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Further identification of races of Cladosporium fulvum (Fulvia fulva) on tomato originating from the Netherlands, France and Poland

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Abstract

Races of *Cladosporium fulvum*, which can overcome the resistance of the genes Cf_2, Cf_4, Cf_5, Cf_8, Cf_9 and Cf_{11} have appeared in the Netherlands, France and Poland. Known isolates from the Netherlands and France and three new isolates from Poland have been investigated for the presence of virulence genes using a set of genotypes carrying resistance genes Cf_2 to Cf_{11}. Several Dutch isolates of races, earlier designated as 2.4, 2.4.5 and 2.4.5.9, were found to break down the resistance gene Cf_{11}. These races must therefore be designated as 2.4.11, 2.4.5.11 and 2.4.5.9.11 respectively. In the new Polish isolates virulence genes, overcoming the resistance genes Cf_2, Cf_4, Cf_8, Cf_9 and Cf_{11} were found. Since all races able to grow on genotypes with Cf_4, could also grow on genotypes carrying Cf_8, it was impossible to discriminate between the genes Cf_4 and Cf_8. These Polish isolates were designated as races 4.11, 2.4.11 and 2.4.9.11. The consequences of the occurrence of these races for tomato breeding are discussed.

*Additional keywords:*gene-for-gene interaction, leaf mold disease, *Lycopersicon esculentum*, resistance, susceptibility.

Introduction

Cladosporium fulvum (*Fulvia fulva*), the causal agent of leaf mold disease in tomato (*Lycopersicon esculentum* Mill.) has been subject of intensive research for several decades. Many resistance genes have been reported, but new races, overcoming the resistance genes used, have evolved rapidly (Boukema, 1981; Leski, 1977; Laterrot et al., 1985; Laterrot, 1986). These new races are detrimental to the tomato production. Therefore they provoke a continuous need to introduce new resistance genes into commercial cultivars. Still the use of resistant cultivars has reduced the occurrence of *Cladosporium fulvum* in commercial tomato production in the Netherlands to an acceptably low level.

Recently, two new isolates have been obtained from glasshouses for commercial

Table 1. Virulence spectrum of some races of *Cladosporium fulvum* from the Netherlands, France and Poland on tomato genotypes.

Tomato genotypes	Resistance genes	Races of *Cladosporium fulvum*											
		Netherlands								France	Poland		
		2	4	5	2.4	2.4.11	2.4.5	2.4.5.11	2.4.5.9.11	2.5.9	4.11	2.4.11	2.4.9.11
Moneymaker	Cf_0	S*	S	S	S	S	S	S	S	S	S	S	S
Vetomold	Cf_2	S	R	R	S	nd	S	nd	S	S	R	S	S
Purdue 135	Cf_4	R	S	R	S	nd	S	nd	S	R	S	S	S
Vagabond	Cf_2, Cf_4	R	R	R	S	S	S	S	S	R	R	S	S
Ontario 7717	Cf_5	R	R	S	R	R	S	S	S	S	R	R	R
Ontario 7818	Cf_6	R	R	R	R	R	R	R	R	R	R	R	R
Ontario 7522	Cf_8	R	S	R	S	nd	S	nd	S	R	S	S	S
Ontario 7719	Cf_9	R	R	R	R	R	R	R	S	S	R	R	S
Ontario 7716	Cf_{11}	R	R	R	R	S	R	S	S	R	S	S	S
Estrella		nd	nd	nd	R	R	S	S	S	nd	nd	R	R
Ostona		nd	nd	nd	R	R	R	R	S	S	nd	R	S
Abunda		nd	nd	nd	R	R	R	R	S	nd	nd	R	?

* S = susceptible, R = resistant, nd = not determined, ? = segregation for susceptible and resistent plants.

tomato production in Poland. A third isolate appeared in a cultivar trial at SHRO, Poland. To identify the virulence genes present in these isolates, they were tested on a set of differentials carrying single resistance genes. For comparison also some races, which are important for tomato breeding in the Netherlands, and 2.5.9, recently isolated in France, were included.

Materials and methods

Tomato genotypes and Cladosporium fulvum isolates. The tomato genotypes were from the IVT tomato collection. The choice of genotypes was based on the presence of one or two known resistance genes for *Cladosporium fulvum* (Kanwar, 1981). The races of *Cladosporium fulvum* 2, 4, 2.4, 2.4.11, 2.4.5, 2.4.5.11, and 2.4.5.9.11 were from IPO. Race 2.5.9 was kindly provided by Dr H. Laterrot, Station d'Amélioration des Plantes Maraîchères, Montfavet, France. The Polish isolates were from SHRO, Poland.

Culture of Cladosporium fulvum. The fungus was grown on potato dextrose agar (PDA) at 22 °C. Cultures were maintained by transferring spores with a droplet of sterile water to fresh PDA. For inoculation large quantities of spores were obtained by growing the fungus in flat bottles of 100 ml with a layer of PDA. These cultures sporulated abundantly in about ten days.

