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Tolerance to a whitefly-transmitted virus causing muskmelon yellows disease in Spain

J. Esteva and F. Nuez

Department of Biotechnology, Polytechnical University, E-46020, Valencia, Spain

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Summary. Muskmelon yellowing disease was one of the most serious problems affecting muskmelon crops along the south-east coast of Spain throughout the 1980s. The causal agent of this disease is a virus that we call muskmelon yellows virus (MYV); MYV is transmitted by the greenhouse whitefly *Trialeurodes vaporariorum* Westwood. It has proven impossible to find sources of resistance to MYV within a wide collection of Spanish muskmelon landraces and exotic varieties. However, 'Nagata Kin Makuwa' and PI 161375, lines of Asiatic origin, show tolerance to this disease. These two lines were studied together with two others ('Galia' and 'Piel de Sapo' type) that are very susceptible to MYV. The crosses between them (susceptible × tolerant) and the segregant generations derived from these crosses were also investigated. The studies were carried out in two different places and years. The expression of tolerance is influenced by the environment. A parabolic type relationship exists between the average value of percentage of tolerant plants and their variation. This allowed us to quantify the expected response in the segregant generations. The results observed in these generations agreed with a simple genetic control of tolerance. This tolerance, combined with protective measures which delay the infection, can contribute notably to mitigating the effects of MYV.

Key words: Muskmelon – Yellowing disease – Virus – Inheritance of tolerance

Introduction

The south-east coast of Spain is one of the principal areas for greenhouse vegetable cultivation in Europe. The production of muskmelon is high in this area, and during the 1980s the most serious problem affecting this crop was a yellowing disease, which had not previously been observed in Spain. The first symptoms of the disease on muskmelon appear either as interveinal chlorotic spotting or as a golden-yellow basal stain at the union between the leaf surface and the petiole. Ultimately, the entire leaf, except for the veins, shows a bright-golden yellowing. The earlier the infection, the more fruit set and development are disturbed.

The causal agent of this muskmelon yellows disease is transmitted by the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Esteva et al. 1987; Soria and Gómez-Guillamón 1989, 1990), but not by sap inoculation (Soria and Gómez-Guillamón 1989). In diseased leaves of muskmelon artificially inoculated by *T. vaporariorum*, long flexuous viral particles have been detected, that have a length of 900 nm (Jordá and Alfaro 1989). Therefore, it seems evident that the causal agent of muskmelon yellowing disease is a virus that has not yet been completely characterized. We call it muskmelon yellows virus (MYV) in the present work.

Several yellowing diseases of cucumber and muskmelon transmitted by the greenhouse whitefly have been described in the literature. Some of them (Van Dorst et al. 1983; Hristova and Natskova 1986) have been identified as being caused be beet pseudo-yellows virus (BPYV), whose biological properties have been described by Duffus (1965) in California. Others have been reported from Japan and France (Yamashita et al. 1979; Lot et al. 1982). These have been referred to as cucumber yellows (CuYV) and muskmelon yellows viruses, respectively, and both have been associated with the presence of long flexuous virus particles in diseased plants. Based upon studies on transmission, host range and symptoms of infected plants, Zenbayashi et al. (1984) considered

Correspondence to: J. Esteva

Table 1. Number of tolerant (PT) and non-tolerant (PNT) plants in the lines studied and in the generations derived from the crosses between them

Generation	Algarrobo						Ejido	
	First year				Second year		First year	
	PNT[a]	PT[a]	PNT[b]	PT[b]	PNT	PT	PNT	PT
Nagata K. M. (N)	10	16	7	6	10	5	18	3
PI 161375 (PI)	6	9	4	21	9	4	16	8
Piel de Sapo (PS)	15	0	17	0	15	0	50	0
Galia (G)	20	0	13	0	15	0	50	0
Amarillo Canario	25	0	20	0	15	0	50	0
F_1 : (PS × N)10	10	0			11	4	7	2
F_2: (PS × N) × (PS × N)	81	28			81	56	98	23
BC: (PS × N) × PS					55	3		
BC′: (PS × N) × N					32	7		
F_1: (G × N)	7	3					7	1
F_2: (G × N) × (G × N)	141	11					77	32
BC: (G × N) × G	37	10						
F_1: (PS × PI)			10	1	15	0	9	2
F_2: (PS × PI) × (PS × PI)			68	39	85	22	81	18
BC: (PS × PI) × PS			45	5	40	6		
BC′: (PS × PI) × PI					49	8		
F_1: (G × PI)			11	0			5	2
F_2: (G × PI) × (G × PI)			123	15			87	22
BC: (G × PI) × G			52	0				

[a] and [b] denote distinct greenhouses

CuYV to be identical to BPYV. The MYV described in Spain could be close to CuYV or BPYV.

