Isolation of a Mutant of Cowpea Mosaic Virus which is Unable to Grow in Cowpeas

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SUMMARY

Following nitrous acid mutagenesis of cowpea mosaic virus (CPMV) virion RNA, a mutant has been isolated which is able to grow in Phaseolus vulgaris but is unable to grow in the 'Early Red' or 'Blackeye Early Ramshorn' varieties of cowpea (Vigna unguiculata). Dot blot analysis failed to detect virus-specific RNA in 'Blackeye Early Ramshorn' plants which had been inoculated with mutant 7A-4, indicating that no CPMV RNA synthesis had occurred. Supplementation experiments showed that the mutation was located in B RNA.

Cowpea mosaic virus (CPMV) is an isometric, positive-stranded RNA virus with a bipartite genome. The genome RNAs are separately encapsidated and can be readily separated by centrifugation through CsCl gradients (van Kammen, 1968). Both RNAs are required for infection although the larger, B RNA can replicate independently in protoplasts (Goldbach et al., 1980). In infected cells the genome RNAs are translated into polyproteins which are subsequently cleaved into functional polypeptides by at least two virus-coded proteases (Goldbach et al., 1981; Rezelman et al., 1980; Rottier et al., 1980). The smaller M RNA has been shown to code for the coat proteins: the larger B RNA codes for the proteases, the RNA polymerase and a protein covalently bound to the 5' end of the virion RNA, termed VPG (Daubert et al., 1978; Franssen et al., 1982, 1984; Goldbach et al., 1980, 1981; Gopo & Frist, 1977; Pelham, 1979; Stanley et al., 1980; Zabel et al., 1984). In comparison with our knowledge of the structure of CPMV and of the synthesis and processing of virus proteins, little is known about such 'biological' aspects of CPMV as the determinants of host range.

The natural host for CPMV is the cowpea (Vigna unguiculata) in which it can cause a severe reduction in yield (van Kammen & de Jager, 1978). A number of mutants of CPMV have been isolated following nitrous acid mutagenesis of virion RNA. This paper describes the characterization of a mutant which is restricted in its ability to replicate in cowpeas.

The hosts used in this study were two varieties of cowpea and Phaseolus vulgaris vat. 'Pinto'. Wild-type isolate of CPMV results in the appearance of necrotic lesions in the primary leaves. Local lesions in P. vulgaris were the source of inoculum in every experiment and isolates were maintained by local lesion transfer. Inoculation of cowpea, variety 'Early Red', with the wild-type isolation of CPMV results in necrotic lesions in the primary leaves. No systemic symptoms were observed, although de Jager & Wesseling (1981) have isolated spontaneously occurring variants which cause a systemic mosaic and variants causing systemic lethal necrosis. Inoculation of the variety 'Blackeye Early Ramshorn' with wild-type CPMV results in diffuse chlorotic spots on the inoculated primary leaves and a mosaic in the systemically infected secondary leaves.

A stock of the Nigerian isolate of CPMV, obtained from Dr C. P. de Jager, was propagated in the cowpea variety 'Blackeye Early Ramshorn' and purified as described by van Kammen (1967). Virion RNA was purified by the method of Zimmern (1975) and was mutagenized with nitrous acid exactly as described by de Jager (1976). The mutagenized RNA was used to inoculate 'Pinto' leaves, aberrant local lesions were selected and those whose phenotype appeared to be stable through at least four lesion transfers were selected for further study.

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**Short communication**

Table 1. Growth of mutant isolate 7A-4 in *V. unguiculata* var. 'Early Red'

<table>
<thead>
<tr>
<th>Inoculum*</th>
<th>No. of lesions on inoculated leaves (mean of at least five plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7A-4</td>
<td>2</td>
</tr>
<tr>
<td>Wild-type</td>
<td>&gt;450</td>
</tr>
<tr>
<td>Wild-type M component</td>
<td>2</td>
</tr>
<tr>
<td>Wild-type B component</td>
<td>36</td>
</tr>
<tr>
<td>Wild-type M + B component</td>
<td>&gt;450</td>
</tr>
<tr>
<td>7A-4 + wild-type M</td>
<td>6</td>
</tr>
<tr>
<td>7A-4 + wild-type B</td>
<td>364</td>
</tr>
</tbody>
</table>

* Inoculum for each plant was prepared by grinding a single local lesion from an infected 'Pinto' leaf in 0-2 ml buffer. For infection with mutant alone, 0.05 ml homogenate was diluted with 0.05 ml buffer. For supplementation tests, 0.05 ml M or B component was added to 0.05 ml homogenate; 0.1 ml inoculum was applied to each plant.

Table 2. Growth of mutant isolate 7A-4 in *V. unguiculata* var. 'Blackeye Early Ramshorn': infectivity assay of leaf homogenates on *P. vulgaris* var. 'Pinto'

<table>
<thead>
<tr>
<th>Cowpeas inoculated with</th>
<th>Infectivity of leaf extracts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7A-4</td>
<td>0</td>
</tr>
<tr>
<td>7A-4 + wild-type M component</td>
<td>2</td>
</tr>
<tr>
<td>7A-4 + wild-type B component</td>
<td>347</td>
</tr>
<tr>
<td>Wild-type M component</td>
<td>0</td>
</tr>
<tr>
<td>Wild-type B component</td>
<td>185</td>
</tr>
<tr>
<td>Wild-type M + B component</td>
<td>327</td>
</tr>
</tbody>
</table>

* Samples of tissue were removed from 'Blackeye Early Ramshorn' leaves 10 days after inoculation, homogenized in 0.01 M-phosphate buffer pH 7.0, frozen and thawed and used to infect 'Pinto' half-leaves. Figures are means of at least three replicates (de Jager, 1976). Inocula were as for Table 1.

