Infection of Protoplasts from Chenopodium quinoa with Cowpea Mosaic and Cymbidium Ringspot Viruses

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SUMMARY

Mesophyll protoplasts were prepared from Chenopodium quinoa plants and infected with cowpea mosaic virus (CPMV) or cymbidium ringspot virus (CyRSV) using inocula containing either polyethylene glycol or poly-L-ornithine. The level of infection was estimated by fluorescent antibody staining and by spot hybridization assay. About 25% of the protoplasts became infected with CPMV and about 35% with CyRSV. Yields of progeny RNA were about 700 ng of CPMV RNA and 3 μg of CyRSV RNA per 10⁶ protoplasts. Cytopathic structures found in C. quinoa protoplasts inoculated with CPMV or CyRSV were similar to the structures found in CPMV-infected cowpea protoplasts or in CyRSV-infected C. quinoa plants respectively.

Protoplasts from several plant species can be infected with plant viruses (for review, see Harrison & Mayo, 1983; Takebe, 1983). Although there is one report of infection of Chenopodium hybridum protoplasts with brome mosaic virus (Okuno & Furusawa, 1979), to our knowledge no attempt has yet been made to exploit Chenopodium quinoa, a well known host for many plant viruses, for protoplast work. We studied the possibility of obtaining protoplasts from this species, maintaining them in culture and infecting them with the comovirus cowpea mosaic (CPMV) and the tombusvirus cymbidium ringspot (CyRSV).

C. quinoa plants were grown in a peat-vermiculite mixture in a glasshouse at 25 °C. During winter, natural light was supplemented by 16 h illumination with 400 W sodium lamps giving 8 klx or 100 μE/m²/s. Plants suitable for protoplast isolation were approximately 2 weeks old, with five or six leaves. Only middle leaves, 2-5 to 3 cm long, were used. The leaves were surface-sterilized by briefly rinsing in 70% ethanol and immersing in a 5% solution of commercial bleach with 0.03% ‘Hederol’ detergent for 10 min, followed by three washes in sterile distilled water. The lower epidermis was removed by peeling. Tissue was plasmolysed in 0.6 M-mannitol for 30 min and then digested with 2% Cellulase Onozuka R-10 (Kinki Yakult Manuf. Co.), 0.1% Macerozyme R-10 (Kinki Yakult Manuf. Co.) in 0.6 M-mannitol, pH 5.5, at 30 °C in a shaking water-bath at 60 excursions/min. Although after 3 h the leaves appeared digested and the resulting isolated protoplasts became infected upon inoculation, these protoplasts tended to stick irreversibly to the incubation vials during culture. This was prevented by digesting leaves for 5 to 6 h. The protoplasts were separated from tissue debris by filtration through a 66 μm nylon mesh and washed three times with 0.6 M-mannitol. Protoplast yields were from 4 × 10⁶ to 6 × 10⁶ protoplasts per gram of tissue.

Freshly prepared protoplasts (Fig. 1a) were inoculated with CPMV or CyRSV using polyethylene glycol (PEG) as described by Maule (1983) and 20 μg of virus per 10⁶ protoplasts. Protoplasts were washed three times with 0.6 M-mannitol containing 10 mm-CaCl₂ and cultured at 3 × 10⁵ to 5 × 10⁶ cells/ml of incubation medium (Rottier et al., 1979) at 25 °C with continuous lighting at 2.5 klx. Protoplast viability, assessed using phenosafranine (Widholm, 1972), had dropped to 80 to 85% after 48 h and to 50 to 55% after 66 h. After longer periods of

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incubation the protoplasts were extensively clumped. Virus infection was assessed by indirect fluorescent antibody staining (Maule et al., 1980) and by spot hybridization (Thomas, 1980).

*C. quinoa* protoplasts could also be infected with CPMV and CyRSV when poly-L-ornithine (PLO) was included in the inocula. Under the conditions tested (1 µg/ml PLO, 10 µg/ml virus and 10 mM-potassium citrate, pH 5.2, in 0.6 M-mannitol), the proportion of protoplasts infected using inocula containing PLO was similar to that using inocula containing PEG. Generally, the PEG method was used in preference because of its simplicity.

