific hybridization between *Cucumis melo* and *C. metuliferus*. Isr. J. Plant Sci. 43:113-123.
2. Bourdin, D., and Lecoq, H. 1994. Increase in cucurbit aphid-borne yellows virus concentration by co-infection with sap-transmissible viruses does not increase its aphid transmissibility. J. Phytopathol. 141:143-152.
3. Clark, M. F., and Adams, A. M. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.
4. Duffus, J. E., Larsen, R. C., and Liu, H. Y. 1986. Lettuce infectious yellows virus - a new type of whitefly-transmitted virus. Phytopathology 76:97-100.
5. Epinat, C., and Pitrat, M. 1994. Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis*) in muskmelon (*Cucumis melo*). I. Analysis of a 8 × 8 diallel table. Agronomie 14:239-248.
6. Epinat, C., and Pitrat, M. 1994. Inheritance of

resistance to downy mildew (*Pseudoperonospora cubensis*) in muskmelon (*Cucumis melo*). II. Generation means analysis of 5 genitors. Agronomie 14:249-257.
7. Epinat, C., Pitrat, M., and Bertrand, F. 1993. Genetic analysis of resistance of five melon lines to powdery mildews. Euphytica 65:135-144.
8. Gilbert, R. Z., Kyle, M. M., Munger, H. M., and Gray, S. M. 1994. Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* L. HortScience 29:107-110.
9. Kishaba, A. N., Bohn, G. W., and Toba, H. H. 1971. Resistance to *Aphis gossypii* in muskmelon. J. Econ. Entom. 64:935-937.
10. Lecoq, H. 1990. Aphid borne viruses infecting cucurbits in southern France: Ecology, epidemiology and approaches for control. Pages 83-84 in: Congr. Mediterr. Phytopathol. Union, 8th.
11. Lecoq, H., Bourdin, D., Wipf-Scheibel, C., Bon, M., Lot, H., Lemaire, O., and Herrbach, E. 1992. A new yellowing disease of cucurbits caused by a luteovirus, cucurbit aphid-borne yellows virus. Plant Pathol. 41:749-761.
12. Lecoq, H., Dafalla, G. A., Mohamed, Y. F., Ali, H. M., Wipf-Scheibel, C., Desbiez, C., Eljack, A. E., Omara, S. K., and Pitrat, M. 1994. Survey of virus diseases infecting cucurbit crops in eastern, central and western Sudan. Univ. Khartum J. Agric. Sci. 2:67-82.
13. Lecoq, H., Gilbert-Albertini, F., Wipf-Scheibel, C., Pitrat, M., Bourdin, D., Belkhala, H., Katis, N., and Yilmaz, M. 1994. Occurrence of a new yellowing disease of cucurbits in the Mediterranean basin caused by a luteovirus, cucurbit aphid-borne yellows virus and prospects for control. Pages 461-463 in: Congr. Mediterr. Phytopathol. Union, 9th.
14. Lecoq, H., Pitrat, M., Bon, M., Wipf-Scheibel, C., and Bourdin, D. 1992. Resistance in melon to cucurbit aphid-borne yellows virus, a luteovirus infecting cucurbits. Pages 191-196 in: EUCARPIA Cucurbitaceae Symp., 5th.
15. Lemaire, O. J., Gubler, W. D., Valencia, J., Lecoq, H., and Falk, B. W. 1993. First report of cucurbit aphid-borne yellows luteovirus in the United States. Plant Dis. 77:1169.
16. Lot, H., Delecolle, B., and Lecoq, H. 1982. A whitefly transmitted virus causing muskmelon yellows in France. Acta Hortic. 127:175-182.
17. McCreight, J. D., Pitrat, M., Thomas, C. E., Kishaba, A. N., and Bohn, G. W. 1987. Powdery mildew resistance genes in muskmelon. J. Am. Soc. Hortic. Sci. 112:156-160.
18. Pitrat, M., and Lecoq, H. 1984. Inheritance of zucchini yellow mosaic virus resistance in *Cucumis melo* L. Euphytica 33:57-61.
19. Soria, C., Gomez-Guillamon, M. L., and Duffus, J.E. 1991. Transmission of the agent causing a melon yellowing disease by the greenhouse whitefly *Trialeurodes vaporariorum* in southern Spain. Neth. J. Plant Pathol. 97:289-296.
20. Waterhouse, P. M., Gildow, F. E., and Johnstone, G. R. 1988. Luteovirus group. No. 339 in: AAB Descrip. Plant Viruses.