岡大資生研報 (Bull. Res. Inst. Bioresour. Okayama Univ.) 4 : 201-213 (1996)

Characterization of Bean Yellow Mosaic Virus from *Ixia hybrida*.

Toshiya TSUJI*, Takanori MAEDA, Hideki KONDO and Narinobu INOUYE

A strain (Ixia-B) of bean yellow mosaic virus (BYMV) isolated from *Ixia hybrida* was characterized and compared with other isolates of BYMV and clover yellow vein virus (CYVV). Ixia-B was transmitted by aphids, *Myzus persicae* in a non-persistent manner and by sap-inoculation to 11 of 46 species in 5 of 10 families tested, and had a similar host range to that of some BYMV isolates, although some differences were detected. Sap from diseased *C. quinoa* was infective after 10 min heating at 55°C but not 60°C, after a dilution to 10^{-3} but not 10^{-4}, and after 2 days but not 4 days at 20°C. The virus particles were filamentous rods of about 13 × 820 nm. Ixia-B contained a single protein species with a molecular weight of 34,000 and a single viral RNA with approximately 9,000 bases. In ultrathin sections of leaf tissues from infected plants, the virus particles, cylindrical cytoplasmic inclusions and dense bodies were observed in the cytoplasm. The antiserum to Ixia-B produced by immunizing a rabbit had a titer of 1/512. A close serological relationship was revealed between Ixia-B and two strains of BYMV from crocus and gladiolus, but no relationship to clover yellow vein virus was found in agar gel diffusion tests. However, Ixia-B could be distinguished from two strains of BYMV by the formation of spurs among them in agar gel and by the differences in the patterns of peptide mapping of coat proteins. From these findings, Ixia-B was identified as a strain of BYMV.

Key words : *Ixia hybrida*, Bean yellow mosaic virus, Potyvirus

INTRODUCTION

Ornamental *Ixia* plants have become important as cut flowers in our

Research Institute for Bioresources, Okayama University, Kurashiki 710, Japan
Received February 1, 1996
*Present address : Graduate School of Medicine, Okayama University, Okayama 700, Japan

market in recent years. In the spring of 1992, a potyvirus was isolated from *Ixia* plants showing mild mosaic on the leaves and color breaking on the flowers, collected from a commercial greenhouse in Kurashiki. Virus diseases have been prevalent in ornamental plants of Iridaceae such as iris[17,18,20], gladiolus[15,24,31], crocus[28], and freesia[2-7,9,14,19,24,25,32,33], but no viruses have been reported yet in *Ixia* plants in Japan. The virus from *Ixia* was identified as a strain of bean yellow mosaic virus (BYMV). This paper describes the host range, symptomatology, vector transmission, physical properties, purification, viral coat protein, serology, electron microscopy and ultrastructures of the infected cells.

MATERIALS AND METHODS

1. *Virus isolates*

The virus used in this study was isolated from *Ixia hybrida* showing mild mosaic on the leaves and color breaking on the flowers, collected from a commercial nursery in Kurashiki, in April 1992 (Plate I-1, 2). The virus isolate (Ixia-B) was used after five successive single lesion transfers on *Chenopodium quinoa*. Bean yellow mosaic virus (BYMV) isolated from crocus (Cro-4)[23], BYMV-G from gladiolus[22], clover yellow vein virus (CYVV) from *Calanthe discolor* (Cal-35)[21], and a potyvirus from freesia (Fre-1)[19] were also used for comparison with Ixia-B.

2. *Host range*

Several plants were inoculated mechanically with sap from leaves of *C. quinoa* showing chlorotic lesions, in a greenhouse. To confirm the virus infection, back inoculations were made on *C. amaranticolor*.

3. *Aphid transmission*

Aphid transmission tests were done in a non-persistent manner in a greenhouse using *Myzus persicae* reared on virus-free *Brassica rapa*. The aphids were starved for 1 hr before an acquisition access feed of 30 sec on the diseased *Vicia faba* and then allowed inoculation feed of 30 sec on healthy *Vicia faba* seedlings.

4. *Virus purification*

The virus was purified from infected leaves of *C. quinoa* according to the modification of the method of Hammond and Lawson[16]. Inoculated leaves of *C. quinoa,* harvested about 1 wk after inoculation, were homogenized in 3 volumes of chilled 0.5 M K_2 H/K H_2PO_4, pH 8.4, containing 0.5% (w/v) Na_2SO_3, and the homogenate was filtered through double layers of cheesecloth. The extracts were centrifuged at 3,300 $\times g$ for 10 min before addition of 2% (v/v) Triton X-100, 0.1 M NaCl and 4% (w/v) polyethylene

glycol 6000 to the supernatant. The mixture was stirred for 1 hr and centrifuged at 8,000 $\times g$ for 20 min. The pellet was resuspended in BK buffer (0.1 M boric acid, 0.1 M KCl ; pH 8.0) by gentle shaking. After addition of 2% (v/v) Triton X-100, the mixture was stirred for 15 min, prior to centrifugation at 8,000 $\times g$. The supernatant was layered on 30% sucrose cushion in BK buffer and centrifuged at 85,600 $\times g$ for 2.5 hr. The pellet was resuspended in BK buffer, then layered on CsCl-sucrose density gradients[28] and centrifuged at 139,000 $\times g$ for 3 hr. The virus zone was collected, diluted with BK buffer and centrifuged at 65,000 $\times g$ for 90 min, and the pellet was resuspended in 0.1 M BK buffer and then centrifuged at 8,000 $\times g$ for 20 min.

