# Biological Properties of Potyvirus Isolates from Hot Pepper (*Capsicum annuum* L.) from the Major Growing Areas of Ethiopia[1]

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## Abstract

Potyviruses cause major diseases on hot pepper (*Capsicum annuum* L.) in Ethiopia. Two selected isolates: 374/94 from Bako and 430/94 from Zwai were biologically characterised and compared with reference strains of other potyviruses: Ethiopian pepper mottle virus PN1 (EPMV-PN1), chilli veinal mottle virus 1037 (CVMV-1037) and pepper veinal mottle virus (PVMV). Among the 29 plant species mechanically inoculated, only 18 solanaceous hosts were infected with at least one isolate. Systemic symptoms were usually observed 10-14 days after inoculations, while local lesions appeared in 3-4 days on few hosts. Differences in the type of species infected and symptoms produced were observed between the Ethiopian isolates. However, some degree of relationships were revealed between isolate 374/94 and PVMV on the one hand and 430/94 and EPMV-PN1 on the other. These relationships were further confirmed by the reactions of pepper and tomato breeding lines and a set of international pepper cultivars to the isolates and the reference strains. Furthermore, the isolates 374/94 and 430/94 had capsid proteins similar in size with PVMV (34.5 kDa) and EPMV-PN1 (33.5 kDa), respectively, as determined by both sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and electro-blot immunoassay (EBIA).

## Introduction

Viruses infecting peppers world-wide have been previously reviewed by Green and Kim (1991) and recently by Edwardson and Christie (1997). The former report listed 50 viruses affecting the crop, whereas the latter reported about a 100 among which 55 are involved in natural infections. Usually, severe yield losses occur when the crop is infected by two or more viruses, especially when infection occurs at an early growth stage. Among the virus families, the Potyviridae contains the largest number of members that infect the crop (Green and Kim 1991; Edwardson and Christie 1997).

The potyviruses reported from peppers from different parts of the world are chilli vein banding mottle virus (CVbMV) (Siriwong *et al.* 1995), chilli veinal mottle virus (CVMV) (Ong *et al.* 1979), pepper mild mosaic virus (PMMV) (Ladera *et al.* 1982), pepper mottle virus (PepMOV) (Purcifull *et al.* 1975), pepper severe mosaic virus (PSMV) (Feldman and Garcia 1977), Peru tomato virus (PTV) (Fernandez-Northcote and Fulton 1980), PVMV (Brunt and Kenten 1972), potato virus Y (PVY) (Cook 1963) and tobacco etch virus (TEV) (Laird *et al.* 1964). In Ethiopia, EPMV and PVMV (Agranovsky 1993) and PVY (Marchoux 1976; Agranovsky 1993) were already reported from hot pepper. Moreover, the occurrence, distribution and economic importance of these and other viruses in the major growing areas of the crop were studied recently (Yaynu *et al.* 1999). In this paper the biological characteristics of selected isolates from the crop from different regions and their status compared to the reference potyvirus strains; EPMV-PN1, CVMV-1037 and PVMV is reported.

## Materials and Methods

### Virus Isolates

Isolates 374/94 and 430/94 were obtained from hot pepper from Bako in western Ethiopia and Zwai in the Rift Valley, respectively, in surveys carried out in 1994 (Yaynu *et al.* 1999). EPMV-PN1 was isolated previously from hot pepper at Melkasa in the Rift Valley and was provided by A.A. Agranovsky, Moscow State University, Russia. CVMV-1037 and PVMV originally isolated from Taiwan and Ghana, respectively, were obtained from the BBA, Institute of Biochemistry and Plant Virology, Braunschweig, Germany. The isolates 374/94, 430/94, EPMV-PN1 and PVMV were maintained on *Datura metel* L. and CVMV-1037 on *Nicotiana glutinosa* L. under green house conditions.

### Host Range

Twenty-nine plant species which included weeds, crop species and standard indicator hosts commonly occurring in the vegetable growing areas in Ethiopia, were inoculated to study their reaction to the isolates and reference potyvirus strains. In order to further compare the Ethiopian potyvirus isolates from hot pepper with the reference potyvirus strains; EPMV-PN1, CVMV-1037 and PVMV and to identify sources of resistance to the Ethiopian potyviruses, pepper and tomato breeding lines and a set of international pepper cvs. were evaluated.

