STATISTIC ASSIGNMENT OF *FUSARIUM OXYSPORUM* F.SP. *MELONIS* STRAINS TO RACES 0, 1, 2 AND 1-2

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SUMMARY

Cultivars and differential genotypes of muskmelon were artificially inoculated with isolates belonging to the physiological races 0, 1, 2, 1-2y and 1-2w of Fusarium oxysporum f.sp. melonis and 31 isolates of the same forma specialis from northern Italy. The results showed that: cultivars 'Aril', 'Delada' and 'Supermarket' can be used in pathogenicity tests instead of the differential genotypes because they have the same reaction to infection; the best separation of fungal isolates into the five races was achieved thirty days after inoculation, when almost all the plants of the susceptible cultivar had been affected; multivariate statistical analyses (i.e. cluster and discriminant analyses) assign isolates more reliably to the right race than the empirical criteria commonly used.

RIASSUNTO

ATTRIBUZIONE DI CEPPI DI *FUSARIUM OXYSPORUM* F.SP. *MELONIS* ALLE RAZZE 0, 1, 2 E 1-2 MEDIANTE METODI STATISTICI. Cultivar e genotipi differenziali di melone sono stati inoculati artificialmente con isolati delle razze fisiologiche 0, 1, 2, 1-2y e 1-2w e con 31 isolati di Fusarium oxysporum f.sp. melonis provenienti dall'Italia settentrionale. I risultati ottenuti hanno mostrato che: le cultivar "Aril", "Delada" e "Supermarket" possono essere impiegate nelle prove di patogenicità in sostituzione dei genotipi differenziali, in quanto mostrano la stessa reazione alle infezioni; la migliore attribuzione degli isolati fungini alle cinque razze è stata ottenuta dopo trenta giorni dall'inoculazione, quando quasi tutte le piante della cultivar suscettibile sono risultate affette; analisi statistiche multivariate ("cluster analysis" e analisi discriminante) possono essere impiegate per attribuire un isolato fungino alla giusta razza fisiologica con una maggior attendibilità rispetto ai criteri empirici normalmente adottati.

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INTRODUCTION

Fusarium oxysporum f.sp. melonis (Leach and Currence) Snyder and Hansen (F.o.m.), the muskmelon-wilt fungus, includes several physiological races (0, 1, 2, 1-2y and 1-2w) (Risser et al., 1976; Armstrong and Armstrong, 1978). Race determination is based on pathogenicity tests on the differential genotypes of the host (Cucumis melo L.) (Risser and Mas, 1965; Risser et al., 1969; Leary and Wilbur, 1976). In these tests, muskmelon seedlings are inoculated by dipping the roots into a conidium suspension, and then transplanted in sterilized soil and observed till the appearance of disease symptoms (Zink et al., 1983; Latin and Snell, 1986; Katan et al., 1994). Every effort has to be made to achieve uniform conditions in performing such tests (Armstrong and Armstrong, 1975; Armstrong and Armstrong, 1978; Windels, 1991), with emphasis on inoculum concentration (Douglas, 1970; Banihashemi and De Zeeuw, 1975) and the age of the plants (Armstrong and Armstrong, 1975; Latin and Snell, 1986). On the other hand, little attention has been given to the criteria to use in distinguishing races (Armstrong and Armstrong, 1975): race identification is based on either a qualitative reaction of the differential muskmelon cultivars (Leary and Wilbur, 1976; Risser et al., 1976) or a subjective response evaluation (Armstrong and Armstrong, 1978). Our aim was, therefore, to find a more reliable procedure for assigning F.o.m. isolates to the right physiological race, looking especially for the set of differential genotypes to use, the time from inoculation when the incidence of affected plants may be recorded, and the use of multivariate statistical techniques in separating races.

MATERIALS AND METHODS

Muskmelon genotypes. In a preliminary test, five commercial muskmelon cultivars ('Aril', 'Cameo',

'Delada', 'Saby' and 'Supermarket'), selected on the basis of their resistance to the F.o.m. races, were compared to three frequently employed differential cultivars ('Charentais T', 'Charentais fom1' and 'Charentais fom2') (Tab. 1). In the tests that follow only three commercial cultivars were used: 'Aril', 'Delada' and 'Supermarket'.