Inoculation and resistance tests. All experiments were carried out at IPO. Seedlings of the tomato genotypes were inoculated by spraying them with a suspension of about 10^6 spores ml^{-1} at the stage of two expanded true leaves. After inoculation, plants were kept at 100% relative humidity under plastic cover for at least two days. They were subsequently incubated in a glasshouse at 20 °C for two more weeks. Artificial light was supplemented and humidity was maintained at $>70\%$ by a humidifier. The plants were scored visually in two classes, resistant (R)) and susceptible (S) on the basis of disease development.

Experimental design. In two trials genotypes with the resistance genes Cf_0 through Cf_{11}, except for Cf_1, Cf_3, Cf_7 and Cf_{10} were screened with single isolates of several races of Dutch, French and Polish origin. In the third trial two, seven and three isolates of races, earlier indicated as 2.4, 2.4.5 and 2.4.5.9 respectively (Boukema, 1981), were compared with some other races on a set of relevant tomato differentials. Also the cultivars Estrella, Ostona and Abunda were included in this trial. All trials were performed in three replicates according to a randomized block design with three plants per plot.

Results

The combined results of the three trials are presented in Table 1. The reaction of the tomato differentials to the races 2, 4, 5 and 2.5.9 were identical with those described earlier (Boukema, 1981; Laterrot, 1986). These interactions once more support the gene-for-gene hypothesis and identify both the resistance genes present in the tomato genotypes and the virulence genes present in the *Cladosporium fulvum* races. In the first two trials, the Dutch isolates, earlier designated as 2.4.5. and 2.4.5.9 (Boukema, 1981), showed odd interactions with 'Ontario 7716', carrying resistance gene Cf_{11}. Based on these observations a third trial was set up to screen different isolates of the races, earlier designated as 2.4, 2.4.5 and 2.4.5.9, to determine their interaction with Cf_{11}. The results are included in Table 1: one out of two isolates identified earlier as 2.4, three out of seven isolates known as 2.4.5 and all 2.4.5.9 isolates could also overcome the resistance of gene Cf_{11}.

Two Polish isolates showed a combination of virulence genes, not yet observed before. Based on their differential interaction with tomato genotypes with known Cf genes, the Polish isolates must be designated 4.11, 2.4.11 and 2.4.9.11. The latter had been isolated from cv. Ostona, which was included in the third trial. Based on the differential interactions of 'Ostona' with the races tested, the resistance was found to be governed at least by Cf_9. Cf_6 was the only gene found to confer resistance to all isolates tested. However, sometimes some mycelium was observed, indicating a moderate level of resistance. Responses of Cf_8 and Cf_4 were identical for all isolates tested.

Discussion

The new Polish isolates have the virulence gene 11 in common. One of them was isolated from cv. Ostona, a hybrid cultivar carrying at least Cf_9. In the literature confusion exists about the significance of Cf_{11}. Though Kanwar et al. (1980a) consider it a distinct gene, they hesitate whether it is located on chromosome 9 or 12. Besides, it hardly provided protection against race 12 (= 2.4, Kerr et al., 1980). In the Netherlands attack of 'Ontario 7716' by race 2.4.5 and by 2.4.5.9 but not by race 2 had led to the suggestion that the function of Cf_{11} might be identical to Cf_4Cf_5 (Boukema, personal communication). The present interactions of several Dutch and Polish strains with Cf_{11} make clear that Cf_{11} is not identical to Cf_4Cf_5. So, Cf_{11} is a unique resistance gene. Consequently, some isolates of races, earlier identified as 2.4 or as 2.4.5 and the 2.4.5.9 isolates, contain the virulence gene 11 and must be designated 2.4.11, 2.4.5.11 and 2.4.5.9.11 respectively.

With respect to Cf_4 and Cf_8 the situation might be comparable. However, based on this and earlier studies there is no reason to distinguish a virulence factor 8. In all cases examined up till now, the reaction of Cf_8 is identical to Cf_4. Yet, linkage studies with morphological genetic markers have positioned both genes on different chromosomes (Kanwar et al., 1980a). However, the conclusive allelic test of Cf_4 with Cf_8 has not been executed. As long as no races are found which discriminate between Cf_4 and Cf_8 the existence of virulence gene 8 is not proven.

It is noteworthy that races show up, carrying a virulence gene of which the corresponding resistance gene in the host population is not present. 'Estrella' was the source of some of the 2.4.5.11 isolates and 'Ostona' of 2.4.9.11, while 2.4.5.9.11 was isolated from a Cf_9 line. The susceptibility of 'Estrella' to 2.4.5 and of 'Ostona' to 2.5.9 indicate that they do not contain Cf_{11}, neither did the Cf_9 line. Concerning 2.4(.11) and 2.4.5(.11), there seems to be an about equal chance that the virulence gene 11 is present or absent. Apparently, races can easily convert into a race carrying virulence gene 11. The opposite, c.q. the reversion into avirulence gene 11, might also occur. Consequently the value of resistance gene Cf_{11} for practical breeding purposes is very limited.