5 . *Electron microscopy*

Preparations of crude sap from infected leaves and purified virus were observed after negative staining with 1% aqueous phosphotungstic acid (PTA), pH 7.0, in a Hitachi H-7100 electron microscope. For ultrathin sectioning, small tissue of infected leaves were prefixed in 6% glutaraldehyde in 0.1 M PB for 2 hr, post-fixed in 1% osmium tetroxide for 2 hr at 4 °C, dehydrated in a graded series of ethanol and embedded in epoxy resin. Ultrathin sections were cut with glass-knives and stained with uranyl acetate and lead citrate.

6 . *Serology*

Antiserum to Ixia-B was prepared by injecting a rabbit intravenously with 500 μg of purified virus and then followed by five intramuscular injections of 1 mg virus emulsified in Freund's incomplete adjuvant at 10 days intervals. The rabbit was bled 2 wks after the final injection. The antiserum was absorbed with concentrated extracts from non-inoculated *C. quinoa* leaves before use. SDS-agar gel double diffusion tests were made in a medium consisting of 0.8% agar (Difco, Agar Noble), 0.85% sodium chloride, 0.3% sodium dodecyl sulfate (SDS), and 0.2% sodium azide (w/v) in 0.05 M PB, pH 7.0.

7 . *SDS-polyacrylamide gel electrophoresis (SDS-PAGE)*

SDS-PAGE was done by the method of Laemmli[26]. For estimation of molecular weight, SDS-degraded virus protein was co-electrophoresed with the Pharmacia marker proteins (phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin) at 50 V for 6 hr.

8 . *Peptide mapping*

Coat proteins of Ixia-B, Cro-4, BYMG, and Cal-35 were prepared from virus particles by the method of MacDonald and Bancroft[27]. The coat proteins were digested partially with papain (EC 3.4.22.2 : E. Merck AG.), chymotrypsin (EC 3.4.21.4 : Sigma Chemical) and pepsin(EC 3.4.23.1 : Sigma

Chemical) as described by Cleveland *et al.*[8], and protein digests were analyzed by SDS-PAGE.

9. *Preparation of viral RNA*

Viral RNAs of Ixia-B, Cro-4, BYMG and Cal-35 isolates were extracted from purified viruses by the proteinase K procedure[30] and analyzed by agarose gel electrophoresis.

RESULTS

1. *Host range and symptoms*

The virus infected 12 out of 47 species in 6 out of 11 families tested. It caused systemic infections in 7 species in 4 families including *Freesia* sp., *Nicotiana clevelandii*, *C. amaranticolor*, *Spinacia oleracea* (Nihondane), *Vicia faba*, *Phaseolus vulgaris* (Kintoki) and *Trifolium incarnatum*, and produced local lesions on the inoculated leaves of *C. quinoa*, *Beta vulgaris*, *Tetragonia expansa* and *Gomphrena globosa*, and latent local infections in *N. tabacum* (Samsun), *S. oleracea* (Orion), and *P. vulgaris* (Kurosando, Top Crop, Hatsumidori). Systemic symptoms of mild mosaic in *Freesia* sp. (Plate I-3), yellowing and stunt were produced in *N. clevelandii*, chlorotic spots in *C. amaranticolor*, severe mosaic and stunt in *P. vulgaris* (Kintoki) (Plate I-4), mild mosaic in *V. faba* (Plate I-5), severe mosaic in *T. incarnatum*, and systemic infection of *S. oleracea* (Nihondane) without symptoms. The virus failed to infect *N. tabacum* (White Burley), *N. rustica*, *N. glutinosa*, *Petunia hybrida*, *Lycopersicon esculentum*, *Solanum melongena* (Kokuyou), *Capsicum annuum*, *Physalis floridana*, *Datura stramonium*, *Hyoscyamus nigar*, *Cucumis sativus* (Syogoin), *C. melo* (Sanuki), *Cucurbita maxima* (Houkou-Aokawa), *Citrullus lanatus* (Kodama), *Pisum sativus* (Kinusaya, Fukkoku-Oosaya), *Vigna unguiculata* (Kurodane-Sanjaku, Akadane-Sanjaku), *V. angularis* (Tanbadainagon), *V. radiata*, *Glycine max*, *T. repins*, *T. pratense* (Hamidori, Kenland), *Astragalus sinicus*, *Medicago sativa*, *Cassia occidentalis*, *Raphanus sativus* (Miyashige Shirimaru), *Brassica rapa*, *B. campestris*, *Zinnia elegans*, *Helianthus annuus*, *Callistephus chinensis*, *Dianthus barbatus* (Victory), *Zea mays* (Astro, Honey) and *Sesamum indicum* (Shirodane, Kurodane).