Table 1. Muskmelon genotypes employed in pathogenicity tests, resistance genes to F.o.m. and supplying seed firms.

Genotype	Supplying firms	Gene for resistance	
		Fom 1	Fom 2
Aril	Nunhems S.r.l.	–	x
Cameo	Asgrow Italia	–	x
Delada	Nunhems S.r.l.	x	–
Saby	Pioneer Hi-Bred S.p.A.	x	x
Supermarket	Pioneer Hi-Bred S.p.A.	–	–
Charentais T	①	–	–
Charentais fom1	①	x	–
Charentais fom2	①	–	x

①: Supplied by Dr. G. Risser.
x: Present.
–: Absent.

***F.o.m.* isolates.** Isolates of races 0, 1, 1-2y, 1-2w (ATCC isolates 28856, 28857, 28859 and 28858 respectively) and 2 (supplied by Banihashemi), together with 31 F.o.m. isolates from soils previously planted with muskmelons in the Cremona and Mantua districts (northern Italy) (Languasco et al., in press), were used.

Pathogenicity tests. The inoculum for pathogenicity tests was obtained by washing the surface of 10-day-old fungal colonies grown on Potato Dextrose Agar 1.5% (PDA) at 25±1°C, with 10 ml of sterile water. The resultant suspension was filtered through a layer of cheesecloth and the conidial concentration of the filtrate determined by a haemocytometer. The conidial concentration was successively adjusted to 10^6 conidia ml^{-1} (Banihashemi and De Zeeuw, 1975). 700 seeds of each muskmelon genotype were disinfected with a 2% sodium hypochlorite solution for 5 min and put to germinate at 25±1°C in trays containing sterilized agriperlite. The seedlings were uprooted at the expansion of the first true leaf stage (Latin and Snell, 1986) and the roots cut with sterilized scissors. Inoculation was carried out by dipping the roots in the conidial suspension for 10 min (Zink et al., 1983) and, once the seedlings had been transplanted into multiple-cell plastic trays, discharging 1 ml of inoculum into each cell. 12 seedlings were inoculated for each fungal isolate and 3 treated with sterile water as a control. The inoculated seedlings were transplanted into oven-sterilized soil in a greenhouse at 20±2°C. Disease symptoms were recorded daily for a month: wilted, stunted, chlorotic and/or necrotic plants were considered affected. Disease incidence was expressed as a percentage of affected seedlings. Dead plants were removed from the greenhouse to prevent cross-contamination. The muskmelon genotypes were classified according to the percentage of affected seedlings, as suggested by Armstrong and Armstrong (1978): resistant 0-46%, intermediate 47-56% and susceptible 57-100%.

Statistical analysis. Cluster analysis was performed as a set of statistical methods useful in singling out clusters of similar units regarding a set of considered variables. It is a method often used in biology for classification and taxonomy (numerical taxonomy) (Bagci et al., 1991; Cinco et al., 1993; Chakraborty et al., 1996).

The aim of the analysis is to identify groups (or clusters) of similar objects, that is, the cases considered are grouped on the basis of their similarity; initially group membership and the number of groups is unknown for all cases. The first step in performing the analysis is the choice of the variables that can be used to identify groups, then the selection of a distance measure to obtain a distance matrix and, finally, the choice of the method for combining objects into clusters.

In this study the 36 isolates of F.o.m. were separated using SPSS Professional Statistics for Windows (SPSS Incorporated, 1994) on the basis of disease incidence (%) observed on three muskmelon cultivars at 10, 20 and 30 days from inoculation. Data were not standardized before analysis, because they were expressed as a percentage of affected seedlings. The Squared Euclidean distance was used as a measure of the diversity between two objects (distance measurements are small when objects are similar); the distance matrix obtained was used to carry out a Hierarchical Cluster Analysis. This kind of analysis starts from a situation in which all cases are considered to be separate clusters and then clusters are formed by grouping cases into bigger and bigger clusters until all the cases are members of a single cluster. The median method was used to decide which clusters should be combined at each step: it weights the two clusters that must be combined equally by computing the centroid (mean) of the cluster being joined, therefore all the clusters have an equal effect on the characterization of the larger clusters into which they are merged, regardless of their dimension. A visual representation of the steps performed by hierarchical clustering is the dendrogram, which identifies the clus-

ters being combined and the values of the distance coefficients at each step. The dendrogram must be read from left to right: vertical lines denote clusters that are merged at each step, the length of the horizontal lines indicate the distance at which clusters were joined.

