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Isolation and identification of pathogenic strains of tomato mosaic virus by host passage

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Abstract

Three isolates of tomato mosaic virus, A.8, SJ-64 and SL[a], assumed to contain the pathogenic strains 1 and 2, were each subjected to selection pressure by passage through different hosts in concurrent series. The sub-isolates obtained were tested at intervals on differential tomato lines heterozygous for each of the resistance genes Tm-1 and Tm-2 and for the combination of Tm-1 and Tm-2.

Three preliminary passages through *Solanum pennellii* followed by 22 passages through the tomato line 'CStMW-18' (Tm-1/Tm-1) resulted for A.8, SJ-64 and SL[a] in sub-iolates of strain 1. Three preliminary passages through *L. peruvianum* P.I. 128655 followed by 22 passages through the tomato line 'Pérou-2' (Tm-2/Tm-2) resulted for A.8 and SL[a] in sub-isolates of strain 2 and for SJ-64 in a sub-isolate of strain 1.2. Twenty passages through 'Pérou-2' followed by two additional passages through the tomato line 'Craigella Tm-1/+Tm-2/+' resulted for SJ-64 and SL[a] in sub-isolates of strain 1.2 and for A.8 in a sub-isolate of strain 2.

Additional keywords: resistance, hypersensitivity, susceptibility, tolerance, adaptation.

Introduction

In work on resistance breeding to tomato mosaic, a disease mostly caused by tomato mosaic virus (ToMV), the existence of pathogenic differences between strains is at present common knowledge. McRitchie and Alexander (1963) working in Ohio (USA) were the first to demonstrate differences in pathogenicity between four strains in *Lycopersicon esculentum* 'Bonny Best' and 'CStMW-18' and a number of *L. peruvianum* accessions. The latter were self-incompatible and represented heterogeneous (populations. Pelham (1968, 1972) proposed a system for strain classification based on the interaction between the four Ohio strains and tomato hybrids of known resistance genotype according to a gene-for-gene concept. The resistance genes in tomato include a gene for tolerance Tm-1 (Holmes, 1957), dominant for the suppression of symptoms and inhibiting virus multiplication in the homozygous condition and two allelic Tm-2 genes (Clayberg, 1961; Laterrot and Pécaut, 1969), dominant for resistance based on hypersensitivity. This hypersensitivity is mostly not apparent from

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necrotic lesions in inoculated leaves, but from necroses formed with systemic spread of the infecting virus.

In Pelham's system the strains were given an arabic numeral corresponding with the gene they were able to overcome. Thus, the Ohio strains I and II were reclassified as strain 0 causing mosaic symptoms only in the universal suscept, the Ohio strains III and IV as strains 1 and 2, respectively, for causing mosaic symptoms in the tomato lines heterozygous for the resistance genes Tm-1 and Tm-2, respectively. Pelham (1972) also obtained strain 1.2 with the combined pathogenicity of the Ohio strains III and IV.

Rast (1968), using clonal test plants of McRitchie and Alexander's differentials supplemented with *Solanum pennellii* (Smith, 1961), distinguished three pathogenically different groups of isolates and provisionally classified them as groups 1, 2 and 3. When tested on Pelham's tomato hybrids (Rast, 1975) the isolates of groups 1 and 2 were classified with strains 0 and 1, respectively, and most isolates of group 3 with strain 2. Previously, these isolates of group 3 had several times been passed through susceptible plants of *L. peruvianum* P.I. 128655. This passage increased their virulence to that host and was repeated until a consistent proportion of the *L. peruvianum* hosts reacted with mosaic symptoms. The same passage decreased their infectivity to *L. esculentum* 'CStMW-18' and *S. pennellii,* indicating a selective propagation of strain 2 in *L. peruvianum.*

A number of group 3 isolates could not be classified with strain 2 as expected because the symptoms in Pelham's tomato hybrids were suggestive of strain 1 rather than strain 2. They caused mosaic symptoms in the Tm-1/+ genotype and a systemic necrosis in the Tm-2/+ genotype instead of mosaic symptoms. This necrosis affected more Tm-2/+ plants and was more severe than would normally result from random infections by strain 1 alone. However, contrary to the other isolates of group 3, these isolates had never been passed through *L. peruvianum* P.I. 128655, in which they caused mosaic symptoms suggestive for the presence of strain 2.