2. *Aphid transmission*

The virus was easily transmitted by *Myzus persicae* in a non-persistent manner after acquisition and inoculation access feeds of 30 sec.

3. *Stability in crude sap*

Sap from *C. quinoa* showing local lesions was infective to *C. amar-*

anticolor after 10 min at 55°C but not at 60°C, after dilution to 10^{-3} but not to 10^{-4}, and after 2 days at 20°C but not 4 days.

4. *Electron microscopy*

In leaf-dip preparations from the infected plants, flexuous rod (Plate I-6) and fragments of cylindrical inclusions were observed (Plate II-1). Out of 206 particles measured, 77% were between 780 and 860 nm long, with a modal length at 820 nm.

5. *Ultrastructure of infected cells*

In ultrathin sections of mesophyll cells of the infected leaves, cylindrical inclusions with pinwheels, laminated aggregates and dense bodies (crystalline aggregates) were observed in the cytoplasm (Plate II-2, 3). These inclusions were very similar in appearance to those induced by BYMV and CYVV, members of Subgroup II of *Potyvirus* according to the classification of Edwardson and Christie[10,11]. Dispersed or aggregated virus particles were also seen in the cytoplasms.

6. *Serology*

The homologous titer of antiserum to Ixia-B was 1/512, in micro-precipitin tests. The serological relationships among Ixia-B, BYMV (Cro-4, BYMV-G), CYVV and a potyvirus from *Freesia* (Fre-1) were studied in SDS-agar gel double immuno-diffusion tests, using antisera to Ixia-B, BYMV (Cro-4) and CYVV. Precipitin lines of Ixia-B and Fre-1 were fused with each other, whereas the pricipitin lines of Ixia-B formed spurs over those developed by BYMV (Cro-4) and BYMV-G, when Ixia-B antiserum was used (Plate II-4). CYVV did not react with the same antiserum (Plate II-4). When anti-BYMV (Cro-4) serum was used, BYMV (Cro-4) spurred over Ixia-B, BYMV-G and Fre-1, but did not react with CYVV (Plate II-5). Anti-CYVV serum reacted with homologous antigen, but did not react with heterologous antigens, Ixia-B, BYMV (Cro-4), BYMV-G and Fre-1 (Plate II-6).

7. *Coat protein*

In SDS-polyacrylamide gel electrophoresis, Ixia-B, BYMV(Cro-4), BYMV-G, CYVV and Fre-1 were found to contain one major polypeptide with a molecular weight of 34,000, 34,500, 34,000, 33,000 and 34,000, respectively (Plate III-1). The minor bands was also detected in purified Ixia-B, which was considered to be an enzymatically degraded product of coat proteins.

8. *Peptide mapping*

Limited digestion of the coat proteins of Ixia-B, BYMV (Cro-4 and BYMV-G) and CYVV with papain, chymotrypsin and pepsin produced the band patterns shown in Plate III-2, 3, 4. All isolates produced distinct band patterns.

9. *Viral RNA*

The viral RNA from the purified Ixia-B migrated as a single band with approximately 9,000 bases in agarose gel electrophoresis. The viral RNAs of Ixia-B, BYMV (Cro-4 and BYMV-G) and CYVV were indistinguishable each other in their sizes (Plate III-5).

DISCUSSION

In *Ixia hybrida*, only bean yellow mosaic virus (BYMV) are reported to occur by Bellardi *et al.*[1]. As viruses occurring in freesia (Iridaceae), BYMV[19,25], cucumber mosaic virus[24], freesia mosaic virus (FMV)[13], freesia streak virus (FSV)[3-7], tobacco rattle virus[9] and a closterovirus[14] have been known. FSV was first named by Brunt[3], but later FSV was concluded to be identical to FMV[13]. FMV has a particle length of 800-850 nm and it is not related to BYMV serologically[13,14]. In this study, a virus (Ixia-B) causing mild mosaic on the leaves and color breaking on the flower of *Ixia hybrida* reacted with antiserum[23] to BYMV (Cro-4), but not with antisera to FMV donated from Ir. Maat, D. Z. (The Netherlands) or to clover yellow vein virus (CYVV). Therefore, Ixia-B virus was identified as an isolate of BYMV on the basis of host range, particle morphology, and stability in sap, in addition to serological tests.