The clusters obtained from cluster analysis were further analysed using discriminant analysis (Fernández and Hanlin, 1996). This technique is not concerned with what directly determines the clusters, but rather with their characteristics after they have been identified. It uses the group membership of the cases to derive linear combinations of the quantitative variables (discriminant functions) and to use them as the basis for the classification of cases into one of the groups so that the probability of misclassification is minimized. The classification method used by SPSS is based on the Bayes rule, which estimates the probability of a case (an isolate) belonging to one of the groups considered on the basis of its D statistic. D is the result of the discriminant equation: $D = B_0 + B_1X_1 + B_2X_2 + ... + B_nX_n$, where X_n are the independent variables (the proportions of affected seedlings of each cultivar) and B_n are the coefficients estimated from the data set, so that the D values differ as much as possible between groups. The interpretation of the coefficients is similar to that in multiple regression: the absolute magnitude of each coefficient indicates the relative importance of the variable for the separation of cases. The D values of each case were used as coordinates to plot the distribution of cases on a two-dimensional space determined by the first two discriminant functions. In this work the advantage of using the discriminant analysis after the cluster analysis is that more information about the groups that have just been formed may be available, particularly the probability of a case belonging to a particular group, and the evaluation of the correct classification for each case.

RESULTS

'Charentais T' and 'Supermarket' showed the same reaction to the infection by F.o.m. races (Tab. 2); 'Charentais fom1' was the same as 'Delada', and 'Charentais fom2' the same as 'Aril'. 'Cameo' and 'Saby' were different from the other cultivars, their reaction being, respectively, intermediate to race 2 and resistant to races 0, 1 and 2.

During the thirty days after inoculation, disease incidence increased progressively with a different pattern for each cultivar: 'Supermarket' suffered more than 'Delada' and 'Aril'. Ten days after inoculation, the differences between 'Supermarket' and the other two cultivars were already evident (21.8, 3.2 and 1.9% plants affected, respectively); after twenty days, differences between 'Aril' and 'Delada' were evident too (61.3, 37.1 and 11.9% plants affected, respectively), and after thirty days the differences were even greater (98.9, 53.2 and 18.6%) (Fig. 1).

Ten days after inoculation 'Aril' was resistant to all the fungal isolates tested, 'Delada' appeared susceptible to only one F.o.m. isolate while 'Supermarket' showed sensitivity to six F.o.m. isolates and an intermediate reaction to two more (Tab. 3). At this time Armstrong and Armstrong's criterion for separating F.o.m. isolates was unable to assign a race for each of them (Tab. 3). The cluster analysis performed allowed us to subdivide the F.o.m. isolates into four clusters: thirty isolates (83.3%) were assigned to the first, four isolates (11.1%) to the second and one isolate (2.8%) to each of the last two. This set of data, however, did not permit us to keep the five isolates of the F.o.m. races apart, as they were all grouped in the first cluster (Tab. 3).

Table 2. Incidence (%) of affected plants in three differential muskmelon genotypes and five cultivars thirty days after inoculation with five F.o.m. races. R (resistant), S (susceptible) and I (intermediate) distinguish the type of response according to Armstrong and Armstrong (1978).

Muskmelon	Race of Fusarium oxysporum f.sp. melonis									
genotype	0		1		2		1-2y		1-2w	
Charentais T	100	S	91	S	100	S	–	–	94	S
Charentais fom1	19	R	85	S	6	R	–	–	81	S
Charentais fom2	31	R	10	R	88	S	–	–	91	S
Aril	30	R	10	R	90	S	90	S	90	S
Cameo	100	S	75	S	55	I	100	S	88	S
Delada	10	R	80	S	10	R	100	S	80	S
Saby	0	R	0	R	0	R	90	S	70	S
Supermarket	90	S	90	S	100	S	100	S	90	S

– : not tested.