Inoculation of the cowpea variety 'Early Red' with the wild-type strain of CPMV resulted in the production of large numbers (>450 per plant) of small brown lesions, 0.5 to 1 mm in diameter on the primary, inoculated leaves. No systemic symptoms were observed. Inoculation of 'Early Red' with the mutant isolate 7A-4 resulted in no lesions or, in some experiments, a very small number of lesions in the inoculated leaves. As when wild-type virus was used, no systemic symptoms were observed.

These observations indicate that the isolate 7A-4 possesses a mutation that restricts symptom induction in 'Early Red'. In order to determine on which of the RNA components the mutation was located, supplementation experiments were carried out as described by de Jager (1976). 'Early Red' plants were inoculated with 7A-4 alone or with 7A-4 supplemented either with M or with B particles from purified wild-type virus. In no case did any of the plants show systemic symptoms. Lesions produced on the inoculated leaves were counted and the results are shown in Table 1. Purified M and B particles were slightly infective due to cross-contamination but when they were mixed full infectivity was restored. Inoculation with 7A-4 either alone or supplemented with M particles induced only a few lesions, whereas when 7A-4 was supplemented with B particles, large numbers of lesions were induced. This suggests that the mutant isolate 7A-4 possesses a mutation in the B RNA which restricts symptom expression in the 'Early Red' variety of cowpea.

No symptoms were observed when 'Blackeye Early Ramshorn' plants were inoculated with isolate 7A-4. Since it is possible that it is symptom expression rather than virus production which is restricted following inoculation with this mutant, the amount of virus present in plants infected with 7A-4 was determined by infectivity assays on 'Pinto' leaves as described by de Jager (1976). The results obtained are shown in Table 2. No infective virus could be detected following inoculation with isolate 7A-4.
Supplementation tests were carried out to determine which component carries the mutation that restricts the growth of 7A-4 in 'Blackeye Early Ramshorn'. The results are shown in Table 2. Inoculation with M particles alone did not result in the production of infective virus. A considerable amount of virus was produced in plants inoculated with B particles, presumably due to residual contamination with M particles, although less than was produced following inoculation with a mixture of M and B particles. Supplementation of the 7A-4 inoculum with M particles did not result in the production of large amounts of virus, whereas supplementation with B particles resulted in the production of more virus than did inoculation with B particles alone, and in a similar amount to that produced following inoculation with a mixture of M and B particles. Thus, the mutation restricting the multiplication of 7A-4 in 'Blackeye Early Ramshorn' is located in the B component.
Although infective virus was not produced following inoculation of 'Blackeye Early Ramshorn' with 7A-4, it was decided to investigate whether any virus-specific RNA was produced. 'Blackeye Early Ramshorn' leaves were inoculated with either 7A-4, 7A-4 supplemented with wild-type M or B components, or with wild-type virus. As controls, leaves were inoculated with purified M, B or M plus B particles. Six days post-inoculation, samples of tissue were removed with a no. 10 cork borer and homogenized in 0·5 ml 0·01 M-phosphate buffer pH 7. A dilution series of the homogenates was applied to a sheet of nitrocellulose which was baked, and then probed with nick-translated replicative forms of phage M13 clones which contained sequences specific either to B or to M component RNA (Lomonossoff & Shanks, 1983; G. P. Lomonossoff, personal communication). As can be seen from Fig. 1, no virus-specific RNA could be detected in leaves inoculated with either 7A-4 alone or with 7A-4 supplemented with wild-type M component. However, virus-specific RNA was detected following inoculation with 7A-4 which had been supplemented with wild-type B component. Thus, the mutant isolate 7A-4 has a defect, located in the B component, that restricts its ability to produce virus-specific RNA in cowpea 'Blackeye Early Ramshorn' leaves. No virus-specific RNA was detected when plants were inoculated with B component alone, although in the infectivity assay infective virus was detected. This apparent discrepancy is probably because the dot blot assay was carried out 6 days after inoculation and the infectivity assay 10 days after inoculation. The amount of virus produced as a result of contaminating M particles was not detectable 6 days post-inoculation. Also, there was considerable variation between experiments in the amounts of infective virus produced following inoculation with B particles alone.

These findings indicate that a virus-coded gene, located in B component RNA, is involved in determining the host range of CPMV. A defect in this gene restricts growth in cowpeas but not growth in P. vulgaris var. 'Pinto'. The B component has been shown to code for the virus-coded polypeptide of the RNA replication complex, VPg, two virus-specific proteases and a polypeptide of 58000 molecular weight, of unknown function (Franssen et al., 1984; Zabel et al., 1984). It is possible that isolate 7A-4 has a defect in the 58000 mol. wt. polypeptide which restricts its growth in cowpeas. Another possibility is that there is a defect in the 110000 mol. wt. polypeptide which is the virus-encoded component of the RNA replication complex (Dorssers et al., 1984). This defect would prevent the correct interaction with the cowpea-coded polypeptides whilst allowing a functional RNA replication complex to form in P. vulgaris var. 'Pinto'.

Another possibility is that the mutant is defective in a gene which is responsible for the cell-to-cell spread of the virus in the infected cowpea plant. If this is the case, then the mutant should replicate in infected cowpea protoplasts.

I should like to thank Dr G. P. Lomonossoff for much helpful advice and comment and for critically reading the manuscript. I should also like to thank Dr C. P. de Jager for advice on mutant isolation techniques and for the provision of virus stocks. I thank Dr J. W. Davies for the provision of facilities in his department. During the course of this work I was in receipt of a John Innes Charity Fellowship.

REFERENCES


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