All muskmelon cultivars grown on the south-east coast of Spain are susceptible to MYV. In the investigation presented here we show the results found from our search for sources of resistance/tolerance to MYV, as well as the genetic control of the tolerance that was found.

Materials and methods

We tested 250 spanish muskmelon landraces and 10 muskmelon accessions of exotic origin under field conditions. Those that showed tolerance were the lines of Asiatic origin, 'Nagata Kin Makuwa' and PI 161375. These were crossed with two lines highly susceptible to MYV: the 'Piel de Sapo' type and the 'Galia' type. Respective crosses were made to obtain seed of the F_1, F_2, BC and BC′ generations (Table 1). The inheritance of tolerance was studied in these materials over three trials in the spring; two of them were in the same year but in different places (Algarrobo-Málaga and Ejido-Almería), and the third was the following year in Algarrobo-Málaga. Two greenhouses were used for the trial carried out in Algarrobo the first year (Table 1). The cv 'Amarillo Canario', which is very susceptible to MYV, acted as control. The trials were conducted under natural conditions of infection using high populations of whiteflies.

Each plant was rated as tolerant or non-tolerant by visual assessment of symptom severity after the first fruit harvest. The percentage of tolerant plants in each generation was used to measure the degree of tolerance.

Table 2. Biometrical model for inheritance of MYV tolerance

Generation[a]	Parameter[b]					
	m	(d)	(h)	(e)	(d × e)	(h × e)
+ Tolerant parent (P1)	1	1	0	1	1	0
+ Non-tolerant parent (P2)	1	−1	0	1	−1	0
+ F_1: (P2 × P1)	1	0	1	1	0	1
+ F_2: (P2 × P1) × (P2 × P1)	1	0	½	1	0	½
+ BC′: (P2 × P1) × P1	1	½	½	1	½	½
+ BC: (P2 × P1) × P2	1	−½	½	1	−½	½
+ + Tolerant parent (P1)	1	1	0	−1	−1	0
+ + Non-tolerant parent (P2)	1	−1	0	−1	1	0
+ + F_1: (P2 × P1)	1	0	1	−1	0	−1
+ + F_2: (P2 × P1) × (P2 × P1)	1	0	½	−1	0	−½
+ + BC′: (P2 × P1) × P1	1	½	½	−1	−½	−½
+ + BC: (P2 × P1) × P2	1	−½	½	−1	½	−½

[a] + and + + indicate different environments
[b] Terminology from Mather and Jinks (1982)

The data were analysed by fitting a biometrical model (Table 2) to the degree of tolerance for each generation using least square regression procedures. Two analysis are presented (Table 3). Dependent on the data being considered, the analysis corresponds to one place only (Algarrobo-Málaga) over 2 con-

Table 3. Estimate of statistical parameters of biometrical model

Cross[a]	m	(d)	(h)	(e)	(d × e)	(h × e)
PS × N[b]	22.9 ± 4.5	21.4 ± 4.7	−7.6 ± 0.1	6.3 ± 0.1	5.5 ± 0.8	−17.1 ± 3.7
PS × N[c]	18.5 ± 3.6	17.0 ± 3.9	−4.4 ± 2.7	10.6 ± 0.8	9.9 ± 0.1	−20.3 ± 6.3
G × N[c]	17.7 ± 3.0	15.9 ± 3.8	4.8 ± 8.3	7.0 ± 0.2	8.8 ± 0.0	−4.1 ± 1.0
PS × PI[b]	28.5 ± 5.1	24.1 ± 4.3	−18.3 ± 0.5	10.2 ± 1.8	11.6 ± 0.8	−5.8 ± 5.4
PS × PI[c]	27.8 ± 3.7	26.2 ± 4.1	−11.2 ± 5.5	11.0 ± 0.3	9.5 ± 0.7	−12.9 ± 0.6
G × PI[c]	25.6 ± 3.6	26.2 ± 4.2	−12.8 ± 3.8	9.4 ± 0.1	9.5 ± 0.6	−24.2 ± 7.2

[a] See nomenclature in Table 1
[b] Estimates obtained from Algarrobo-Málaga (first and second year) data
[c] Estimates obtained from the first year (Algarrobo-Málaga and Ejido-Almería) data

secutive years or 1 year only (the first) in both places (Algarrobo-Málaga and Ejido-Almería).