And although these isolates had been repeatedly transferred from single lesions in *Nicotiana glutinosa* to *N. tabacum* 'Samsun', they probably still represented strain mixtures.

In this work three such isolates of group 3 were subjected to a selective host passage in order to better separate the pathogenic strains 1 and 2 and so to demonstrate that both strains were really involved with the necrotic symptoms observed in the Tm-2/+ genotype.

Materials and methods

The investigation was carried out in a glasshouse under natural light conditions at night and day temperatures of 18° and 23° C, respectively. Plants were grown in normal potting soil either individually in plastic pots of 10 cm diameter or in rows of 4-10 plants each, up to a total of 40 plants in plastic trays measuring 60 × 40 × 12 cm.

The test plants included the *L. esculentum* breeding lines 'Craigella' developed by Pelham (resistance genotype +/+, Tm-1/+, Tm-2/+ and Tm-1/+ Tm-2/+), Walter's 'CStMW-18' (Tm-1/Tm-1) (see Holmes, 1957), Laterrot and Pécaut's 'Pérou-2' (Tm-2/Tm-2) (1969) and Pelham's 'GCR 254' (Tm-1/Tm-1 Tm-2/Tm-2), the *L. peruvianum* accession P.I. 128655, Rick's *S. pennellii* (see Smith, 1961), *N. glauca, N. glutinosa,*

N. tabacum 'Xanthi nc', 'Samsun' and 'White Burley' (the so-called 'necrotic' line or 'Dutch A').

'CStMW-18', 'Pérou-2' and 'Craigella Tm-1/+ Tm-2/+' were used as passage hosts and together with the other 'Craigella' lines and 'GCR 254' also for monitoring the pathogenic changes in the virus isolates resulting from host passage. Clonal test plants of *S. pennellii* and *L. peruvianum* P.I. 128655 were also used as passage hosts. All other test plants mostly served the general purposes of virus propagation and/or assays.

Inocula were freshly prepared from infected leaves ground in water, except for the original isolates, which were used as purified suspensions (Venekamp et al., 1973) in a suitable dilution with water. Before inoculation the test plants were dusted with 600 mesh carborundum.

With individual test plants the inoculum was applied to the leaves with a plug of cotton wrapped around one end of a 6-8 cm bamboo pricker, meanwhile supporting the leaves with a disk of filter paper. The differential tomato lines, grown together in plastic trays, were inoculated at the second true leaf stage by rubbing the cotyledons and leaflets gently with inoculum-wetted fingers. All plants in a tray were treated with the same inoculum. After inoculation the excess carborundum was removed from the plants with a spray of water. Symptoms of differential tomato lines were recorded after 10 and 21 days.

The virus isolates selected for this study, A.8, SJ-64 and SL^a, were typical for ToMV causing local, necrotic lesions in 'White Burley'. All three had several times been subjected to a single lesion isolation, in which lesions from *N. glutinosa* were each sub-cultured in *N. tabacum* 'Samsun' in between successive transfers. They were kept as purified suspensions at $-18°$ C.