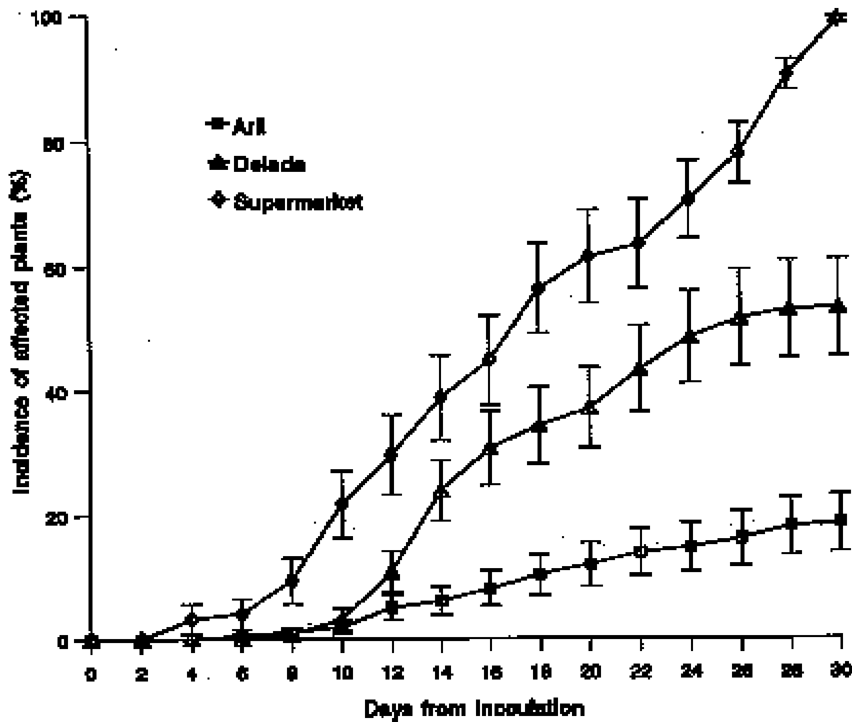

Fig.1. Disease incidence in the three muskmelon cultivars 'Aril', 'Delada' and 'Supermarket' during the pathogenicity test. Every point represents the mean of the percentages of plants infected by all the thirty-six F.o.m. isolates. Vertical bars indicate the standard error for each mean.

Twenty days after inoculation symptoms were more frequent and 'Aril', 'Delada' and 'Supermarket' appeared susceptible to three, thirteen and twenty-two F.o.m. isolates respectively (Tab. 4). According to the percentages of wilted seedlings suggested by Armstrong and Armstrong (1978), twelve F.o.m. isolates were not classified and five isolates received an uncertain assignment (Tab. 4). The disease incidence led to the following distribution: one isolate (2.8%) into the first cluster, four isolates (11.1%) into the second, nineteen isolates (52.8%) into the third and twelve (33.3%) into the fourth; the separation of the isolates of the F.o.m. races began to become evident, but not yet enough as the isolates of races 0 and 2 were both included in the first cluster (Tab. 4).

Thirty days after inoculation 'Supermarket' appeared susceptible to all the F.o.m. isolates while 'Aril' and 'Delada' showed sensitivity to four and twenty F.o.m. isolates respectively (Tab. 5). Cluster analysis placed fifteen isolates (41.7%) in the first cluster, one (2.8%) in the second, three (8.3%) in the third and seventeen (47.2%) in the fourth; the distribution of the isolates of F.o.m. races in the four clusters was still larger: the isolates of races 0, 1 and 2 were assigned to the first, fourth and second cluster respectively, while the isolates of races 1-2y and 1-2w were joined in the third (Tab. 5). Cluster analysis produced the dendrogram shown in figure 2. The first clustering step led to the specification of four groups: fourteen isolates belonged to the first, the second group consisted of two isolates, including the isolate of race 1-2y, the third of three isolates and the fourth of twelve isolates from northern Italy and the isolate of race 1. Afterwards the isolate of race 0 was added to the first group (distance coefficient = 706), the isolate of race 1-2w to the second (distance coefficient = 500), while the third and the fourth groups were joined together (distance coefficient = 481). In a further step isolate B7 was associated to the group embracing race 1 (distance coefficient = 1151). At this point the clustering was considered to be concluded since the next aggregations took place at very large distances. In this scheme race 2 established a cluster apart (cluster 2). The least distance coefficient between two adjacent clusters was 3258 (clusters 3 and 4), against the longest distance of 7396, at which all the isolates were grouped in the same cluster.

Separation of races 1-2y and 1-2w into distinct clusters was impossible even with a cluster analysis set to make up five clusters.