BYMV has occurred widely in ornamental plants belonging to Iridaceae[1,12,17,23,25,31] as well as plants of Leguminosae[12,22] and others. FMV has been reported to infect 7 species in 5 families, but not infect *C. quinoa*, *Gompherena globosa* or *Pisum sativum*[13]. Ixia-B virus apparently differs from FMV, as FMV could not infect *Vicia faba*, *Phaseolus vulgaris*, *C. quinoa*, or *G. globosa*.

The particle of BYMV is flexuous rod, about 750 nm long generally[2], but varies from 523 to 950 nm by the isolates[12]. The particle length of Ixia-B virus from Ixia in Japan was 820 nm, longer than the 722 nm of BYMV from *Ixia* in Holland reported by Ballardi *et al.*[1]. Some potyviruses including some isolates of BYMV[12] and leek yellow stripe virus[29] have been shown to have particles about 820 nm long.

In SDS agar-gel diffusion tests, Ixia-B, BYMV (Cro-4) and Fre-1 from

freesia reacted with Ixia-B antiserum and BYMV (Cro-4) antiserum, but spur formation occurred between Ixia-B and two isolates of BYMV (Cro-4, BYMV-G). The serological results indicated that Ixia-B differs from two isolates of BYMV in amino acid sequence. Moreover, we conducted peptide mapping to prove this. When coat proteins of Ixia-B and BYMV isolates were partially digested with several proteinases, they could be distinguished from each other. This supports the differences between Ixia-B and BYMV isolates, revealed by the serological tests.

REFERENCES

1. Bellardi, M. G., Pisi, A. and Masenga, V. 1987. Bean yellow mosaic virus infecting Ixia bulbs imported from Holland. Phytopatoh. Medit. 26 : 165-169.
2. Bos, L. 1970. Bean yellow mosaic virus. CMI/AAB Descriptions of Plant Viruses. No.40 : pp. 4.
3. Brunt, A. A. 1968. Freesia. Rep. Glasshouse Crops Res. Inst., 1967 : 101.
4. Brunt, A. A. 1969. Freesia streak virus (FSV). Rep. Glasshouse Crops Res. Inst., 1968 : 104-105.
5. Brunt, A. A. 1970. Freesia. Rep. Glasshouse Crops Res. Inst., 1969 : 131-132.
6. Brunt, A. A. and Atkey, P. T. 1971. A new group of filamentous aphidborne viruses? Rep. Glasshouse Crops Res. Inst., 1970 : 152-153.
7. Casper, R. and Brunt, A. A. 1971. Das Freesia streak virus-ein in Deutschland neues Freesienvirus. Nachrichtenbl. Deutsch. Pflanzenschutzd. 23 : 89-90.
8. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. and Laemmli, U. K. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. 255 : 1102-1106.
9. Derks, A. F. L. M., Hollinger, T. C., Segers, L. C. and Asjes, C. T. 1986. "Department of plant pathology" written by Aartrijk, T. van. In Jaarverslog 1985. Laboratorium voor Bloembollenderzaek, Lisse. Netherlands. 55-78, 105-112.
10. Edwardson, J. R. and Christie, R. G. 1978. Use of virus induced inclusions in classification and diagnosis. Ann. Rev. Phytopathol. 16 : 31-55.
11. Edwardson, J. R., Christie, R. G. and Ko, N. J. 1984. Potyvirus cylindrical inclusions-Subdivision IV. Phytopathology 74 : 1111-1114.
12. Edwardson, J. R. and Christie, R. G. 1991. Bean yellow mosaic virus. The Potyvirus group. 1 : 291-348. Monograph No. 16-I. Agri. Exp. Sta., Inst Food and Agric. Sci. Univ. Florida.
13. Edwardson, J. R. and Christie, R. G. 1991. Freesia mosaic virus. The Potyvirus group. 2 : 507-509. Monograph No. 16-II. Agri. Exp. Sta., Inst Food and Agric. Sci. Univ. Florida.
14. Fox. M. J. and Wilson, U. E. 1985. Investigation of virus infection of freesia. Acta Horticulturae 164 : 291-295.
15. Fukumoto, F., Ito, Y. and Tochihara, H. 1982. Viruses isolated from gladiolus in Japan. Ann. Phytopathol. Soc. Jpn. 48 : 68-71.
16. Hammond, J. and Lawson, R. H. 1988. An improved purification procedure for preparing potyvi-

ruses and cytoplasmic inclusions from the same tissue. J. Virol. Methods 20 : 203-217.

