Expression of Potato Virus X Resistance Gene Rx in Potato Leaf Protoplasts

By SALLY E. ADAMS,† R. A. C. JONES‡ and R. H. A. COUTTS*

Department of Pure and Applied Biology, Imperial College of Science and Technology, Prince Consort Road, London SW7 2BB, U.K.

(Accepted 1 August 1986)

SUMMARY

Protoplasts derived from shoot cultures of potato cv. Cara, which carries immunity gene Rx, supported only limited virus multiplication after inoculation with particles or RNA of isolate DX, a group 3 strain of potato virus X, as compared to similarly inoculated protoplasts of cultivars King Edward and Pentland Ivory which lack Rx. The Cara protoplasts were, however, able to support extensive replication of the resistance-breaking strain HB. This strain-specific resistance did not appear to be mediated by a failure or inhibition of the uncoating mechanism or of virus assembly.

INTRODUCTION

Genetically controlled resistance that decreases or prevents replication and symptom expression is of obvious value in the control of economically important plant virus diseases. Although potato breeders have incorporated resistance genes into potato clones and cultivars, little is known about the products of these genes or how they exert an influence over the virus or its effects at the molecular level (Harrison, 1980; Barker & Harrison, 1984).

Valuable information has been obtained using plant protoplasts in the study of the biochemical basis of resistance to viruses. Hypersensitivity was not expressed in isolated protoplasts, suggesting that it is a tissue-related phenomenon and that cell to cell contact is required for its expression (Otsuki et al., 1972; Motoyoshi & Oshima, 1977; Koike et al., 1977; Gonda & Symons, 1979; Oldfield & Coutts, 1980; Barker & Harrison, 1984; Adams et al., 1985). Complete resistance of plants to virus infection also appeared not to function in some virus–plant protoplast combinations (Huber et al., 1977; Furusawa & Okuno, 1978). However, there are instances in which resistance does seem to occur at the cellular level. Thus, protoplasts isolated from certain cultivars of tomato, cucumber, cowpea and potato have been shown to be at least partially resistant to infection with certain strains of tobacco mosaic virus (Motoyoshi & Oshima, 1977, 1979), cucumber mosaic virus (CMV) (Coutts & Wood, 1977; Maule et al., 1980), cowpea mosaic virus (CPMV-SB) (Kiefer et al., 1984; Sanderson et al., 1985) and potato virus Y (PVY) (Barker & Harrison, 1984) respectively.

The hypersensitive response to infection with potato virus X (PVX) in potato plants is determined by two dominant genes, Nx and Nb (Cockerham, 1955, 1970). A third gene, Rx, confers extreme resistance (immunity) to all strains of PVX so far tested (Cockerham, 1970; Jones, 1985) except a resistance-breaking strain HB, from South America (Moreira et al., 1980). Although Foxe & Prakash (1986) report otherwise, we have previously found that gene Nx, which determines a hypersensitive response to group 3 strains of PVX, is not phenotypically expressed in potato protoplasts (Adams et al., 1985). The aim of this work was to discover whether the resistance conferred by gene Rx was expressed at the single cell level and if so, at what stage of the infection process the resistance was operating.

† Present address: Department of Biochemistry, Oxford University, South Parks Road, Oxford OX1 3QU, U.K.
‡ Present address: Plant Pathology Branch, Department of Agriculture, Baron-Hay Court, South Perth 6151, Western Australia.

0000-7253 © 1986 SGM
Table 1. Protoplast infection with isolate DX RNA*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Cultivar</th>
<th>Protoplasts fluorescing (%)†</th>
<th>Virus/10⁶ viable protoplasts (µg)‡</th>
<th>Virus/infected protoplast (pg)‡</th>
<th>Viable protoplasts (%)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>King Edward</td>
<td>70</td>
<td>10–16</td>
<td>14–23</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Cara</td>
<td>10</td>
<td>1–1.5</td>
<td>10–15</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>Pentland Ivory</td>
<td>70</td>
<td>9–11</td>
<td>13–16</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Cara</td>
<td>14</td>
<td>1–2</td>
<td>7–14</td>
<td>56</td>
</tr>
</tbody>
</table>

* All determinations were carried out at 40 h post-inoculation.
† Determined by fluorescent antibody staining.
‡ Determined by ELISA.
§ Assessed using phenoasafarine by the method of Widholm (1972).

METHODS

Plant material and virus inocula. Shoot cultures of potato (Solanum tuberosum L. ssp. tuberosum) cultivars King Edward (Nx:nb), Pentland Ivory (nx: Nb) and Cara (Rx) were established and maintained as previously described (Adams et al., 1985). Chenopodium amaranticolor plants, the assay host for PVX, were grown in potting compost (John Innes No. 2) in a glasshouse at 16 to 25 °C with supplementary illumination from mercury vapour lamps giving 7000 to 8000 lx at pot level for 16 h/day. Particles of PVX isolate DX, a group 3 strain of PVX (Jones, 1985) and isolate HB (Moreira et al., 1980) were purified and RNA was extracted from them as described previously (Adams et al., 1985).