If cluster analysis was performed considering, at the same time, the disease incidences at 10, 20 and 30 days from inoculation the clustering of isolates and races was the same as when the analysis was performed considering disease incidence at 30 days from inoculation.

The clustering of the F.o.m. isolates of Table 5 was further tested by discriminant analysis. The first two calculated discriminant functions accounted for 93.97% and 6.01% of the total variation respectively.

Table 3. Incidence (%) of affected plants in three muskmelon differential cultivars 10 days after inoculation with five races and thirty-one isolates of F.o.m.. R (resistant), S (susceptible) and I (intermediate) distinguish the type of response according to Armstrong and Armstrong (1978).

Isolate	Muskmelon cultivar						F.o.m. race[1]	Cluster[2]
	Aril		Delada		Supermarket			
R 0	0	R	10	R	50	I	0 or –	1
R 1	0	R	10	R	0	R	–	1
R 2	10	R	0	R	30	R	–	1
R 1-2y	10	R	10	R	0	R	–	1
R 1-2w	20	R	0	R	20	R	–	1
A5	0	R	0	R	0	R	–	1
B7	0	R	0	R	8	R	–	1
C2	0	R	0	R	67	S	0	2
D6	0	R	0	R	100	S	0	2
E2	30	R	58	S	83	S	1	3
G1	0	R	0	R	0	R	–	1
G2	0	R	0	R	33	R	–	1
G3	0	R	0	R	0	R	–	1
G5	0	R	0	R	17	R	–	1
G6	0	R	0	R	0	R	–	1
G8	0	R	0	R	0	R	–	1
G9	0	R	0	R	0	R	–	1
G11	0	R	0	R	0	R	–	1
H1	0	R	0	R	0	R	–	1
H2	0	R	0	R	0	R	–	1
H3	0	R	0	R	0	R	–	1
I1	0	R	0	R	0	R	–	1
I3	0	R	0	R	0	R	–	1
I4	0	R	0	R	25	R	–	1
M1	0	R	0	R	0	R	–	1
M2	0	R	0	R	0	R	–	1
N1	0	R	0	R	0	R	–	1
N2	0	R	0	R	0	R	–	1
N3	0	R	0	R	0	R	–	1
N7	0	R	0	R	0	R	–	1
Q3	0	R	25	R	100	S	0	4
S5	0	R	0	R	0	R	–	1
T3	0	R	0	R	83	S	0	2
U2	0	R	0	R	83	S	0	2
U4	0	R	0	R	50	I	0 or –	1
W6	0	R	0	R	25	R	–	1

[1] Race attribution according to Armstrong and Armstrong (1978).
[2] Clustering of F.o.m. isolates.
– : impossible attribution.

Table 4. Incidence (%) of affected plants in three muskmelon differential cultivars 20 days after inoculation with five races and thirty-one isolates of F.o.m.. R (resistant), S (susceptible) and I (intermediate) distinguish the type of response according to Armstrong and Armstrong (1978).

Isolate	Muskmelon cultivar						F.o.m. race[1]	Cluster[2]
	Aril		Delada		Supermarket			
R 0	30	R	10	R	80	S	0	3
R 1	10	R	80	S	50	I	1 or –	1
R 2	50	I	10	R	100	S	0 or 2	3
R 1-2y	80	S	100	S	90	S	1-2	2
R 1-2w	60	S	60	S	70	S	1-2	2
A5	10	R	0	R	67	S	0	3
B7	40	R	67	S	100	S	1	2
C2	10	R	58	S	100	S	1	3
D6	0	R	100	S	100	S	1	3
E2	70	S	75	S	100	S	1-2	2
G1	0	R	50	I	100	S	0 or 1	3
G2	0	R	50	I	100	S	0 or 1	3
G3	10	R	75	S	100	S	1	3
G5	0	R	42	R	100	S	0	3
G6	0	R	50	I	100	S	0 or 1	3
G8	0	R	8	R	100	S	0	3
G9	10	R	83	S	100	S	1	3
G11	0	R	42	R	83	S	0	3
H1	0	R	0	R	8	R	–	4
H2	10	R	0	R	0	R	–	4
H3	10	R	0	R	0	R	–	4
I1	0	R	0	R	0	R	–	4
I3	0	R	0	R	8	R	–	4
I4	0	R	83	S	100	S	1	3
M1	0	R	0	R	8	R	–	4
M2	0	R	0	R	0	R	–	4
N1	0	R	0	R	0	R	–	4
N2	0	R	0	R	0	R	–	4
N3	10	R	0	R	0	R	–	4
N7	0	R	0	R	0	R	–	4
Q3	0	R	100	S	100	S	1	3
S5	0	R	0	R	0	R	–	4
T3	0	R	83	S	100	S	1	3
U2	20	R	0	R	100	S	0	3
U4	0	R	0	R	50	I	0 or –	3
W6	0	R	92	S	92	S	1	3