The host passage was started by inoculating each of these original isolates to five clonal test plants of *S. pennellii* and five of *L. peruvianum* P.I. 128655. Plants of *S. pennellii* which the first time remained symptomless were assayed on *N. glutinosa* to indicate the plants infected and to be used for sub-inoculations to fresh plants of the same *S. pennellii* clones. Similarly, plants of *L. peruvianum* reacting with symptoms were used to inoculate fresh *L. peruvianum* plants. After three successive transfers, when the clonal test plants reacted uniformly with mosaic symptoms, one plant of *S. pennellii* and one of *L. peruvianum* were selected for inoculations to seedlings of either 'CStMW-18' or 'Pérou-2', respectively. The passage of A.8, SJ-64 and SL^a through the latter hosts in concurrent series was subsequently repeated 22 times. The sub-isolates obtained after 20 passages through 'Pérou-2' were passed twice through 'Craigella Tm-1/+ Tm-2/+'. The progress in the pathogenic changes was assessed by testing the original isolates and their respective sub-isolates on the differential tomato lines. This was done after the three preliminary passages through *S. pennellii* and *L. peruvianum,* after 10 and 20 passages through 'CStMW-18' and 'Pérou-2' and again after the two additional passages through these hosts and through 'Craigella Tm-1/+ Tm-2/+'. For SL^a and its sub-isolates *N. glauca* was included as a test plant as it reacts with local necrotic lesions to SL^a in its original form (Rast, 1975).

Results

The reactions of the differential tomato lines, especially those carrying the Tm-2 gene often varied in type and severity of symptoms and in the proportion of plants affected in a batch. Symptomless plants (0) which produced no lesions (0^-) when assayed on *N. glutinosa* were regarded as resistant. Assays yielding up to 10 lesions (0^+) per *N. glutinosa* leaf were arbitrarily also considered to indicate resistance, more than 10 lesions ($0^{+\,+}$) indicating susceptibility. Necrotic symptoms in Tm-2-carrying plants mostly consisted of a systemic top necrosis. A distinction was made between mild (N1) and moderate necrosis (N2) which allowed further growth and severe necrosis (N3) which stunted growth permanently and often caused death of the plants (N3!) soon after inoculation. A dwarfing type of growth (D) in combination with mosaic symptoms often resulted from a stem necrosis below the cotyledons. Necrotic symptoms were considered to indicate resistance only when no further symptoms developed or when no transmission of symptoms occurred with sub-inoculation. A systemic necrosis followed by mosaic symptoms was explained as partial susceptibility. Mosaic symptoms, regarded to indicate susceptibility, varied from a mild mottling (M1) to a distinct mosaic without (M2) or with (M3) attendant leaf narrowing. For a clear presentation of the results it was necessary to summarize the actual symptom variation observed in a batch of test plants by one or two combinations of symptom symbols.

In evaluating the symptoms observed in a host carrying the Tm-2 gene for resistance it was assumed that mosaic symptoms as compared to necrosis represent a more advanced stage in the adaptation of the infecting virus. Consequently, with increasing pathogenicity of the virus the host will first remain symptomless, then react with necrosis and eventually develop mosaic symptoms. In a host carrying the Tm-1 gene for tolerance and initially reacting symptomlessly to infection, an increase in pathogenicity of the virus will be expressed in increasingly distinct mosaic symptoms.

The results of the tests with A.8, SJ-64 and SL^a on differential tomato lines after the three preliminary passages through *S. pennellii* and *L. peruvianum* are presented in Table 1. While for A.8 and SL^a the effects of the passage through *S. pennellii* could not be tested for lack of test plants, the passage resulted for SJ-64 in the development of distinct mosaic symptoms in both Tm-1 genotypes. The sub-isolate of SJ-64, causing no longer a lethal necrosis in 'Craigella Tm-2/+', appeared therefore more closely related to strain 1 than the original isolate. The passage through *L. peruvianum* in general increased pathogenicity to the Tm-2 genotypes and decreased pathogenicity to 'Craigella Tm-1/+'. For A.8 the passage resulted in the appearance of mosaic symptoms in 'Craigella Tm-2/+' and 'Pérou-2', being preceded in the former by a mild necrosis. For SJ-64 the only pathogenic change occurred in 'Pérou-2' which reacted with a mild necrosis. For SL^a the necrosis in 'Craigella Tm-2/+' became more severe and mosaic symptoms preceded by a mild necrosis appeared in 'Pérou-2'. Because of the necrotic symptoms in the Tm-2 genotypes neither of the sub-isolates from *L. peruvianum* were sufficiently adapted to be classified as strain 2.