Protoplast isolation, inoculation and culture. Methods used for the isolation of potato protoplasts from shoot cultures and their inoculation with 50 µg DX nucleoprotein or 100 µg RNA per 2 x 10⁶ protoplasts were as described by Adams et al. (1985). An identical procedure was employed for the inoculation of 2 x 10⁶ potato protoplasts with 100 µg HB RNA. Mock inoculations were performed in the absence of virus or RNA. After inoculation, protoplasts were incubated in Gamborg's B5 medium (Flow Laboratories) containing 0.4 M-mannitol, 200 µg/ml carbenicillin (Beecham Pharmaceuticals) and 75 units/ml nystatin (Squibb, Princeton, N.J., U.S.A.). Protoplasts were incubated at 20 °C in continuous fluorescent light at 2000 lx for 40 h or exceptionally for 70 h.

Assessment of protoplast infection. Protoplast viability was determined by staining with phenoasafarine as described by Widholm (1972). Fluorescent antibody staining of infected protoplasts, estimation of virus content by ELISA and infectivity assays were as described by Adams et al. (1985). Western blotting was done with a monoclonal antibody to PVX (MAC 72), provided as a tissue culture supernatant by Dr L. Torrance, MAFF, Harpenden, U.K., which detects isolates DX and HB (Torrance et al., 1986). Protoplast samples were prepared essentially as described by Nassuth (1982). The extracted proteins were separated by discontinuous SDS–PAGE in 10 % gels (Laemmli, 1970), transferred to nitrocellulose membranes (Towbin et al., 1979) and the capsid detected using MAC 72 as the first antibody and an anti-rat antibody conjugated to horseradish peroxidase as the second antibody. The enzymic reaction was developed as described by De Blas & Cherwinski (1983).

RESULTS

PVX infection of potato protoplasts with and without gene Rx

Protoplasts of cv. Cara (Rx) inoculated with isolate DX RNA were compared with similarly inoculated protoplasts of cvs. King Edward and Pentland Ivory (Table 1). The lesser accumulation of progeny virus, as assessed by fluorescent antibody staining and ELISA, indicated that the resistance of Cara to DX functions in protoplasts. The reproducibility of these observations was confirmed by repeating these experiments several times (data not shown). Furthermore, similar results were obtained using DX virus particles as the inoculum; for example, in one experiment, protoplasts of King Edward had accumulated 9 to 17 µg virus per 10⁶ viable protoplasts 40 h post-inoculation, whereas only 1 µg virus per 10⁶ protoplasts could be detected in similarly inoculated Cara protoplasts. The levels of infection were 50 % and approximately 5 % for King Edward and Cara protoplasts respectively. However, estimation of the number of infected Cara protoplasts was difficult in all cases as the specific fluorescence of these protoplasts was always considerably less intense than that of infected King Edward or Pentland Ivory protoplasts. Thus, although the amount of virus per infected protoplast calculated in Table 1 is similar for Cara, King Edward and Pentland Ivory, the weaker staining of Cara protoplasts indicated that many Cara protoplasts must contain insufficient antigen to stain.
Fig. 1. Time course of accumulation of isolate DX capsid protein, as monitored by ELISA, in King Edward protoplasts inoculated with DX RNA (○) or DX particles (●), and in Cara protoplasts inoculated with DX RNA (□) or DX (■).

Fig. 1 shows a time course of accumulation of virus antigen as determined by ELISA. Cara protoplasts accumulated 10% or 15% of the amount of antigen that accumulated in King Edward protoplasts inoculated with DX RNA or DX virus particles respectively after 48 h. The amounts of virus antigen that accumulated in DX- or DX RNA-inoculated Cara protoplasts remained low even after 70 h of culture (data not shown). Thus, it is unlikely that a susceptible but delayed reaction occurred. The synthesis of capsid protein in DX RNA-inoculated Cara and King Edward protoplasts was also investigated by Western blotting which again demonstrated that less virus antigen was present in Cara than King Edward protoplast extracts (data not shown).

The infectivity extractable from Cara and Pentland Ivory protoplasts 40 h after inoculation with DX RNA (Table 1, expt. 2) was assayed by inoculation to six leaves of C. amaranticolor. Extracts of 12 500 protoplasts from Cara and Pentland Ivory in 0.1 ml of 0.1 M-sodium phosphate buffer (pH 7.0) induced an average of five and 148 lesions/leaf respectively. Thus, there was a 30-fold difference between the numbers of local lesions induced by the two extracts. Interpolation on a graph of log₁₀ lesion number against concentration gave estimates of 0.2 and 7.0 µg virus per 10⁶ viable protoplasts for Cara and Pentland Ivory respectively. These values were comparable with those obtained by ELISA and indicated that the amounts of viral antigen detected by ELISA corresponded to the amounts of infectious virus particles.