Results and discussion

We did not found sources of resistance in the screened collection; however among the lines tested of exotic origin, 'Nagata Kin Makuwa' and PI 161375 showed symptoms that were less severe than those found in the remainder of the collection.

The response of the 'Galia' and 'Piel de sapo' lines as well as that of the control was always uniform, confirming that all the plants were non-tolerant in the three trials carried out to study the inheritance of tolerance (Table 1). The response of the 'Nagata Kin Makuwa' and PI 161375 lines was not uniform, although tolerant plants were observed in both places and in both years. This also happened in the majority of the F_1, F_2, BC and BC′ generations (Table 1). The consistent presence of tolerant plants in these groups suggests that tolerance is genetically controlled.

Biometric analysis similarly explains the tolerance heredity of 'Nagata Kin Makuwa' in the three situations studied (Table 3). Dominance effects are of little importance compared to additive effects. The influence of environmental effects and interactions would explain the environmental differences that were observed for identical generations. The same could be considered for the prediction made by the model for the variation structure of PI 161375, although in this case the dominance (towards non-tolerance) is of greater importance than in 'Nagata Kin Makuwa'.

A model like this does not necessarily imply a polygenic control. In fact, it rather suggests a simple genetic basis would be better: given that tolerance is not dominant in either of the sources, the number of tolerant plants in some generations is excessive for a polygenic control.

As we have already shown, the proportion of tolerant plants even in non-segregant generations was variable when environments were compared, with the exception of non-tolerant parents and controls (Table 1). This variability varies according to genotype, and an increasing monotonous sequence can be observed between the average value of tolerance degree ($\bar{x}$) and the variance (s^2) (Table 4). The relationship between both statistics could be parabolic (Fig. 1). The parabola of fit obtained by means of least square regression is

$$s^2 = -0.038128\,\bar{x}^2 + 13.361\,\bar{x} \qquad [1]$$

and the coefficient of multiple determination is

$$R^2 = 0.867$$

Thus, tolerance degree distribution could be binomial. If, for convenience, we use a normal approximation, the response of a given genotype would be quantified by its average value and by its variance as indicated in the examples in Fig. 2. Segregant generation distribution would then be a consequence of the distributions of each one of the genotypes that form it and the proportion they represent in the said segregant generation. It is then possible to check whether a monogenic model with two alleles agrees with the variation observed in the segregant generations. We can assign the average tolerance degree of non-segregant generations (Table 4) to each of the three genotypes in the model, providing they belong to an equivalent genotype. As we know the different proportions of the different genotypes in a segregant generation, its average tolerance degree ($\bar{x}$) can be predicted as the weighted mean of the average tolerance degree corre-

Table 4. Average value and variance of tolerance degree in non-segregant generations

Generation	Average value ($\bar{x}$)	Variance (s^2)
Nagata Kin Makuwa	38.82	400.61
PI 161375	52.02	629.10
Piel de Sapo	0.00	0.00
Galia	0.00	0.00
F_1: Piel de Sapo × Nagata Kin Makuwa	16.29	204.03
F_1: Galia × Nagata Kin Makuwa	21.25	153.12
F_1: Piel de Sapo × PI 161375	9.09	82.62
F_1: Galia × PI 161375	14.28	408.12

Table 5. Expected tolerance degree according to a monogenic model, confidence intervals and observed tolerance degree in segregant generations

Generation[a]	Trial		Expected tolerance degree	Confidence interval[b]	Observed tolerance degree
F_2 : (PS × N) × (PS × N)	Algarrobo	1st year	17.85	0–47.34	25.69
F_2 : (PS × N) × (PS × N)	Ejido	1st year	17.85	0–47.34	19.00
F_2 : (PS × N) × (PS × N)	Algarrobo	2nd year	17.85	0–47.34	40.87
BC : (PS × N) × PS	Algarrobo	2nd year	8.12	0–28.3	5.47
BC': (PS × N) × N	Algarrobo	2nd year	27.53	0–63.61	17.94
F_2 : (G × N) × (G × N)	Algarrobo	1st year	20.33	0–51.68	7.23
BC : (G × N) × G	Algarrobo	1st year	10.62	0–33.61	21.27
F_2 : (G × N) × (G × N)	Ejido	1st year	20.33	0–51.68	29.36
F_2 : (PS × PI) × (PS × PI)	Algarrobo	1st year	17.55	0–46.80	36.45
BC : (PS × PI) × PS	Algarrobo	1st year	4.54	0–19.71	10.00
F_2 : (PS × PI) × (PS × PI)	Ejido	1st year	17.55	0–46.80	18.18
F_2 : (PS × PI) × (PS × PI)	Algarrobo	2nd year	17.55	0–46.80	20.56
BC : (PS × PI) × PS	Algarrobo	2nd year	4.04	0–19.71	13.04
BC': (PS × PI) × PI	Algarrobo	2nd year	30.50	0–68.38	14.03
F_2 : (G × PI) × (G × PI)	Algarrobo	1st year	20.14	0–51.35	10.86
BC : (G × PI) × G	Algarrobo	1st year	7.14	0–26.09	0.00
F_2 : (G × PI) × (G × PI)	Ejido	1st year	20.14	0–51.35	20.86

