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Inhibition of different cowpea viruses by non-virulent cowpea mosaic virus is dependent on the type of immunity of the plant to the inhibitory virus

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The interference between two comoviruses, viz. cowpea mosaic virus (CPMV) and cowpea severe mosaic virus (CPSMV) in a cowpea host which was immune or extremely resistant to CPMV, has been reported (Sterk and De Jager, 1987). The authors found that the number of local CPSMV-infections was reduced when CPMV was present in the CPSMV-inoculum and that this occurred only when the inhibiting CPMV or CPMV-RNA, although avirulent in the test plant, was otherwise infectious. This indicated some form of interaction between CPMV and the immune test plant.

Description of the inhibition phenomenon may provide further information about this interaction and, thereby, about the immunity mechanism. One of the aspects of the interference is its specificity, both with regard to test virus and to the type of immunity of the test plant against the inhibitor. In the following study we show that the interference is aspecific with reference to the test virus but dependent on the type of immunity of the test plant.

As earlier (Sterk and De Jager, 1987), we used a CPMV isolate (no 6) from Nigeria as the inhibitor and the Vs-isolate of CPSMV as the standard test virus. Three other test viruses were:

1. Blackeye cowpea mosaic virus (BlCMV), W-isolate, described by Dijkstra et al., 1987;
2. sunn-hemp mosaic virus (ShMV), isolated in our laboratory from an inadvertantly diseased Pinto bean plant, possibly infected through the seed. This isolate was morphologically and serologically identical to a ShMV-isolate maintained in our laboratory in sunn-hemp, but it induced more conspicuous local symptoms than the latter in our test plants.
3. cucumber mosaic virus (CuMV), isolated from Bambarra groundnuts at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Test plants were: cowpea (*Vigna unguiculata* (L.) Walp.) accessions TVu 470 and TVu 1948, obtained from IITA, Ibadan, and cowpea cv. Arlington (PI 293453), kindly provided by the United States Department of Agriculture, Plant Introduction Station, Experiment, Georgia, USA.

Eight to 10 days after sowing the two primary leaves of the test plants were inoculated, one with a 1 : 1 dilution of the test virus preparation with 0.01 *M* phosphate buffer

Table 1. Results of interference tests with four different test viruses on three cowpea genotypes possessing different types of immunity to the inhibitor, cowpea mosaic virus.

Test virus[1] and concentration[2]	Cowpea test plant				
	TVu 470 (dominant immunity)		Arlington (dominant immunity)		TVu 1948 (recessive immunity)
	−UV	+UV	−UV	+UV	−UV
Exp. 1.					
CPSMV (5000×)	100[3]				
BlCMV (10×)	100				
Exp. 2.					
CPSMV (6)	100		100*		
ShMV (0.5, 25)			75*		0*
Exp. 3.					
CPSMV (3)	100		100		
ShMV (0.5, 50)	95		75		0
CuMV (10×)	95*				0*
Exp. 4.					
CPSMV (3)	100*	0	100	0	
BlCMV (10×)	95*	0			
ShMV (0.5, 2.5)			100	0	0
CuMV (10×)	95	0			0

[1] CPSMV = cowpea severe mosaic virus; BlCMV = Blackeye cowpea mosaic virus; ShMV = sunn-hemp mosaic virus; CuMV = cucumber mosaic virus.

[2] Concentrations are given in parenthesis. Figures followed by × are dilution factors of fresh sap from plants infected with the test virus. Other figures give concentrations of purified virus in $\mu g\ ml^{-1}$. For ShMV, the first figure is the virus concentration used on TVu 470 and TVu 1948 and the second one that on cv. Arlington. Concentration of the inhibitor was c.130 $\mu g\ ml^{-1}$ in all tests.

[3] Figures are approximate percentages of inhibition: 100 × (1 − lesion number on leaves with test virus plus inhibitor/lesion number on leaves with test virus only). An asterisk (*) indicates that a representative leaf pair of that particular test is shown in Fig. 1.

alone and the other with a similar dilution of the test virus preparation with the same buffer containing an excess of CPMV. Test virus preparations were either purified virus in phosphate buffer or virus-containing crude plant juice, with final concentrations or dilution factors as mentioned in Table 1.

To enable evaluation of the results, the infection sites in the inoculated leaves were made clearly visible as starch lesions by treatment with iodine, as described earlier (Sterk and De Jager, 1987). No attempt was made to count the starch lesions since inhibition was either complete, unmistakably high, or clearly absent.