Protoplast viability after inoculation with PVX

The percentage of viable protoplasts (Table 1) decreased after inoculation with either DX or DX RNA. Although the rate at which deterioration occurred varied between experiments, no marked differences were observed between Cara, King Edward or Pentland Ivory protoplasts.
Binding of PVX virions to potato protoplasts

The binding of DX virions to King Edward and Cara protoplasts was compared by assaying protoplasts by ELISA immediately after inoculation and washing. In three separate experiments, King Edward and Cara protoplasts bound 0.6 to 1.0 and 0.7 to 1.0 μg virus particles per 10^6 protoplasts respectively. Thus, no significant differences between the two cultivars were detected.

Ability of Cara protoplasts to replicate strain HB

In order to demonstrate that Cara protoplasts are metabolically capable of supporting virus multiplication, they were inoculated with RNA extracted from particles of strain HB. Some 50% of the protoplasts became infected and the yield of progeny virus was 4.5 to 12.5 μg/10^6 viable protoplasts.

DISCUSSION

Although the hypersensitive response controlled by gene Nx does not function at the single cell level (Adams et al., 1985) we found that the extreme resistance conferred by Rx did. Protoplasts from Cara inoculated with DX always produced substantially less progeny virus than similarly inoculated protoplasts of King Edward and Pentland Ivory. However, some replication of isolate DX did occur in the Cara protoplasts. In contrast, in whole plants of Cara no infection was detected in leaves after sap- or graft-inoculation with DX (Jones, 1985).

Extensive replication of virus in a few cells, but to a limited extent as compared to the potential if accumulation occurs in all of the cells, has been reported in some virus–host systems (Sulzinski & Zaitlin, 1982). The amount of virus per stained Cara protoplast calculated in Table 1 might suggest that a few cells supporting extensive virus replication could account for the DX accumulation noted. This appears to be the case in PVY-infected potato protoplasts carrying gene Ry, where a few brightly fluorescing cells were seen (Barker & Harrison, 1984). However, the fluorescence observed in Cara protoplasts was of a generally low to medium intensity, and was always less intense than that of infected King Edward or Pentland Ivory protoplasts. It would therefore seem likely that other Cara protoplasts also contained virus antigen, but at too low a concentration to allow visual detection. Thus, the protoplasts from Cara may not vary from those of King Edward or Pentland Ivory in their ability to resist infection but only in their ability to resist virus replication. This situation would resemble that reported for CMV in cucumber (Maule et al., 1980) and CPMV-SB in cowpea protoplasts (Kiefer et al., 1984). However, it is not possible to measure directly either the intensity of stained Cara protoplasts or the number of protoplasts which might contain antigen but not be stained. Thus, we cannot exclude the former possibility that protoplasts which contain gene Rx are able to resist both infection and virus replication.

No differences were found in the ability of DX particles to bind to Cara or to King Edward protoplasts. This suggests that the resistance present is not a result of differing requirements for efficient inoculation. Furthermore, as Cara protoplasts were able to support extensive cytoplasmic replication of HB after inoculation with HB RNA, it is unlikely that these protoplasts were metabolically deficient or that the restriction of DX replication is PVX-specific.

There are obviously several stages in the virus replication cycle at which resistance could be operating. However, the decrease in multiplication found in DX RNA-inoculated Cara protoplasts indicates that the resistance is unlikely to be due to failure or inhibition of the uncoating mechanism. Inhibition of virus assembly is also unlikely to be the operational step in resistance of Cara protoplasts to DX. Infectivity assays demonstrated clear differences between the amounts of infectious DX that accumulated in Cara as compared to Pentland Ivory protoplasts and that the viral antigen detected by ELISA was assembled into functional, infectious virus particles. Therefore, as previously suggested for CMV in cucumber (Maule et al., 1980) and CPMV-SB in cowpea protoplasts (Kiefer et al., 1984; Sanderson et al., 1985), the resistance mechanism operating in Cara protoplasts probably affects virus replication during transcription and/or translation.
Resistance of potato leaf protoplasts to PVX

We thank Beecham Pharmaceuticals PLC for a gift of carbenicillin. During these studies S.E.A. was in receipt of an SERC-CASE award in conjunction with the MAFF, Harpenden Laboratory, Hatchings Green, Harpenden, Herts., where the virus purifications were done. Work on PVX isolate HB was done under licence No. PHF 29A/143.

REFERENCES


SULZENSKY, M. A. & ZAITLIN, M. (1982). Tobacco mosaic virus replication in resistant and susceptible plants: in some resistant species virus is confined to a small number of initially infected cells. Virology 121, 12–19.


(Received 19 May 1986)