[1] Race attribution according to Armstrong and Armstrong (1978).
[2] Clustering of F.o.m. isolates.
– : impossible attribution.

Table 5. Incidence (%) of affected plants in three muskmelon differential cultivars 30 days after inoculation with five races and thirty-one isolates of F.o.m.. R (resistant), S (susceptible) and I (intermediate) distinguish the type of response according to Armstrong and Armstrong (1978).

Isolate	Muskmelon cultivar						F.o.m. race[1]	Cluster[2]
	Aril		Delada		Supermarket			
R 0	30	R	10	R	90	S	0	1
R 1	10	R	80	S	90	S	1	4
R 2	90	S	10	R	100	S	2	2
R 1-2y	90	S	100	S	100	S	1-2	3
R 1-2w	90	S	80	S	90	S	1-2	3
A5	20	R	0	R	100	S	0	1
B7	50	I	100	S	100	S	1 or 1-2	4
C2	30	R	100	S	100	S	1	4
D6	0	R	100	S	100	S	1	4
E2	90	S	100	S	100	S	1-2	3
G1	10	R	100	S	100	S	1	4
G2	10	R	100	S	100	S	1	4
G3	20	R	100	S	100	S	1	4
G5	0	R	92	S	100	S	1	4
G6	10	R	92	S	100	S	1	4
G8	10	R	100	S	100	S	1	4
G9	20	R	100	S	100	S	1	4
G11	20	R	83	S	100	S	1	4
H1	0	R	0	R	100	S	0	1
H2	10	R	0	R	100	S	0	1
H3	10	R	0	R	100	S	0	1
I1	0	R	0	R	100	S	0	1
I3	10	R	100	S	100	S	1	4
I4	0	R	83	S	100	S	1	4
M1	0	R	0	R	100	S	0	1
M2	0	R	0	R	100	S	0	1
N1	10	R	0	R	100	S	0	1
N2	0	R	0	R	100	S	0	1
N3	10	R	0	R	100	S	0	1
N7	0	R	0	R	100	S	0	1
Q3	0	R	100	S	100	S	1	4
S5	0	R	0	R	100	S	0	1
T3	0	R	92	S	100	S	1	4
U2	20	R	0	R	100	S	0	1
U4	0	R	0	R	100	S	0	1
W6	0	R	92	S	92	S	1	4

[1] Race attribution according to Armstrong and Armstrong (1978).
[2] Clustering of F.o.m. isolates.