Infected leaves from *D. metel* for isolates 374/94, 430/94, EPMV-PN1 and PVMV and *N. glutinosa* for CVMV-1037 were ground in 0.03 M Na-phosphate buffer at a ratio of 1:10 as described earlier (Yaynu *et al.* 1999). The inocula were mechanically inoculated on leaves at 2-3 leaf-stage of 4 plants each of the following species: *Gomphrena globosa* L. (Amaranthaceae), *Chenopodium amaranticolor* Coste & Reynier, *C. foetidum* Schrad., *C. foliosum* Aschers, *C. quinoa* (Chenopodiaceae), *Cucumis sativus* L., (Cucurbitaceae), *Zea mays* L., (Gramineae), *Phaseolus vulgaris* L., *Vicia faba* L., (Leguminosae), and many solanaceous hosts listed in Table 1. The presence of viruses in symptomless plants was checked by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams 1977).

### Aphid Transmission

Isolates 374/94 and 430/94 were transmitted by aphids from infected hot pepper cv. Mareko Fana to virus-free test plants of *D. stramonium*, *D. metel* and Mareko Fana and again from the infected plants of these back to virus-free test plants of each species. Thirty apterous *Myzus persicae* Sulz. colonies per plant were starved for 3-4 h, allowed an acquisition access feeding period of 5-10 min on plants infected with 374/94 and 430/94 and transferred to virus-free test plants. Aphids were killed with Pirimicarb (Pirimor, ICI) or Pyrethrum (Spruzit, Neudorff) after an inoculation access feedings of two days. Acquisition feedings on virus-free plants of all species were included as controls.

### Virion Length Determination

For measurements of particle length, virions were trapped by immunosorbent electron microscopy (ISEM) from extracts of plants infected with isolates 374/94, 430/94, EPMV-PN1 and CVMV-

1037. The length of about 100 particles of each isolate were measured at a magnification 50,000x using a Zeiss EM906 electron microscope with an on-line attached image analysing system (Digivion, SIS, Muenster, Germany).

## Cytopathology of Infected Tissues

The cytopathological effects induced by isolates 374/94, 430/94 and EPMV-N1 in *D. metel* and CVMV-1037 in *N. glutinosa* were studied using methods described by Koenig and Lesemann (1985).

## Virus Purification

Isolates 374/94, 430/94 and EPMV-PN1 were cleaned from possible PVY contamination by inoculating to *D. stramonium*. Besides, a pure isolate of 374/94 was obtained following three serial transmissions of a single lesion on *N. occidentalis*. Isolates were then propagated on *D. metel* and leaves showing mosaic symptoms 10-14 days after inoculations were harvested 2-3 days later and stored frozen until use.

Frozen leaves were purified by homogenisation in 4 volumes of 0.5M $K_2HP0_4/KH_2P0_4$ buffer, pH 8.2 containing 0.5% (w/v) $Na_2S0_3$, 0.5% DIECA and 0.05 M EDTA in a blender. One volume of chloroform was added and centrifuged at maximum speed for 45 sec. Then pulps were clarified at 10,000 rpm for 10 min in a Sorvall GSA centrifuge at 4°C. The supernatants were filtered, mixed with 2% triton X-100 and stirred for 90 min at 4°C, distributed into 60-ml centrifuge tubes on top of a 20 ml layer of 30% sucrose solution (in a 0.05 M Na-citrate, pH 7.5) and centrifuged in a Beckman Ti45 rotor at 32,000 rpm for 2.5 h. Each pellet was resuspended in one ml of 0.05 M Na-citrate buffer. To each 4 ml of virus suspension, 0.92 gm of $Cs_2S0_4$ was added, dissolved and the solution was layered on top of a 53% (w/v) $Cs_2S0_4$ solution in the same buffer followed by centrifugation at 35,000 rpm in a Beckman SW55 rotor for 23 h. The isolates were further purified in 10-40% (w/v) sucrose gradients (prepared in 0.05 M Na-citrate buffer) by centrifugation at 28,000 rpm in Bechman SW28 rotor for 2 h. The gradients were fractionated with an ISCO model 640 density gradient fractionator and a model UA-5 UV-analyzer. Virus-containing fractions were diluted in 0.05 M Na-citrate buffer and concentrated by ultracentrifugation at 35,000 rpm for 3 h. Final pellets were resuspended in the same buffer and the purity and concentration of the virus isolates were examined by electron microscope. Yields of purified viruses were estimated spectrophotometrically (UV scan from 200 to 400 nm) in a Hitachi U-3200 spectrphotometer (Noordam 1973).