It is remarkable that new races appear, which combine several existing virulence genes with a new gene. For instance, the new Polish races and the latest appearing Dutch race (2.4.5.9.11) carry a rather complex pattern of virulence genes. As in most cultivars which are currently grown, only one resistance gene is present, there is no strict need for *Cladosporium fulvum* races to combine several virulence genes. The observation that such complex sets of virulence genes are present in one race, implies that the gain in virulence genes does not cause a decrease of fitness. The consequence for practical tomato breeding is the need to introduce new resistance genes, whenever new races with complex virulence genes appear at a large scale. It has been suggested that the use of tomato cultivars with more than one new resistance gene should slow down the appearence of new races with new virulence genes (Laterrot, 1981). It is essential to state that a combination of two new genes is only effective if both genes are not yet overcome by current isolates.

Though presently the tomato production does not encounter unsurmountable problems caused by *Cladosporium fulvum*, this situation might change. For the time being, Cf_6 still confers resistance to all races available. Other resistance genes are available (Kanwar et al., 1980b; Laterrot, 1981) but the level of resistance, conferred by some of these genes, is rather low.

Samenvatting

Verdere identificatie van fysio's van Cladosporium fulvum (Fulvia fulva) op tomaat afkomstig uit Nederland, Frankrijk en Polen

Fysio's van *Cladosporium fulvum*, die de resistentie-genen Cf_2, Cf_4, Cf_5, Cf_8, Cf_9 en Cf_{11} kunnen doorbreken, zijn in Nederland, Frankrijk en Polen opgetreden. Met behulp van een groep genotypen, die de resistentie genen Cf_2 tot en met Cf_{11} dragen, zijn Nederlandse, Franse en enkele nieuwe Poolse isolaten onderzocht op de aanwezigheid van virulentiegenen. Enkele Nederlandse isolaten, eerder aangeduid met 2.4, 2.4.5 en 2.4.5.9, bleken het resistentie-gen Cf_{11} te kunnen doorbreken. Deze moeten daarom aangeduid worden als respectievelijk 2.4.11, 2.4.5.11 en 2.4.5.9.11. In de nieuwe Poolse isolaten werd virulentie gevonden voor Cf_2, Cf_4, Cf_8, Cf_9 en Cf_{11}. Alle fysio's die op genotypen met Cf_4 konden groeien, groeiden ook op genotypen met Cf_8. Daarom kon geen ondersheid gemaakt worden tussen Cf_4 en Cf_8. De Poolse isolaten behoren tot de fysio's 4.11, 2.4.11 en 2.4.9.11. De gevolgen van het voorkomen van deze fysio's voor de tomateveredeling worden besproken.

References

Boukema, I.W., 1981. Races of *Cladosporium fulvum* Cooke (*Fulvia fulva*) and genes for resistance in the tomato (*Lycopersicon* Mill.). Proceedings of the meeting of the Eucarpia Tomato Working Group, Avignon, 18-21 May 1981: 287-292.

Kanwar, J.S., Kerr, E.A. & Harney, P.M., 1980a. Linkage of the Cf_1 to Cf_{11} genes for resistance to tomato leaf mold *Cladosporium fulvum* Cke. Tomato Genetics Cooperative Report 30: 20-21.

Kanwar, J.S., Kerr, E.A. & Harney, P.M., 1980b. Linkage of the Cf_{12} to Cf_{24} genes for resistance to tomato leaf mold *Cladosporium fulvum* Cke. Tomato Genetics Cooperative Report 30: 22-23.

Kanwar, J.S., 1981. Genetics of resistance to leaf mold, *Cladosporium fulvum* Cke. (*Fulvia fulva* (Cke.) Ciferri) in tomato. Dissertation Abstracts International B41: 74005B.

Kerr, E.A., Kerr, E., Patrick, Z.A. & Potter, J.W., 1980. Linkage relation of resistance to *Cladosporium* leaf mold (Cf_2) and root-knot nematodes (Mi) in tomato and a new gene for leaf mold resistance (Cf_{11}). Canadian Journal of Genetics and Cytology 22: 183-186.

Laterrot, H., 1981. Premier bilan de l'étude de l'efficacité envers *Cladosporium fulvum* de 24 nouvelles origines résistantes de tomate. Proceedings of the Meeting of the Eucarpia Tomato Working Group, Avignon, 18-21 May 1981: 293-298.

Laterrot, H., Gerlagh, M., Ester, A. & Stamova, L., 1985. Race 2.5, a new race of *Cladosporium fulvum* (*Fulvia fulva*) on tomato. Netherlands Journal of Plant Pathology 91: 45-47.

Laterrot, H., 1986. Race 2.5.9, a new race of *Cladosporium fulvum* (*Fulvia fulva*) and sources of resistance in tomato. Netherlands Journal of Plant Pathology 92: 305-307.

Leski, B., 1977. Identification of *Cladosporium fulvum* Cooke races from group B and C on tomatoes in Poland. Acta Agrobotanica 30: 181-194.