17. Inouye, N., Maeda, T. and Mitsuhata, K. 1978. Bean yellow mosaic virus isolated from *Iris* sp. Ann. Phytopatoh. Soc. Jpn. 44 : 397 (Abstr.).
18. Inouye, N. and Mitsuhata, K. 1978. Turnip mosaic virus isolated from *Iris* sp. Nogaku Kenkyu 57 : 1-16.
19. Inouye, N. and Mitsuhata, K. 1983. *Freesia* streak virus isolatd from Freesia. Ann. Phytopatoh. Soc. Jpn. 49 : 113-114 (Abstr.).
20. Inouye, N., Miyaji, K. and Mitsuhata, K. 1981. Host range and some properties of iris severe mosaic virus. Ann. Phytopathol. Soc. Jpn. 47 : 182-188.
21. Inouye, N., Maeda, T. and Mitsuhata, K. 1988. A strain of clover yellow vein virus isolated from *Calanthe* sp. Acta Horticulturae 234 : 61-68.
22. Inouye, T. 1968. Studies on host range of PVY-group viruses in leguminous plants in Japan, and the identification of them by the selected differential test plants. Nogaku Kenkyu 52 : 11-29.
23. Kaneshige, H., Maeda, T. and Inouye, N. 1991. Host range and properties of bean yellow mosaic virus (BYMV) infecting crocus, and serological relationships among three strains of BYMV. Nogaku Kenkyu 62 : 225-240.
24. Komuro, Y. 1966. Identification of viruses infecting vegetables and ornamentals in Japan. Ann. Phytopathol. Soc. Jpn. 32 : 114-116.
25. Komuro, Y. and Tochihara, H. 1964. On bean yellow mosaic virus isolated from freesia. Ann. Phytopathol. Soc. Jpn. 29 : 80 (Abstr.)
26. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 : 680-685.
27. MacDonald, J. G. and Bancroft, J. B. 1977. Assembly studies on potato virus Y and its coat protein. J. Gen. Virol. 35 : 251-263.
28. Maeda, T. and Inouye, N. 1993. An improved procedure for purification of plant viruses. Ann. Phytopathol. Soc. Jpn. 59 : 739 (Abstr.).
29. Noda, C. and Inouye, N. 1989. Leek yellow stripe virus isolated from an ornamental *Allium* plant in Japan. Ann. Phytopathol. Soc. Jpn. 55 : 208-215.
30. Sambrook, J., Fritsch, E. F. and Maniatis, T. 1982. Molecular Cloning : A Laboratory Manual 2nd ed.. Cold Spring Harbor Laboratory. Cold Spring Harbor, N. Y.
31. Takahashi, M., Ooishi, C., Inoue, A. and Sorimachi, A. 1965. Identification of CMV and BYMV in gladiolus. Ann. Phytopathol. Soc. Jpn. 30 : 30 (Abstr.).
32. Van Dorst, H. J. M. 1973. Two new disorders in freesias. Neth. J. Pl. Pathol. 79 : 130-137
33. Van Koot, Y., Van Slogteren, D.H.M., Cremer, M.C. and Camfferman, J. 1954. Virusverschijnselen in Freesia's. Tijdschr. PlZiekt. 60 : 157-192.

Ixiaから分離されたbean yellow mosaic virus

辻　俊也・前田孚憲・近藤秀樹・井上成信

1992年に岡山県倉敷市玉島で，葉に斑入りを生じた球根類花卉植物*Ixia hybrida*からpotyvirus（Ixia-B）が分離され，その諸性質からbean yellow mosaic virus（BYMV）と同定された。本ウイルスを11科47種の植物に接種したところ，フリージャ，*Nicotiana clevelandii*，*Chenopodium amaranticolor*，ソラマメ，クリムソンクローバー，インゲンマメ，ホウレンソウに全身感染し，また*C. quinoa*，フダンソウ，ツルナ，センニチコウなどに局部感染したが，エンドウ，ササゲ，ダイズなどには感染しなかった。本ウイルスはモモアカアブラムシにより非永続的伝搬され，*C. quinoa*の病葉粗汁液中の安定性は耐熱性が55～60℃（10分），耐希釈性10^{-3}～10^{-4}，耐保存性2～4日であった。DN法試料の電顕観察で多くのpotyvirusよりやや長い約820 nmのひも状粒子と管状封入体の破片が見られた。感染葉の超薄切片では風車状，層板状の封入体，dense body，細胞質に散在するウイルス粒子が観察された。本ウイルスはfreesia mosaic virusおよびclover yellow vein virusの抗血清と反応せず，また本ウイルス抗血清を用いた寒天ゲル内二重拡散法ではBYMV分離株（Cro-4，BYMV-G）と反応したが，本ウイルスとBYMVのCro-4およびBYMV-G間にspurが形成され，異種抗原の存在が認められた。外被タンパク質の分子量は約34 Kで，ssRNAのサイズは約9 Kbであった。Papain, chymotrypsin, pepsinを用いた外被タンパク質のペプタイドマッピングでは，本ウイルス，Cro-4，BYMV-G，Cal-35の部分分解パターンがそれぞれ異なり，外被タンパク質がアミノ酸配列レベルで異なっていることが示唆された。

キーワード：イキシャ，インゲン黄斑モザイクウイルス，ポティウイルス

Explanation of plates

Plate I.