The test results obtained after the subsequent 10 and 20 passages through 'CStMW-18' and 'Pérou-2' were essentially the same, which means that the pathogenic changes observed had occurred during the first 10 passages. However, the test results after 20 passages were used for presentation in Table 2, as they were completed by assays of symptomless plants. The passage through 'CStMW-18' resulted for all three

Table 1. Reactions of differential tomato lines to the tomato mosaic virus (ToMV) isolates A.8, SJ-64 and SL[a] and their respective sub-isolates as obtained after three preliminary cycles of host passage through *Lycopersicon peruvianum* P.I. 128655 or *Solanum pennellii.*

Isolate/sub-isolate	Differential tomato line and resistance genotype					
	Craigella +/+	Craigella Tm-1/+	CStMW-18 Tm-1/ Tm-1	Craigella Tm-2/+	Pérou-2 Tm-2/ Tm-2	Craigella Tm-1/+ Tm-2/+
A.8: Original isolate	M3	M1	0	N2	0	0
Sub-isolate from *L. peruvianum* P.I. 128655	M3	M2/0	0	N1M3	M2	0
SJ-64: Original isolate	M3	M1	0	N3!	0	0
Sub-isolate from *S. pennellii*	M3	M3	M2	0	0	N2/0
Sub-isolate from *L. peruvianum* P.I. 128655	M3	M1	0	N3!	N1	0
SL[a]: Original isolate	M3	M1	0	N2/0	0	0
Sub-isolate from *L. peruvianum* P.I. 128655	M3	0	0	N3!	N1M2	0

M1 = mild mottling, M2 = distinct mosaic, M3 = distinct mosaic and leaf distortion, N1 = mild necrosis, N2 = moderate necrosis, N3 = severe necrosis, N3! = lethal necrosis, 0 = no symptoms, / = two types of symptoms observed in a batch of test plants.

isolates in the development of distinct symptoms in the Tm-1 genotypes, which were typical of strain 1. The symptomless reaction of 'Pérou-2' and necrotic reactions of 'Craigella Tm-1/+ Tm-2/+' also conformed with strain 1. The mosaic symptoms caused by the sub-isolates of SJ-64 and SL[a] in the Tm-2 genotypes and 'Craigella Tm-1/+ Tm-2/+' respectively, did not entirely fit in with strain 1.

The passage of the three isolates through 'Pérou-2' brought out their strain 2 characteristics more clearly as shown by distinct mosaic symptoms in the Tm-2 genotypes. However, mosaic symptoms observed in the Tm-1 genotypes and/or 'Craigella Tm-1/+ Tm-2/+' were not quite in agreement with the sole presence of strain 2. In this connection it should be noted that the sub-isolate of A.8 from 'Pérou-2', which gave no symptoms in 'Craigella Tm-1/+ Tm-2/+' had produced mosaic symptoms in this host in a previous test performed after 10 passages through 'Pérou-2'.

In order to find out whether strain 1.2 was possibly involved, the virus infecting

Table 2. Reactions of differential tomato lines to the tomato mosaic virus (ToMV) isolates A.8, SJ-64 and SL[a] and their respective sub-isolates as obtained after 20 cycles of host passage through the tomato breeding lines 'CStMW-18' or 'Pérou-2'.

