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Reversible Change in the Nucleoprotein Composition of Bromoviruses after Multiplication in *Chenopodium hybridum* L.

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SUMMARY

Brome mosaic virus (BMV) and cowpea chlorotic mottle virus (CCMV), propagated in two varieties of *Chenopodium hybridum* L., showed a considerable change in the relative proportions of the RNA and nucleoprotein components, as compared to virus propagated in *Hordeum vulgare* and *Vigna unguiculata* respectively. The reversible change was independent of the type of infection in *C. hybridum*: local necrotic, local chlorotic or systemic.

Brome mosaic virus (BMV) causes small chlorotic lesions in the inoculated leaves of the green variety of *Chenopodium hybridum* L. which has green stems and petioles (Kassanis & Lebeurier, 1969), vein yellowing and chlorosis of systemically infected older leaves and yellow mottling accompanied by leaf distortion of systemically infected younger leaves. Cowpea chlorotic mottle virus (CCMV) causes only chlorotic lesions in the inoculated leaves of the green variety of *C. hybridum* L., but no systemic symptoms. The lesions obtained with CCMV are of the same size as with BMV.

Both BMV and CCMV cause only necrotic local lesions in the inoculated leaves of the purple variety of *C. hybridum* L., which has purple stems and petioles (Rochow, 1959; Hiebert, Bancroft & Bracker, 1968), but no systemic symptoms. No virus infectivity could be recovered from leaves of the purple variety without symptoms. BMV and CCMV could be isolated from all leaves carrying symptoms using either the purple or the green variety.

BMV and CCMV were isolated from leaves, inoculated 5 to 6 days previously of the green and the purple *C. hybridum* varieties by precipitation with polyethylene glycol (PEG) and differential centrifugation (Verduin, 1978). About 1 to 5 mg of BMV and CCMV were obtained from 100 g samples of the purple variety and about 10 to 50 mg of BMV and CCMV were obtained from 100 g samples of the green variety. Virus was isolated similarly from systemically infected leaves of the green variety 14 days after infection (60 mg/100 g wet weight tissue). Plants were kept in a greenhouse at 22 °C with additional illumination during winter. The experiments were carried out over a period of 2 years. The stock isolates of BMV and CCMV were passed through local lesions just before starting the experiments and this was repeated after 1 year. No change in lesion type and systemic symptoms were observed.

To analyse the virus RNA, purified BMV and CCMV particles were dissociated in 2% (w/v) SDS, 10% (v/v) glycerol, 1 mM-EDTA and then subjected to electrophoresis in 2·6% (w/v) polyacrylamide gels at 60 °C (Verduin, 1978).

In Fig. 1 the RNA patterns of BMV and CCMV isolated from the green *C. hybridum* varieties are compared with those obtained with BMV isolated from barley (*Hordeum vulgare* L. var. Cambrinus) and with CCMV isolated from cowpea (*Vigna unguiculata* (L) Walp. var. Blackeye Early Ramshorn) and treated in the same way. All virus preparations contained the four RNA species, designated RNA-1, -2, -3 and -4, according to decreasing mol. wt. and characteristic in the bromoviruses (for review see Lane, 1974). There was a