1-2. *Ixia hybrida* naturally infected with Ixia-B, showing mild mosaic on the leaves (fig. 1) and color breaking on the flower (fig. 2).
3. *Freesia* sp. inoculated leaf infected with Ixia-B, showing mild mosaic.
4. Systemic mosaic symptoms induced by Ixia-B on *Phaseolus vulgaris* (cv. Kintoki)
5. Systemic mild mosaic symptoms induced by Ixia-B on *Vicia faba.*
6. Virus particles of Ixia-B in leaf-dip preparation from the infected *Chenopodium quinoa.* Bar represents 100 nm.
7. Negatively stained Ixia-B particles purified from *Vicia faba* leaves. Bar represents 200 nm.

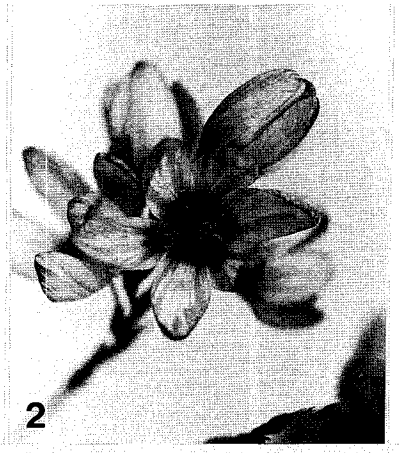
Plate II.

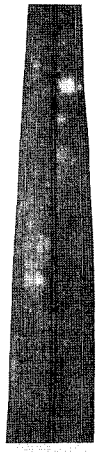
1. Fragment of cylindrical inclusions in negatively stained sap from *Vicia faba* leaves infected with Ixia-B. Bar represents 200 nm.
2. Pinwheels and laminated aggregates of cylindrical inclusions in *Trifolium incarnatum* cells infected with Ixia-B. Bar represents 1,000 nm.
3. Cylindrical inclusions and crystalline inclusions (dense bodies) in *Trifolium incarnatum* cells infected with Ixia-B. Bar represents 1,000 nm.
4-6. SDS-agar gel double diffusion serological tests. Relationship among Ixia-B, BYMV (Cro-4), BYMV-G, CYVV (Cal-35) and Fre-1 (from *Freesia*). Center wells contain antisera to Ixia-B (fig. 4, 1-AS) ; Cro-4 (fig. 5, 4-AS) and Cal-35 (fig. 6, 35-AS), respectively. Outer wells contain purified preparation of Ixia-B, BYMV, Cro-4 (4), BYMV-G (G), CYVV (35) and Fre-1 (F).

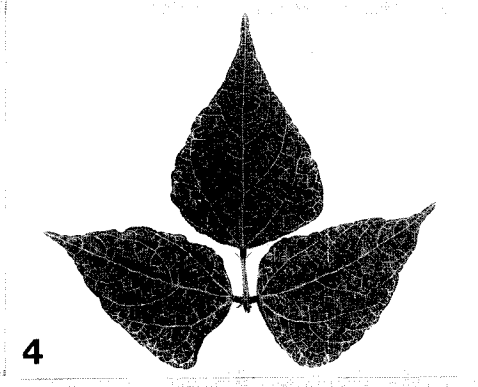
Plate III.

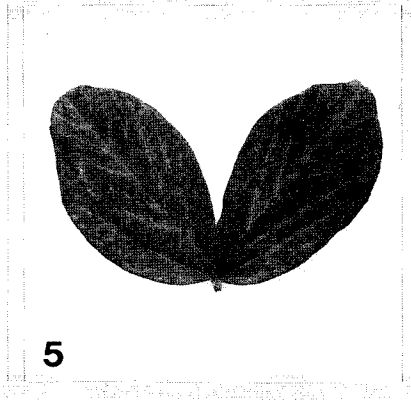
1. SDS-polyacrylamide gel electrophoresis of coat protein subunits from Ixia-B, Cro-4 (BYMV), Cal-35 (CYVV) and Fre-1. Lane M : Marker proteins of molecular weight containing (from top) phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactalbumin (14,400). Lane 2-6 : Coat protein subunits of Ixia-B, Cro-4 (BYMV), BYMV-G, Cal-35 (CYVV) and Fre-1, respectively.
2-4. Patterns of peptide bands produced after electrophoresis of partial papain (fig. 2), pepsin (fig. 3), or chymotrypsin (fig. 4) protease digests of coat protein subunits from Ixia-B, Cro-4 (BYMV), BYMV-G and Cal-35 (CYVV). Lane 1 : Marker proteins of molecular weight ; lane 2 : protease only ; lane 3 : undigested coat protein subunits from Ixia-B ; lane 4-7 : digested coat protein subunits from Ixia-B ; Cro-4 (BYMV) ; BYMV-G and Cal-35 (CYVV), respectively.
5. Agarose gel electrophoresis of viral RNA of Ixia-B, Cro-4 (BYMV), BYMV-G and Cal-35 (CYVV). Lane O : Viral RNA of odontoglossum ringspot virus ; lane M : 0.24-9.5 Kb RNA marker, each RNA contains sequences derived from bacteriophage T7, yeast μ circle and bacteriophage λ DNA ; lane 1-4 : viral RNA of Ixia-B, Cro-4 (BYMV), BYMV-G and Cal-35 (CYVV), respectively.