Isolate/sub-isolate	Differential tomato line and resistance genotype					
	Craigella +/+	Craigella Tm-1/+	CStMW-18 Tm-1/ Tm-1	Craigella Tm-2/+	Pérou-2 Tm-2/ Tm-2	Craigella Tm-1/+ Tm-2/+
A.8: Original isolate	M3	M2	0^+	N3	0^-	0^-
Sub-isolate from CStMW-18 (Tm-1/Tm-1)	M3	M3	M2	N2/0	0^-	N2/0^-
Sub-isolate from Pérou-2 (Tm-2/Tm-2)	M3	M3	$0^{+\,+}$	M3	M2	0^+
SJ-64: Original isolate	M3	M2	M2	N3!	N1M2	0^-
Sub-isolate from CStMW-18 (Tm-1/Tm-1)	M3	M3	M3	N2M2	M1/0^+	N1
Sub-isolate from Pérou-2 (Tm-2/Tm-2)	M3	M2/$0^{+\,+}$	$0^{+\,+}$	M3	M2	M1
SL[a]: Original isolate	M3N1	M2	0^+	N2	0^+	0^-
Sub-isolate from CStMW-18 (Tm-1/Tm-1)	M3	M3	M2	N1/N2	0^-	N1/N1M2D
Sub-isolate from Pérou-2 (Tm-2/Tm-2)	M3	$0^{+\,+}$	0^+	M3	M2	0^+

M1 = mild mottling, M2 = distinct mosaic, M3 = distinct mosaic and leaf distortion, N1 = mild necrosis, N2 = moderate necrosis, N3 = severe necrosis, N3! = lethal necrosis, 0 = no symptoms, 0^- = no symptoms, no virus on assay, 0^+ = no symptoms, 1-10 lesions on assay, $0^{+\,+}$ = no symptoms, more than 10 lessions on assay, / = two types of symptoms observed in a batch of test plants.

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M1 = mild mottling, M2 = distinct mosaic, M3 = distinct mosaic and leaf distortion, N1 = mild necrosis, N2 = moderate necrosis, N3 = severe necrosis, N3! = lethal necrosis, 0 = no symptoms, 0^- = no symptoms, no virus on assay, 0^+ = no symptoms, 1-10 lesions on assay, $0^{+\,+}$ = no symptoms, more than 10 lessions on assay, / = two types of symptoms observed in a batch of test plants.

Table 3. Reactions of differential tomato lines to the tomato mosaic virus (ToMV) isolates A.8, SJ-64 and SL[a] and their respective sub-isolates as obtained after 22 cycles of host passage through the tomato breeding lines 'CStMW-18' or 'Pérou-2' and after 20 cycles through 'Pérou-2' followed by two additional cycles through the tomato breeding line 'Craigella Tm−1/+ Tm-2/+'.

Isolate/sub-isolate	Differential tomato line and resistance genotype					
	Craigella Tm-1/+	CStMW-18 Tm-1/ Tm-1	Craigella Tm-2/+	Pérou-2 Tm-2/ Tm-2	Craigella Tm-1/+ Tm-2/+	GCR 254 Tm-1/Tm-1 Tm-2/Tm-2
A.8: Original isolate	M2	0	N3	0	0	0^-
Sub-isolate from CStMW-18 (Tm-1/Tm-1)	M2	M1/M2	N2M2	0	N2M2D	0^-
Sub-isolate from Pérou-2 (Tm-2/Tm-2)	M2	0	M2	M2	M2	0^-
Sub-isolate from Craigella Tm-1/+ Tm-2/+ (via Pérou-2)	M2	0	M2	M2	M2	0^+
SJ-64: Original isolate	M2/0	M1	N3!	0	0	0^-
Sub-isolate from CStMW-18 (Tm-1/Tm-1)	M2	M1/M2	0	0	N1	0^+
Sub-isolate from Pérou-2 (Tm-2/Tm-2)	M1/M2	M1/0	M2	M2	M1/M2	0^+
Sub-isolate from Craigella Tm-1/+ Tm-2/+ (via Pérou-2)	M2	M1	M2	M2	M2	0^{++}
SL[a]: Original isolate	M1/0	0	N1/N3	0	0	0^-
Sub-isolate from CStMW-18 (Tm-1/Tm-1)	M2	M2	N2M2D	0	N2M2D	0^+
Sub-isolate from Pérou-2 (Tm-2/Tm-2)	M1	0	M2	M2	M1/M2	0^+
Sub-isolate from Craigella Tm-1/+ Tm-2/+ (via Pérou-2)	M2	M1	M2	M1	M2	0^{++}

'Craigella Tm-1/+ Tm-2/+' was transferred to this host for another passage and then tested again, this time also on 'GCR 254' (Tm-1/Tm-1 Tm-2/Tm-2).

