

SHORT COMMUNICATION
INHERITANCE OF RESISTANCE TO ALFALFA MOSAIC VIRUS
IN *LYCOPERSICON HIRSUTUM* f. *GLABRATUM* PI 134417

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SUMMARY

Alfalfa mosaic virus (AMV) is of sporadic occurrence in tomato crops. More recently, important epidemics caused by AMV have been reported in field-grown canning tomatoes in southern Italy. Since no lines or commercial varieties resistant to AMV are available, we have studied the inheritance of resistance to this virus, derived from the accession of *Lycopersicon hirsutum* f. *glabratum* PI 134417. A necrogenic tomato isolate of AMV was used as source of inoculum. Visual observations of symptoms and enzyme-linked immunosorbent assay (ELISA) were used to screen plants after mechanical inoculation. All the F₁ tested were resistant while the segregation ratios of F₂ and BC₁ generations were consistent with a simple dominant inheritance. We propose tentatively to name this gene *Am*.

RIASSUNTO

EREDITÀ DELLA RESISTENZA AL VIRUS DEL MOSAICO DELL'ERBA MEDICA NELL'ACCESSIONE PI 134417 DI *LYCOPERSICON HIRSUTUM* f. *GLABRATUM*. Negli ultimi anni sono state segnalate numerose epidemie causate dal virus del mosaico dell'erba medica (AMV) in colture di pomodoro da industria nel sud dell'Italia. Attualmente non è disponibile nessuna linea o varietà commerciale di pomodoro resistente a tale virus. Il presente lavoro riporta i risultati relativi allo studio dell'eredità della resistenza a tale virus derivata dall'accessione di *Lycopersicon hirsutum* f. *glabratum* PI 134417. A tale scopo è stato utilizzato come fonte di inoculo un isolato di AMV necrogenico su pomodoro. Le piante inoculate meccanicamente sono state valutate per la resistenza mediante saggi ELISA ed osservazioni dei sintomi. Tutte le piante F₁ sono risultate resistenti mentre i rapporti di segregazione ottenuti nelle generazioni F₂ e BC₁ erano in accordo con un'eredità monogenica dominante. Per tale gene è stato proposto il simbolo *Am*.

Key words: *Lycopersicon hirsutum*, inheritance, alfalfa mosaic virus.

Alfalfa mosaic virus (AMV; *Bromoviridae*) is a plant pathogen with worldwide distribution, transmitted non-persistently by at least 22 aphid species and by seed in some alfalfa varieties and solanaceous species (Edwardson and Christie, 1986). It can infect most solanaceous crops in the field, causing severe losses to pepper (Berkeley, 1947; Kovacevski, 1965; Hamm *et al.*, 1995), tobacco (Conti and Vegetti, 1970; Vuitteñez *et al.*, 1974; Tedford and Nielsen, 1990), potato (Black and Prince, 1940) and is of lesser economic importance to eggplant (Crill *et al.*, 1970; Marchoux and Rougier, 1974; Kemp and Troup, 1977).

AMV infections in tomato are generally sporadic, but in the last few years epiphytotic of a necrotic phenotype have occurred in southern Italy and southern France (Ragozzino, 1995; Finetti Sialer *et al.*, 1997; Parrella *et al.*, 1997). Historically, most AMV infections in tomato were associated with the presence of alfalfa crops nearby (Zitter, 1993). However, this was not the case with the latest reports from Italy and France, where natural reservoirs of the virus were not identified.

As with other non-persistently transmitted viruses with a wide host range, control of AMV is difficult. Spraying to reduce aphid populations does not prevent primary infection and usually requires heavy pesticide use. Physical barriers, such as nets, are useful in nurseries or in greenhouse crops, but are unmanageable in the open field. The best way to reduce losses would be to use resistant or tolerant tomato varieties, but to our knowledge, no such varieties are commercially available. However, we have recently reported three potential sources of AMV resistance in *Lycopersicon hirsutum* accessions (Parrella *et al.*, 1997). In the present study, we have determined the inheritance of resistance in one of these resistant *L. hirsutum* accessions to be used in tomato breeding programmes.

L. hirsutum f. *glabratum* PI 134417 was used as donor of resistance to AMV, while the genotypes Mo-

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mor of *L. esculentum* and PI 247087 Australia of *L. hirsutum* f. *glabratum* were used as susceptible controls (Parrella *et al.*, 1997) and were crossed with PI 134417.

For the inheritance study, the following generations were used: F₁ (Momor x PI 134417), F₁ (PI 134417 x PI 247087), F₁ (PI 247087 x PI 134417), F₂ progenies (Momor x PI 134417) and (PI 134417 x PI 247087) obtained by F₁ intercrossing, and the first generation of backcross BC₁ PI 247087 x (PI 134417 x PI 247087).