Plate I.









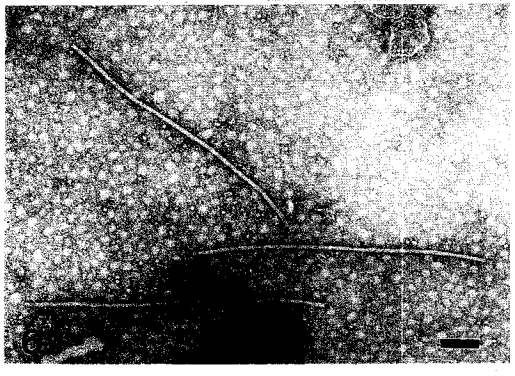
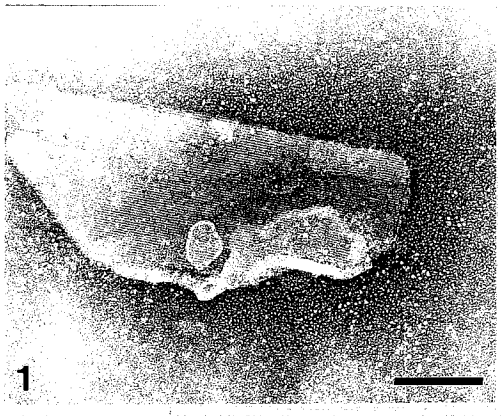
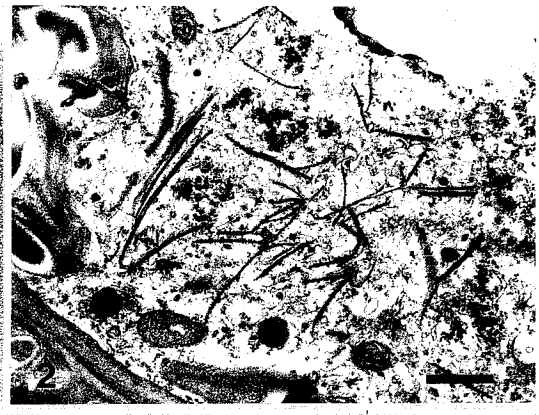
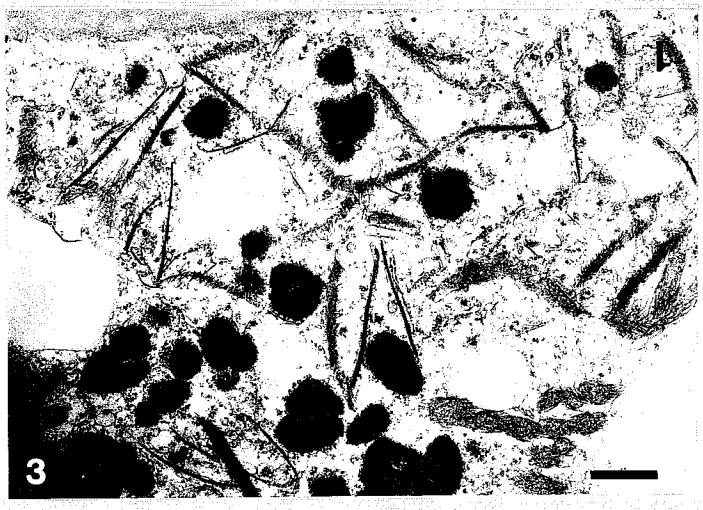
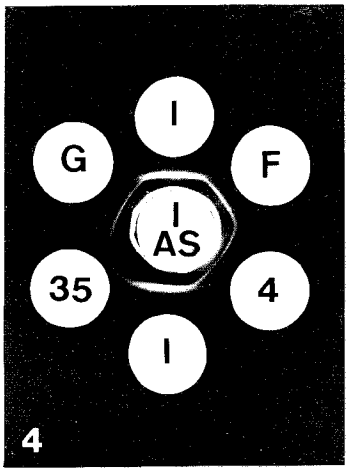


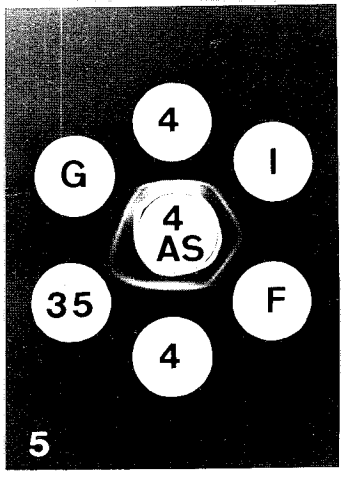
Plate II.