The results of these tests are presented in Table 3 together with those obtained after a total of 22 passages through 'CStMW-18' and 'Pérou-2'. No significant changes were observed after the final passage through 'CStMW-18'. For the sub-isolates of A.8 and SL^a the mosaic symptoms following the necrosis in 'Craigella Tm-2/+' and 'Craigella Tm-1/+ Tm-2/+' would suggest a development towards strain 1.2. However, as no symptoms were produced in 'Pérou-2' they were still to be regarded as strain 1. The sub-isolate of SJ-64 from 'CStMW-18' was a typical representative of strain 1. The sub-isolates from 'Pérou-2' and 'Craigella Tm-1/+ Tm-2/+' hardly differed from each other. For those of A.8 the only difference was found in the few lesions produced by the assays of 'GCR 254' inoculated with the sub-isolate from 'Craigella Tm-1/+ Tm-2/+'. As both sub-isolates caused mosaic symptoms in the other differential tomato lines, but not in 'CStMW-18', they had to be classified as strain 2 and not as strain 1.2.

For the sub-isolates of SJ-64 the only difference was again found in the assays of 'GCR 254'. The sub-isolate from 'Pérou-2', for which the assays yielded only a few lesions, but apart from that caused mosaic symptoms in all other tomato lines, was consequently classified as strain 1.2. The sub-isolate from 'Craigella Tm-1/+ Tm-2/+' was considered typical for strain 1.2 as the assays of 'GCR 254' produced numerous lesions.

For similar reasons the sub-isolates of SL^a from 'Pérou-2' and 'Craigella Tm-1/+ Tm-2/+' were classified as strains 2 and 1.2, respectively.

As for 'GCR 254' it should be noted that reading of symptoms was not possible because of the chlorotic and dwarfed appearance of the plants due to the homozygous condition of a sublethal gene for 'netted virescence' *nv* which is linked to the Tm-2 gene as used in 'GCR 254' (Clayberg, 1961).

The characteristic necrosis caused by SL^a in tomato in winter (Rast, 1975) was only observed with the original isolate in seven out of ten plants of the generally susceptible 'Craigella +/+' (Table 2). *N. glauca,* when tested on two different occasions, reacted with typical, necrotic local lesions only to the original isolate and sub-isolate from 'CStMW-18', but not to that from 'Pérou-2'.

Discussion and conclusions

In general the results obtained by host passage confirmed that the isolates A.8, SJ-64 and SL^a originally comprised strain mixtures. The separation of strains 1 and 2 was probably accomplished by selective propagation in their respective hosts. The observed tendency for strain 1.2 to develop during passage in 'Pérou-2' rather than in 'CStMW-18' is more difficult to explain. Possibly these hosts differed in the capacity for inhibiting the multiplication of other but the favoured strains, especially with regard to the strain 0 fraction in the original mixture. Neither host appeared completely effective in this capacity. The sub-isolates from 'CStMW-18' may still have contained strain 2 as suggested by mosaic symptoms in 'Craigella Tm-2/+' while the sub-isolates from 'Pérou-2', used as a selective host for strain 2, may still have contained strain 1 as they caused mosaic symptoms in 'Craigella Tm-1/+'. From literature 'CStMW-18' is known as a symptomless carrier for strains 0 and 2 (McRitchie and