## Coat Protein Size Determination

Molecular weights (MW) of the coat proteins (CP) of isolates 374/94, 430/94, EPMV-PN1 were determined from purified preparations by SDS-PAGE using a 4% stacking gel on a 12% resolving gel and the buffer systems of Laemmli (1970). Gels were run in a vertical gel electrophoresis (Mighty Small II, Hoefer Scientific Instruments, San Francisco, USA). Purified preparations were denatured by boiling at 95°C for 5 min. Amounts equivalent to 0.25, 0.20 and 0.25 $\mu$g of purified preparations of isolates 374/94, 430/94 and EPMV-PN1, respectively, were loaded into the slots and electrophoresed at 80V and then at 120V until they reached the bottom of the gel. Low MW markers (BioRad) diluted 1:20 in sample buffer were used as standards. Protein bands were observed after staining the gel with coomassie brilliant blue for 3 h and destaining the gel overnight. The MWs of the proteins were determined by first measuring the mobility of the maker proteins in the gel and plotting their migration rate against their MWs (on a logarithmic scale) to obtain a regression line. The MWs of the capsid proteins were estimated by determining the transecting points of their relative mobility with the regression line.

Electro-blot immunoassay (EBIA) experiments were conducted using appropriate dilutions of leaf extracts from potyvirus-infected plants as well as from purified preparations of 374/94, 430/94, EPMV-PN1 and PVMV (Towbin *et al.* 1979). For the preparation of leaf extracts, 1 g of leaf tissue was ground in 2 ml of 2x sample buffer (Laemmli 1970). The resulting extracts were boiled for 5 min, clarified by centrifuging for 5 min at 13,000*g* in a Hettich centrifuge and kept at -20°C until use. Extracts from non-infected plants were used as controls. Preparations of samples from purified isolates were carried out as described for SDS-PAGE. A 10-$\mu$l aliquot of each extract and amounts equivalent to 0.1$\mu$g of purified preparations of each isolate were used. The

samples were separated in a 12% polyacrylamide gel together with biotinylated marker proteins diluted 1:20 in sample buffer. One µl of pyroline stain (2% pyroline + 1% SDS) was loaded per slot just before the end of the electrophoresis for orientation and identification of the lanes later on the nitrocellulose membrane. Following SDS-PAGE, the gel was carefully removed and immediately used for electrophoretic transfer of the protein bands from the gel onto the nitrocellulose membrane (electro-blotting). A transfer buffer of 25 mM Tris base and 192 mM glycine, pH 8.3 containing 20% (v/v) methanol was used. Transfer of the proteins onto nitrocellulose membrane was performed using electro-blotting apparatus (Hoefer Scientific instruments, San Francisco) overnight at 220 mA and at 4 - 10°C.

# Results

## Host Range of Isolates

The reactions of 19 solanaceous species belonging to 7 genera to inoculations with the isolates 374/94 and 430/94 and the reference potyvirus strains; EPMV-PN1, CVMV-1037 and PVMV are listed in Table 1. All solanaceous hosts, except *N. tabacum*, *N. debneyi* and *Solanum demissum* reacted with local and/or systemic symptoms with at least one of the isolates. Systemic symptoms were usually observed 10-14 days after inoculation, while local lesions appeared 3-4 days after inoculations. Some difference in host range and symptoms induced were observed between the isolate 374/94 and isolates 430/94 and EPMV-PN1. For example, on hot pepper cvs. Mareko Fana and Bako Local isolate 374/94 caused mild leaf mottling (Fig. 1a), while the latter two isolates induced severe leaf mosaic followed by deformations (Fig. 1b). EPMV-PN1 and isolate 430/94 caused nearly indistinguishable symptoms in most of the susceptible hosts, although there was a slight difference in the number of plants infected. On the other hand, isolate 374/94 infected *Lycopersicon lycopersicum* and *N. glutinosa* systemically, where as *N. tabaccum* var. White Burley and *N. sylvestris* reacted with irregular local lesions, all of which were immune to strain EPMV-PN1 and isolate 430/94 (Table 1). In *N. occidentalis* isolate 374/94 consistently induced local and systemic necrotic spots, rendering this species a useful assay and local lesion host. The strain CVMV-1037 caused death of inoculated plants of Bako Local and Mareko Fana vars. two weeks after inoculation.