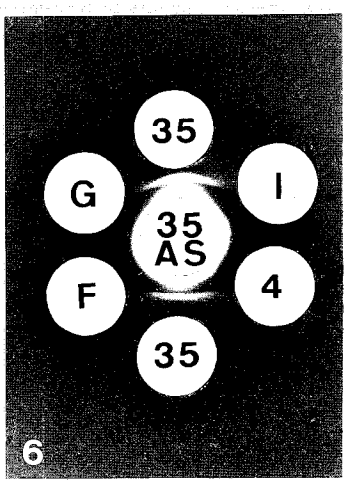
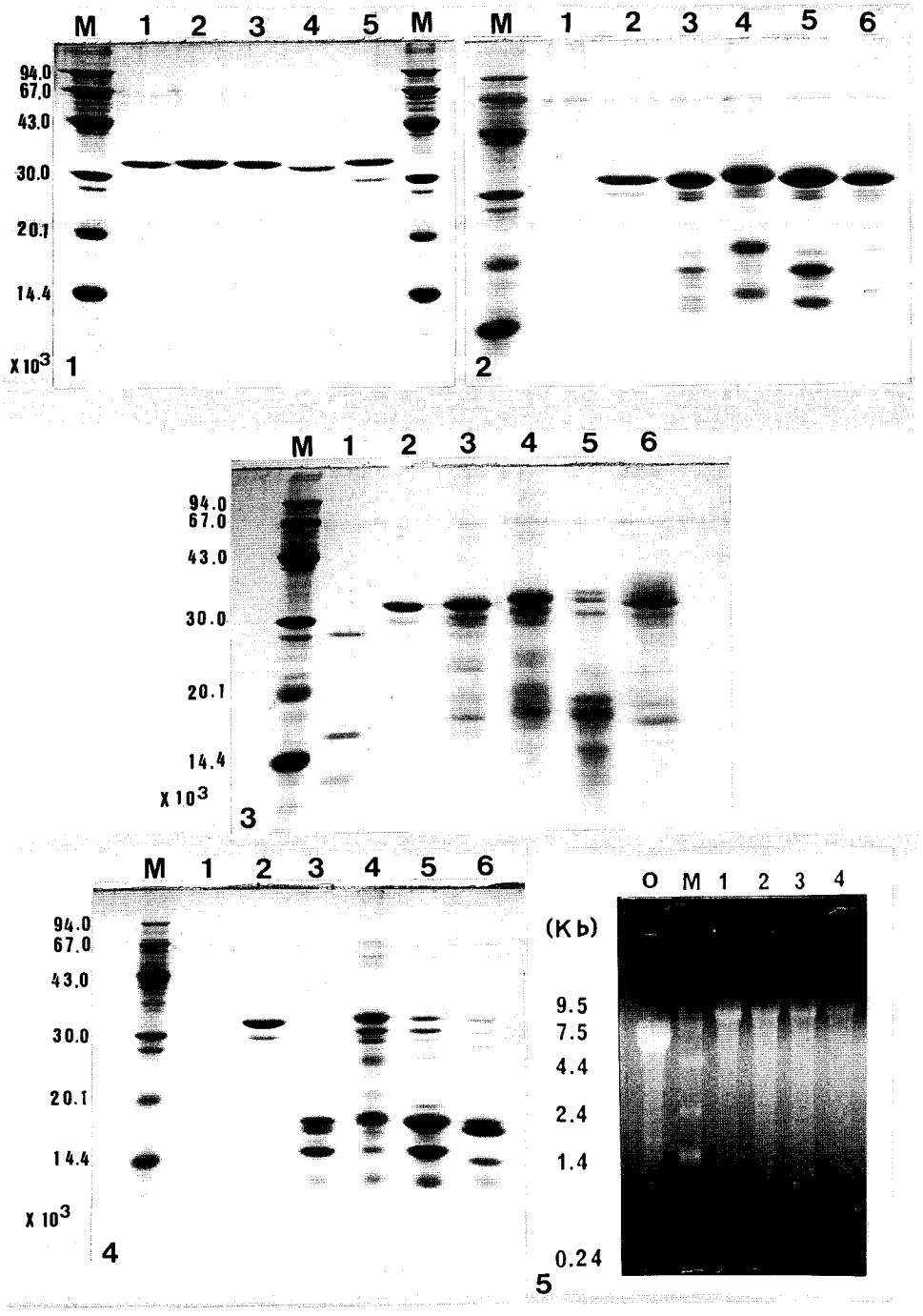


Plate III.


M 1 2 3 4 5 M
94.0
67.0
43.0
30.0
20.1
14.4
X 10³
1
M 1 2 3 4 5 6
2
M 1 2 3 4 5 6
94.0
67.0
43.0
30.0
20.1
14.4
X 10³
3
M 1 2 3 4 5 6
94.0
67.0
43.0
30.0
20.1
14.4
X 10³
4
O M 1 2 3 4
(Kb)
9.5
7.5
4.4
2.4
1.4
0.24
5