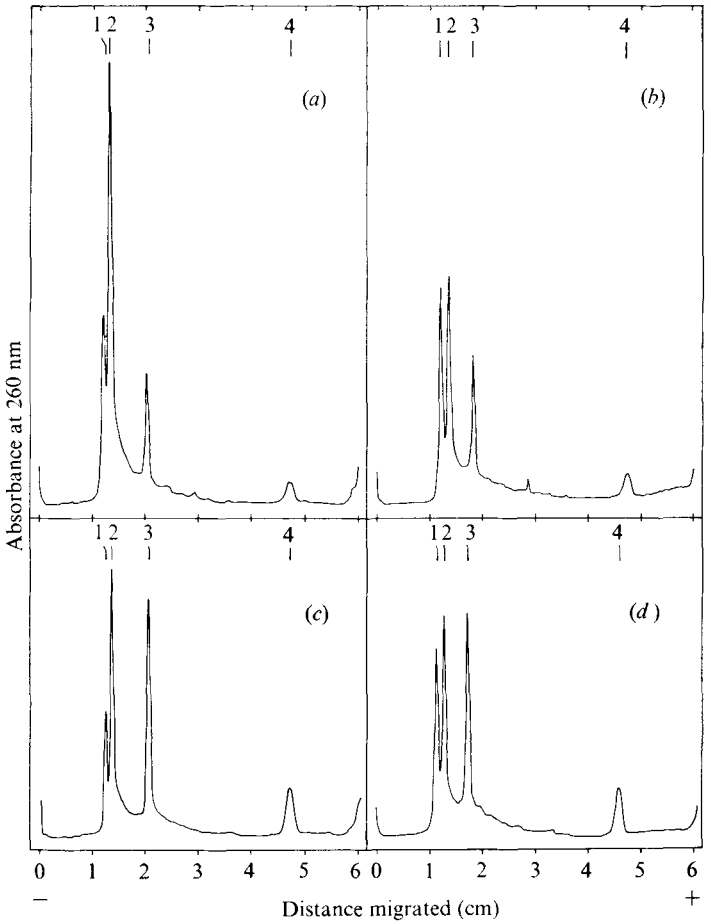

Fig. 1. Polyacrylamide gel electrophoresis patterns of BMV-RNA and CCMV-RNA obtained from virus isolated from different hosts: (*a*) BMV from *Chenopodium hybridum* L., green variety; (*b*) CCMV from *Chenopodium hybridum* L., green variety; (*c*) BMV from *Hordeum vulgare* L. var. Cambrinus; (*d*) CCMV from *Vigna unguiculata* (L.) Walp var. Blackeye Early Ramshorn. Forty mg of virus were dissociated in 2% (w/v) SDS, 10% (v/v) glycerol and 1 mM-EDTA and applied to each 2·6% (w/v) polyacrylamide gel. Electrophoresis was performed for 4 h at 5 V/cm (4 mA/gel) at 60 °C (Verduin, 1978). The positions of the RNAs are indicated by the numbers 1, 2, 3 and 4.

striking difference between the RNA patterns of BMV isolated from *C. hybridum* (Fig. 1*a*) and those of BMV from barley (Fig. 1*c*). The relative amounts of RNA-3 and -4 were less by half in BMV from *C. hybridum* than in BMV from barley. A similar difference was found with CCMV of which the relative amounts of RNA-3 and -4 were less by one third in virus from *C. hybridum* (Fig. 1*b*) than in virus from cowpea (Fig. 1*d*). No difference was found between RNA patterns of isolates from either green or purple varieties of *C. hybridum*.

The change in the relative proportions of the encapsidated RNA species in *C. hybridum* appeared to be independent of the length of the infection period (1 and 2 weeks) at 22 °C. However, the proportions could be changed by varying the temperature during infection. At a lower temperature (15 °C) more encapsidated RNA-(3+4) was found and at a higher temperature (30 °C) less.

The RNA patterns of BMV and CCMV from *C. hybridum* show a striking resemblance with the RNA pattern of broad bean mottle virus (BBMV), another member of the bromo-

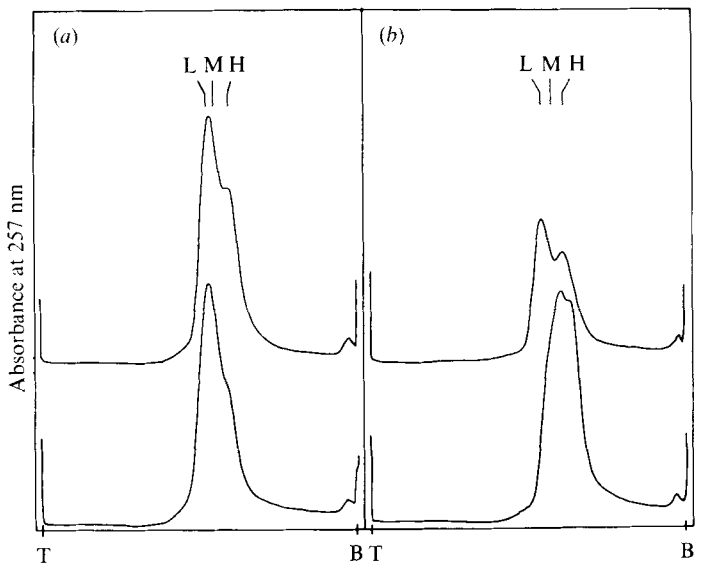

Fig. 2. Absorption patterns of the nucleoprotein distribution in 38 % (w/w) RbCl gradients after equilibrium centrifugation of virus isolated from different hosts: (*a*) lower curve, BMV from *Hordeum vulgare* L. var. Cambrinus; upper curve, BMV from *Chenopodium hybridum* L., green variety; (*b*) lower curve, CCMV from *Vigna unguiculata* (L.) Walp var. Blackeye Early Ramshorn; upper curve, CCMV from *Chenopodium hybridum* L., green variety. Virus (400 μg) was centrifuged for 48 h at 40000 rev/min and 5 °C in a Beckman SW 50.1 rotor. The absorbance of the contents of the tube was monitored at 257 nm and the contents were fractionated. From the refractive index, determined at 25 °C, densities were calculated, using the equation of Ifft, Martin & Kinzie (1970). The positions of the nucleoprotein particles are indicated by L (light), M (medium density) and H (heavy). T = top and B = bottom of the tube. ρ_{av} BMV = 1·337 g/ml and ρ_{av} CCMV = 1·346 g/ml.