[a] See nomenclature in Table 1
[b] $P=0.05$

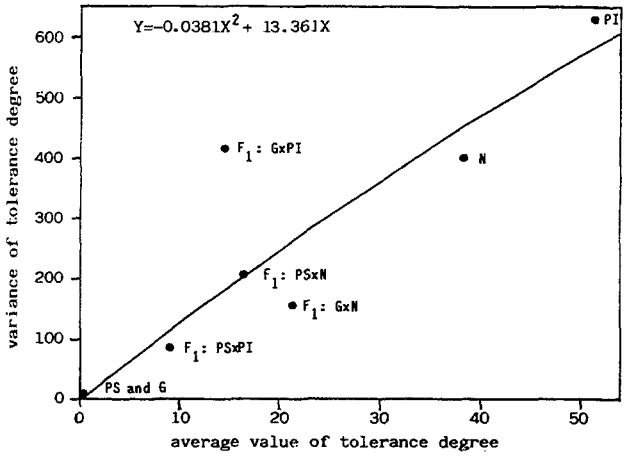

Fig. 1. Parabolic regression of variance of tolerance degree over average value of tolerance degree for non-segregant generations (see nomenclature in Table 1)

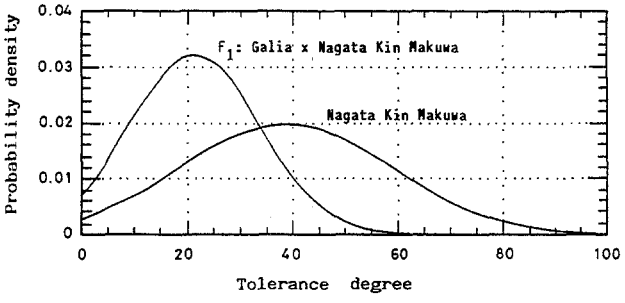

Fig. 2. Density functions of tolerance degree for 'Nagata Kin Makuwa' and for the hybrid F_1 'Galia' × 'Nagata Kin Makuwa', assuming a normal distribution

sponding to the different genotypes. Bearing in mind the normal approximation used, we can build a confidence interval for the average tolerance degree of each generation (average proportion of tolerant plants), such as

$$P(\bar{x}-Z_{\alpha/2}\, s<\mu<\bar{x}+Z_{1-\alpha/2}\, s)=1-\alpha$$

where s would be obtained from [1]. The model is theoretically correct if the tolerance degree observed in each segregant generation is included in its corresponding interval. This happened in all of the cases studied (Table 5). The amplitude of the constructed intervals indicates that there is a low power of test. However, in many cases the position of the observed value is reasonably close to the expected one, if you take the amplitude of the total interval into account.

These analysis could suggest that the tolerance of these materials has a genetic basis that is simpler than it originally appeared to be when observing the variability between environments. The genetic nature of these materials is partially recessive in PI 161375, and heredity is

more or less intermediate in 'Nagata Kin Makuwa' (Table 2). In both lines character penetration is incomplete (Table 1), and expression is notably influenced by the environmental factors.

The disease caused by MYV in Spain takes priority over other yellowing diseases of the cucurbits because of its persistence and the important economic losses it has produced. The damage caused by this disease can be reduced if the infection is delayed. This can be achieved by controlling the vector of the disease when the crop is in the nursery and in the phase immediately after transplanting. In these two early stages it is possible to protect the crop in the greenhouse from whitefly almost totally by using coverings (plastic or anti-insects mesh). It has been shown that these preventative measures have produced positive results. Although the studied tolerance has an incomplete penetration and a variable expressiveness, it is of agronomic interest, given that combined with culture techniques which delay infection the seriousness of the effects of MYV can be notably reduced.

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