Seedlings were transplanted at the cotyledon stage to 10 cm diameter pots with peat bog-moss as substrate and watered weekly with a nutrient solution. All plants were grown in an insect-proof greenhouse (22-26°C and 70-80% humidity) under natural lighting.

The AMV isolate LYH-1 (Parrella *et al.*, 1997) was used as source of inoculum, and was maintained and multiplied in *Nicotiana tabacum* cv. 'Xanthi' n.c. Inoculum was prepared by grinding 1 g of young tobacco leaves in 4 ml of 0.03 M Na₂HPO₄ buffer containing 0.2% sodium diethyldithiocarbamate. Before inoculation, 75 mg ml⁻¹ of carborundum and activated charcoal were added to the sap extract (Marrou, 1967). The slurry was used to inoculate cotyledons and the first two true leaves of 16-day-old seedlings.

Symptoms were observed from one day up to one month after inoculation. Samples from uninoculated leaves were tested 15 and 30 days after inoculation by using DAS-ELISA as described by Clark and Adams

(1977). Plant extracts were prepared by grinding 1 g of leaves in 4 ml of inoculation buffer with a leaf press.

An antiserum (kindly supplied by H. Lot, INRA, Avignon) to an isolate of AMV from lettuce was used. Plates were read with a Tirtetek Multiskan Plus Photometer at 405 nm 1 h after addition of the substrate. Samples were considered positive when the absorbance value was more than twice the mean of the healthy controls.

L. hirsutum f. *glabratum* PI 134417 showed no reaction to infection and no symptoms for at least 1 month after inoculation both in inoculated and uninoculated leaves, and no virus was detected by ELISA 15 and 30 days after inoculation in the same tissues (Table 1). The susceptible controls, Momor and the *hirsutum* accession PI 247087 Australia, reacted with prominent local and systemic necrosis few days after inoculation, followed by death of the plant.

Plants of the three F₁ Momor x PI 134417, PI 134417 x PI 247087 and PI 247087 x PI 134417, behaved like the resistant parent and showed no symptoms in inoculated and uninoculated leaves, which were also negative when checked by ELISA 15 and 30 days after inoculation. The resistance was therefore dominant regardless of the resistant parent.

F₂ plants deriving from the crosses Momor x PI 134417 and PI 134417 x PI 247087 segregated in the ratio 3 resistant to 1 susceptible. Further confirmation of the presence of a single dominant gene was obtained

Table 1. Reaction of parents Momor (susceptible), PI 134417 (resistant), PI 247087 (susceptible) and segregating generations to inoculation with AMV isolate LYH-1, based on symptoms development and ELISA results.

Genotypes	no. plants	Symptoms				ELISA			
		S/R	espected ratio	χ^2	P	+/-	espected ratio	χ^2	P
Momor (P1)	30	30 ^a /0				-			
PI 134417 (P2)	30	0/30				0/30			
PI 247087 (P3)	30	30 ^a /0				-			
F ₁ (P1 x P2)	10	0/30				0/30			
F ₁ (P2 x P3)	30	0/30				0/30			
F ₁ (P3 x P2)	30	0/30				0/30			
BC ₁ (P2 x P3) x P3	100	51/49	1:1	0.04	0.90-0.75	51/49	1:1	0.04	0.90-0.75
F ₂ (P1 x P2)	159	32 ^a /127	1:3	2.56	0.25-0.10	-/127	1:3	2.56	0.25-0.10
F ₂ (P2 x P3)	149	33/116	1:3	0.65	0.50-0.25	33/116	1:3	0.65	0.50-0.25

S/R: no. susceptible plants/no. resistant plants

+/-: no. of plants positive/no. of plants negative

^a: dead plants

from the backcross population PI 247087 x (PI 1344117 x PI 247087) which segregated in the ratio 1 resistant to 1 susceptible. This was considered as evidence that the genetic factor in *L. hirsutum* f. *glabratum* PI 134417 is a single dominant gene, to which the symbol *Am* has been tentatively assigned.

Am is the first gene identified conferring resistance to AMV in tomato. As the gene is simply inherited, it can be easily introgressed using backcrossing or pedigree breeding. No differences were found in segregating ratios in intra- and interspecific crosses. The resistance of *L. hirsutum* PI 134417 was efficient against several virus isolates from different geographic areas in mechanical inoculation tests (data not shown). Further analysis could give more information about the possibility of using this resistance for breeding purposes by testing advance breeding lines in the field.

Recently AMV has been reported as potentially very dangerous in tomato crops in the south of Italy, mainly due to the appearance of a strain inducing necrosis on leaves and fruits (Ragozzino, 1995; Finetti Sialer *et al.*, 1997). The resistance to AMV reported in this study could provide a useful means of reducing such losses.

ACKNOWLEDGEMENTS

The authors thank Prof. G.P. Martelli and Prof. D. Gallitelli for critical reading of the manuscript.

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Received 26 June 1998

Accepted 17 September 1998