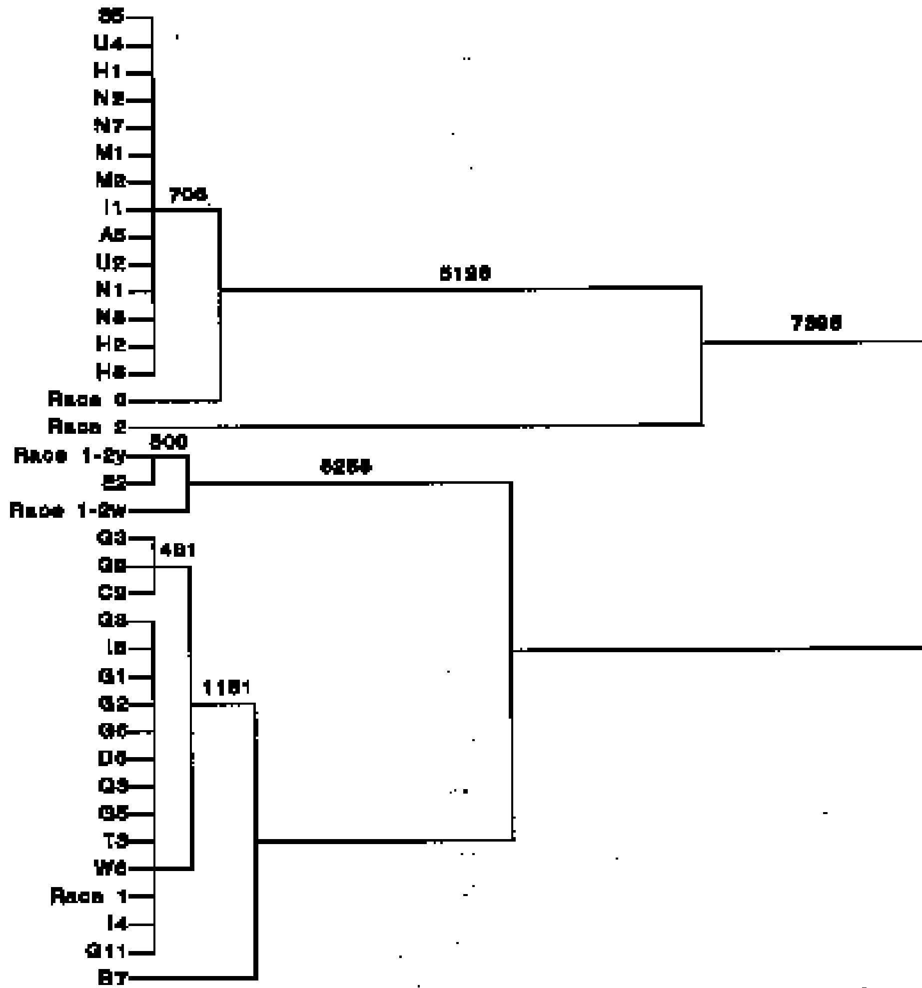

Fig.2. Distance dendrogram based on the analysis of disease incidence 30 days after inoculation of three muskmelon cultivars with thirty-six F.o.m. isolates.

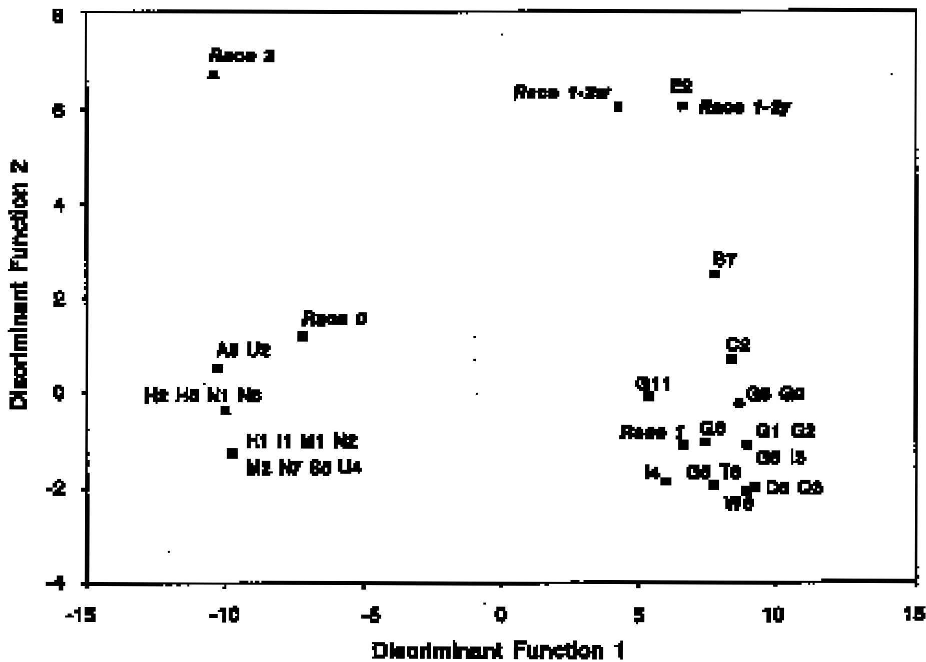

Fig.3. Grouping of F.o.m. isolates based on the discriminant analysis performed on disease incidence 30 days after inoculation of three muskmelon cultivars.

Table 6. Coefficients of the two discriminant functions produced by the discriminant analysis performed on disease incidences 30 days after inoculation of thirty-six F.o.m. isolates on three muskmelon cultivars.