The proportion of protoplasts stained with fluorescent antibody increased from 1 to 5% 12 h post-inoculation to about 25% 36 to 60 h post-inoculation for CPMV, and from 15% at 12 h to about 35% 48 to 60 h post-inoculation for CyRSV. The appearance of the fluorescence in the
stained protoplasts differed strikingly between infections by the two viruses. Fluorescing material in protoplasts infected with CyRSV was diffusely spread in the cytoplasm forming a network between the chloroplasts (Fig. 1b). In protoplasts infected with CPMV, fluorescent masses were localized in discrete areas of the cytoplasm (Fig. 1c) and only occasionally, late in infection, was the fluorescence distributed throughout the cytoplasm as described for CPMV-infected cowpea protoplasts by Hibi et al. (1975).

RNA was extracted from infected protoplasts disrupted in the presence of 5% Sarkosyl (Sigma) in 40 mM-Tris–HCl, 80 mM-NaCl, 4 mM-EDTA, pH 8.5, by treatment with a buffer-saturated mixture of phenol and chloroform (8:1, v/v) and recovered by ethanol precipitation. When RNA was spotted onto nitrocellulose filters (Thomas, 1980) and probed with 32P-labelled cDNA (Maule et al., 1983) prepared to each viral RNA by random priming (Taylor et al., 1976), it was observed that the accumulation of viral RNA paralleled the accumulation of virus antigen (as assessed by fluorescent antibody staining). For quantification of viral RNA, the radioactive areas were cut from the nitrocellulose filters, assayed by scintillation counting, and compared with blots of known quantities of purified RNA. As shown in Fig. 2, CPMV-infected protoplasts contained about 700 ng of progeny viral RNA per 10⁶ protoplasts and CyRSV-infected protoplasts, more than 3 μg of progeny RNA per 10⁶ protoplasts. The molecular weight distribution of virus RNA detected in this assay was assessed using Northern blots (Thomas, 1980). RNA extracted from protoplasts was denatured with 70% (w/v) formamide in the presence of 1 mM-glyoxal (Covey et al., 1983) and electrophoresed in 1% agarose gels. After electrophoresis the gels were blotted onto nitrocellulose filters and probed with 32P-labelled cDNA. Both species of CPMV RNA (B-RNA and M-RNA) were detected in RNA from CPMV-infected protoplasts (Fig. 3a); a major species, corresponding to the genomic RNA, was detected in RNA from protoplasts infected with CyRSV (Fig. 3b), together with smaller species whose significance is at present under investigation (D. Gallitelli, personal communication).

Samples of healthy and infected protoplasts were collected by centrifugation, resuspended in 4% glutaraldehyde in 0.05 M-phosphate buffer pH 7.0, containing 0.6 M-mannitol, and processed for electron microscopy essentially as described by Martelli & Russo (1981). Thin sections of CPMV-infected protoplasts (Fig. 1d) showed the presence of the cytopathic structure typical of infections by this virus both in cowpea mesophyll tissue (De Zoeten et al., 1974) and protoplasts (Hibi et al., 1975). In thin sections of CyRSV-infected protoplasts (Fig. 1e)
peroxisomes appeared to have been transformed into multivesicular bodies (MVB), a characteristic cytopathological feature of infections by this virus (Russo et al., 1983). Also in these protoplasts, a small proportion of the mitochondria contained fibril-containing vesicles between the two mitochondrial membranes (Fig. 1e, inset), a feature peculiar to CyRSV infection of C. quinoa, also found in leaf tissue, but not in several other plants infected with the same virus (A. Di Franco & M. Russo, unpublished results).

In conclusion, we have shown that protoplasts from C. quinoa can be used for virus infection studies, including those of CyRSV for which C. quinoa is a local lesion host. C. quinoa protoplasts are easily produced and are stable. They look promising as an experimental system to be tried with other viruses belonging to different groups.

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REFERENCES


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