Alexander, 1963) and it is not unlikely that both strains were present in the sub-isolates of this host. Indirect evidence to support this view may be derived from the reactions shown by *N. glauca* to SL^a (Rast, 1975). Numerous necrotic local lesions developed with the original isolate, a smaller number with the sub-isolate from 'CStMW-18', but none with the sub-isolate from 'Pérou-2'. As the local lesion reaction is most probably linked to the strain 0 fraction in SL^a it is obvious that the multiplication of this strain was reduced in 'CStMW-18', but not completely suppressed as in 'Pérou-2'. The available evidence therefore suggests that 'Pérou-2' in the absence of strain 0 would be a more suitable host than 'CStMW-18' for the strains 1 and 2 to form a strain of combined pathogenicity.

In this connection reference should be made to McNeill and Fletcher (1971), who inoculated their graft combinations with strain 0, isolated strain 1.2 from the heterozygous Tm-2 graft component and not from the heterozygous Tm-1 component. Assuming that by selection pressure in 'Pérou-2' the strain population consists largely of strain 2, then a single mutation in the remaining strain 1 fraction into strain 1.2 would be sufficient to account for the mosaic symptoms in all the tomato lines used. On the other hand in 'CStMW-18' a predominant strain 1 fraction would continue to induce necrosis in tomato lines carrying the Tm-2 gene, even when a mutation in the minor strain 2 fraction into strain 1.2 would eventually result in mosaic symptoms. For the pathogenic changes observed, whether resulting from selection pressure or host-induced mutations or both, changes may be found in the virus genome by methods used in molecular biology. As a practical consequence for strain classification it may be concluded that necrotic reactions should not be disregarded as they may provide a clue for the identity of the strains involved. For A.8, SJ-64 and SL^a the necrosis caused in 'Craigella Tm-2/+' could not be accepted as a non-specific hypersensitive reaction, simply because from previous knowledge they appeared closely related to other isolates classified as strain 2. The necrosis was thought to be incited by strain 1 which was carried along with systemic invasion by strain 2. This assumption is consistent with observations (Taliansky et al., 1982) that TMV can be made to systemically infect an alien host by an unrelated helper virus adapted to that host.

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Samenvatting

Isolatie en identificatie van pathogene stammen van tomatemozaïekvirus door waardplantpassage

Op drie isolaten van het tomatemozaïekvirus, A.8, SJ-64 en SL^a, die naar alle waarschijnlijkheid de pathogene stammen 1 en 2 bevatten, werd selectiedruk uitgeoefend door een herhaalde waardplantpassage. Voor de afscheiding van stam 1 onderging elk van de isolaten eerst driemaal een passage door *Solanum pennellii* en vervolgens 22 maal door de tomaatselectie 'CStMW-18' (Tm-1/Tm-1). Voor stam 2 passeerde elk iso-

laat eerst driemaal *Lycopersicon peruvianum* P.I. 128655 en vervolgens 22 maal de tomaatselectie 'Pérou-2' (Tm-2/Tm-2). Op gezette tijden werden de sub-isolaten, verkregen uit de gelijktijdig verlopende passageproeven, getoetst op een differentiële reeks tomaatselecties. Deze waren heterozygoot en homozygoot voor de resistentiegenen Tm-1 en Tm-2 en voor de combinatie van Tm-1 en Tm-2.

Voor elk van de isolaten A.8, SJ-64 en SL^a werden na passage door 'CStMW-18' sub-isolaten verkregen van stam 1. Terwijl voor A.8 en SL^a na passage door 'Pérou-2' sub-isolaten van stam 2 werden verkregen, leverde dezelfde passage voor SJ-64 een sub-isolaat op van stam 1.2. Voor SJ-64 en SL^a gaven twee passages door de tomaatselectie 'Craigella Tm-1/2 Tm-2/+' in aansluiting op 20 passages door 'Pérou-2' aanleiding tot de vorming van stam 1.2.

In de discussie wordt op de mogelijke ontstaanswijze van stam 1.2 ingegaan.

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