Muskmelon cultivar	Coefficient of discriminant function	
	Function 1	Function 2
Aril	– 0.45465	0.04962
Delada	1.13952	– 0.04399
Supermarket	– 0.32933	1.01623

Plotting the distribution of isolates on a two-dimensional space showed a clear separation of clusters according to their race (Fig. 3). Parting of clusters on the function 1 axis was mainly due to disease incidence on 'Delada' (standardized canonical coefficient = 1.1395), while on the function 2 axis the largest separation was due to disease incidence on 'Supermarket' (standardized canonical coefficient = 1.0162) (Tab. 6). Discriminant analysis did not change the grouping of F.o.m. isolates generated by cluster analysis. Races 1-2y and 1-2w, 2, 0 and 1 were far from each other and the isolates attributed to each race were very close to them, with the exception of B7. In fact the probability of each isolate belonging to the assigned group was equal to 1, with the exception of B7, which was assigned to race 1 at a 0.93 probability level and to race 1-2 at 0.07.

DISCUSSION

Pathogenicity test management, with particular reference to muskmelon genotypes and trial length, was found suitable for pointing out the different behaviour of the seedlings towards inoculation.

The good parting of the five F.o.m. races obtained in the preliminary test leads us to believe the use of the muskmelon cultivars in such tests was appropriate and the commercial cultivars 'Aril', 'Delada' and 'Supermarket' can replace 'Charentais fom2', 'Charentais fom1' and 'Charentais T', respectively, in pathogenicity tests. This is a positive element since the pathogenicity test is simpler and faster if local muskmelon cultivars can be used, rather than differential genotypes which are often difficult to find and to reproduce in purity. In our tests there was a general agreement between the behaviour of the cultivars towards inoculation and the statements of the seed firms about the presence or the absence of F.o.m. resistance genes in each cultivar.

In previous studies, the pathogenicity tests went on for a very variable period, from 10 to 28 days (Gubler and Grogan, 1976; Latin and Snell, 1986; Gerlagh and Blok, 1988; Jacobson and Gordon, 1988; Katan et al., 1994). Carrying out daily reading of symptomatic plants, it was possible to establish that the most suitable length of time, able to give a complete separation of the considered isolates, was thirty days, when almost all the plants of 'Supermarket' had been affected. In further trials less time should be dedicated to daily recording of the disease symptoms, limiting effort to getting rid of the dead plants so as to prevent cross-contamination, and reading the percentage of diseased or dead plants when disease incidence on the susceptible cultivar is about 100%.

Another remarkable aspect of this study was the application of multivariate statistical techniques. The comparison between the attribution of the F.o.m. isolates to the different races using both cluster and discriminant analyses, and that on the basis of the criterion suggested by Armstrong and Armstrong (1978) showed that the two groupings turned out identical with the single exception of the B7 isolate attribution. In fact, according to Armstrong and Armstrong (1978) the reaction of the cultivar 'Aril' (50% of diseased plants) was intermediate so that it was impossible to attribute this isolate to race 1 or 1-2. Cluster analysis assigned the B7 to race 1, while discriminant analysis assigned it to race 1 with a high probability level (93%) and to race 1-2 with a probability of only 7%.

Hence the cluster analysis was found to be efficient in distinguishing the F.o.m. races and grouping the other isolates around them. It was useful to verify the possibility of using this statistical method especially as it makes it possible to assign F.o.m. isolates to a race on an objective basis, eliminating the uncertainty produced by subjective judgement. In such appraisals, subjectivity arises, essentially, from the need to read a fairly varied symptomatology on affected plants: wilting, stunting, chlorosis and necrosis. This fact, together with the requirement to use thresholds (% of affected plants) to establish the reaction of the host-pathogen couple studied, can greatly influence the expression of the final result. In fact, the wrong evaluation of one or a few plants can often lead to the erroneous assignment of a fungal isolate. The elaboration of the data by cluster analysis allows us to minimize such risks. Moreover the discriminant analysis provided further information, since if the probabilities of belonging to different groups are too close, this would lead one to suppose that some mistake had occurred during the execution of the experiment (for example misunderstood symptoms or experimental conditions). A clear example of the benefit of using these statistical techniques is supplied by the attribution of the B7 isolate.

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