virus group, isolated from *Vicia faba* (Lane, 1974). Such BBMV-RNA preparations contain concentrations of RNA-3 and -4 comparable to those in RNA of BMV and CCMV isolated from *C. hybridum*.

RNA-3 and -4 of the bromoviruses are encapsidated in one particle while the other two RNAs are encapsidated separately (Lane, 1974). All three particles have the same protein coat and can be distinguished by their different buoyant densities. The light (L) and medium dense (M) particles of BMV from barley, containing RNA-2 and RNA-(3+4) respectively have similar buoyant densities and are visualized as one band in a 38 % (w/w) RbCl density gradient after equilibrium centrifugation for 48 h at 40000 rev/min and 5 °C (Fig. 2*a*, lower curve). The heavy particles (H) containing RNA-1 appear as a shoulder of the main peak. With CCMV from cowpea two peaks H and M and a shoulder L are visible (Fig. 2*b*, lower curve). The loss of RNA-3 and -4 in virus particles purified from *C. hybridum* correlates well with the loss of medium dense particles from the RbCl density gradient pattern (Fig. 2*a* and *b*, upper curves). In the nucleoprotein distribution patterns of BMV and CCMV obtained from *C. hybridum*, both L and M peaks appear more pronounced. The average buoyant densities of the different nucleoprotein preparations in RbCl (ρ_{av} BMV = 1·337 g/ml, ρ_{av} CCMV = 1·346 g/ml) remained unchanged and were independent of the virus isolate.

Re-isolation of CCMV, previously isolated and purified from cowpea and which had been added to the leaf homogenate of healthy *C. hybridum*, did not alter the relative RNA or nucleoprotein proportions. This result indicates that the change in the relative proportions

of the RNAs and the nucleoproteins is not due to an isolation artefact. Alterations of RNA component ratios due to extraction techniques were overcome using methods described by Verduin (1978).

Propagation of CCMV and BMV isolates from *C. hybridum* again in cowpea and barley respectively, restored the original RNA and nucleoprotein compositions, which have remained constant for several years upon serial transfer in the same host type.

The standard isolates of BMV and CCMV contain almost equal numbers of the nucleoprotein components L, M and H, which are all necessary for infectivity. The specific infectivities of virus purified from *C. hybridum* were comparable to the infectivities found with virus from barley or cowpea. This rather puzzling result is in contrast to the results obtained upon degradation of RNA-2, where a decrease in the concentration of RNA-2 relative to RNA-1 and -3 greatly decreased specific infectivity (Verduin, 1978).

The results clearly indicate that multiplication of BMV and CCMV in *C. hybridum* induced a reversible change in the relative proportions of the RNA and nucleoprotein components, independent of the type of infection: local necrotic, local chlorotic or systemic. The selection of a strain like the *Lolium* isolate (Hofferek *et al.* 1972) cannot be exlcuded although the standard isolates have been obtained by serial local lesion transfer. Compared with the standard isolate the phenomenon can also be explained by assuming either an increase of the amount of RNA-(1+2) or a decrease in the amount of RNA-(3+4), regulated at the replication level, or upon assembly with the virus coat protein. To discriminate between these possibilities, the relative proportions of the virus RNAs in the plant have to be determined. Smookler (1971) found a basic protein in *Chenopodiales*, which strongly inhibited virus infection of *Phaseolus vulgaris* by tobacco necrosis virus, when mixed with virus prior to infection. This protein may also be involved in the regulation of RNA replication or nucleoprotein assembly of BMV and CCMV.

In view of the low concentrations of encapsidated RNA-(3+4) in the bromoviruses from *C. hybridum* the observation that the 'hybrid' virus (BMV-RNA-(1+2)+CCMV-RNA-3 after multiplication in *C. quinoa* contained no detectable amount of RNA-(3+4) (Bancroft, 1972) can be understood. Recent experiments have indicated that, at least for BMV, low concentrations of encapsidated RNA-(3+4) are observed after multiplication of BMV in *C. quinoa* and *C. amaranticolor*.

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