Results are summarized in Table 1 and Fig. 1. In all experiments with cowpea TVu 470, there was a complete inhibition of the standard test virus CPSMV, indicating that

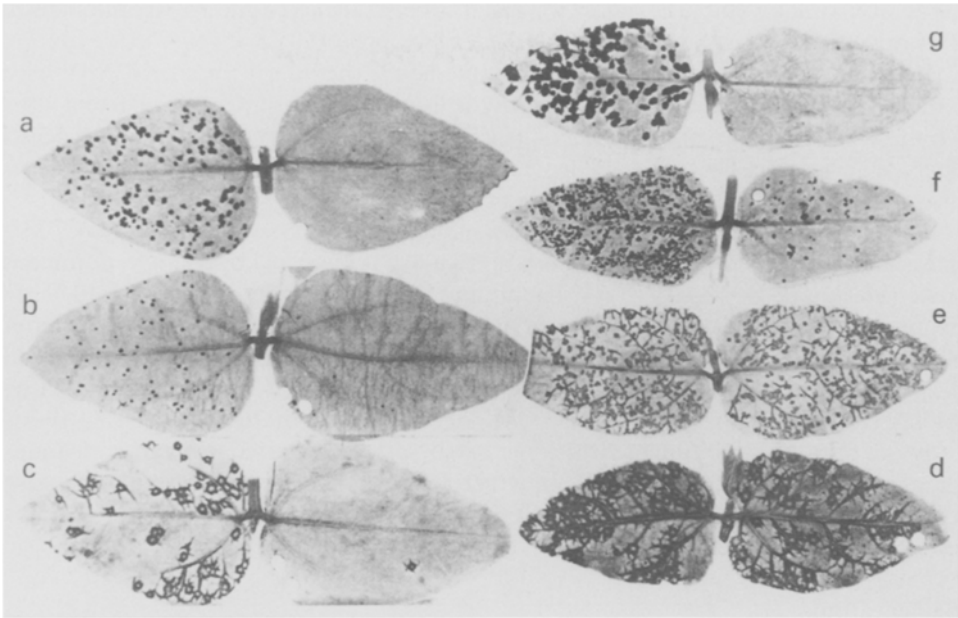

Fig. 1. Representative leaf pairs of interference tests with four different test viruses on three cowpea genotypes, possessing different types of immunity to the inhibitor, cowpea mosaic virus.

Left leaves: inoculum containing test virus only; right leaves: inoculum containing test virus and inhibitor (concentrations of purified virus and dilution factors of sap from infected plants are given in Table 1).

a. Experiment 4: cowpea severe mosaic virus on TVu 470;
b. Experiment 4: Blackeye cowpea mosaic virus on TVu 470;
c. Experiment 3: cucumber mosaic virus on TVu 470;
d. Experiment 3: cucumber mosaic virus on TVu 1948;
e. Experiment 2: sunn-hemp mosaic virus on TVu 1948;
f. Experiment 2: sunn-hemp mosaic virus on cv. Arlington;
g. Experiment 2: cowpea severe mosaic virus on cv. Arlington.

the CPMV preparations used were fully inhibitory. The other three test viruses were almost completely inhibited in this plant.

In cv. Arlington only ShMV and CPSMV could be compared, as the other two viruses gave no (consistent) local reaction. There was a complete inhibition of both CPSMV and ShMV. However, in the latter, it was only so when its concentration was not much different from the former, whereas in other experiments the concentration of ShMV was apparently too high for full inhibition.

In TVu 1948 only ShMV and CuMV induced clear starch lesions. Neither virus showed evidence of any inhibition in this host, although both exhibited a 75-100% inhibition in the other cowpea genotypes.

Experiment 4 included tests in which the inhibitor virus was fully inactivated by a 30-min UV-treatment (Sterk and De Jager, 1987). In the electron microscope, the UV-treated particles appeared to have absorbed more stain than the untreated ones, but their numbers were equal. With all test viruses the UV-treatment of CPMV had abolished

its ability to inhibit (Table 1), suggesting that in all cases the same interference mechanism was operating.

These results show that in CPMV-immune hosts, the interference of CPMV with infection by virulent viruses is aspecific, as the four test viruses belong to different virus groups with widely varying strategies of genome expression and replication. This suggests that the interference does not result from a direct effect of a CPMV-function on a specific stage of the test virus multiplication process.

Results with different test plant genotypes indicate that the type of immunity to CPMV plays an important role. The immunity of TVu 470 is governed by a single, dominant gene (Sterk and De Jager, 1987). The immunities of 'Arlington' and TVu 1948 are reported to be based on one dominant and one recessive gene, respectively (Kiefer et al., 1984; Patel, 1982). We could confirm this using our Nigerian CPMV-isolate. In F2-populations of crosses with susceptible cv. California Blackeye we found healthy: diseased segregation ratios of 251:84 with 'Arlington' and of 76:187 with TVu 1948.

In our tests the inhibition of virulent viruses by CPMV occurred in plants with dominant immunity and was absent in plants with recessive immunity. Obviously, the recessive type of immunity does not allow the CPMV-function to occur which is necessary for interfering with infection by virulent viruses.

Samenvatting

Remming van verschillende virulente 'cowpea'-virussen door niet virulent 'cowpea'-mozaïekvirus is afhankelijk van het type onvatbaarheid van de plant voor het remmende virus

In 'cowpea'-planten die onvatbaar zijn voor het 'cowpea'-mozaïekvirus (CPMV) kan dit virus, mits infectieus, infectie door vier andere, onderling geheel verschillende virussen remmen. Dit vermogen tot aspecifieke remming komt tot uiting in 'cowpea'-planten met onvatbaarheid die bepaald wordt door één dominant gen, maar niet in planten met onvatbaarheid op basis van één recessief gen. De beide typen van onvatbaarheid laten dus in verschillende mate expressie van de genetische informatie van CPMV toe.

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