## Reactions of Pepper and Tomato Breeding Lines

Inoculation with CVMV-1037 resulted in death of three pepper genotypes, while the Ethiopian isolates, in particular 430/94 and EPMV-PN1, generally caused more severe symptoms in most of the breeding lines than CVMV-1037 and PVMV (Table 2). As compared to the other isolates, isolate 374/94 and PVMV induced relatively mild systemic symptoms in pepper lines and were the only isolates that did not infect the pepper line 'Perennial HDV, VC16a' but did infect the tomato line 'TK 70'. The breeding lines 'HAD 268 VC 39a' and 'HAD 832 VC 58a' were immune and the lines 'HAD 248 VC 35a' and 'HAD 249 VC 36a' were either immune or tolerant to all the Ethiopian isolates.

## Reaction of an International Set of Pepper Cultivars

Using a set of international pepper cultivars for the biological characterization and differentiation of the Ethiopian pepper isolates, further interesting virus-host interactions were observed (Table 3). Most remarkably, the isolate 374/94 and PVMV caused only symptomless infections in all susceptible cvs. of *C. annuum*. Isolate 430/94 and EPMV-PN1 also caused very similar systemic symptoms, but in contrast to isolate 374/94 and PVMV, were more severe on the majority of the pepper cvs. They also differed from CVMV-1037 which caused exceptionally severe systemic necrosis, leading to plant death of the cvs: Anaheim F-6, Avelar, New Ace, and PBC-276. Serrano Vera Cruz was immune, while Agronomico 8 was either immune to or only latently infected with all the isolates and viruses, indicating that these two cvs. may also be useful sources of resistance for future breeding work in Ethiopia.

## Aphid Transmission

Using 30 apterous aphids per plant, isolate 374/94 and 430/94 were transmitted in a non-persistent manner in all combinations of sources and

recipient host plant species (Table 4). Symptoms in all recipient hosts were observed about two weeks after aphid inoculation.

## Cytopathology of Infected Tissues

The cytoplasmic cylindrical inclusions (CI) in cells infected with isolate 374/94 comprised pinwheel elements consisting of conspicuous scrolls and short curved laminated aggregates and can be classified as CI type IV. The isolates 430/94 and EPMV-PN1 induced indistinguishable CIs, but distinct from those induced by 374/94. Their CIs comprised pinwheels, very conspicuous laminated aggregates and weakly developed scrolls. Therefore, these two isolates can be assigned to potyviruses inducing CI type III (Edwardson and Christie 1996).

## Virion Length Determination

Filamentous particles of about 12 nm in diameter were consistently observed in leaf extracts infected with all isolates studied. The modal length of isolates 374/94 and 430/94 were 826 nm and 843 nm, respectively and are thus very similar.

## Capsid Protein Size

SDS-PAGE analysis of purified preparations of the isolates 374/94, 430/94 and EPMV-PN1 revealed that each isolate contained one major protein band with MW of 34.5, 33.5 and 33.5 kDa, respectively. In addition, preparations of 430/94 and EPMV-PN1 gave two faint bands of ca. 32.5 kDa, which migrated slightly faster and presumably represent minor proteins resulting from slight proteolytic degradation of the major protein. The extent of proteolytic degradation during virus purification was determined by comparing the CP sizes in both purified virus preparation and infected leaf extracts, using the broad-spectrum MAb P-3-3H8 for detection. Purified preparations of PVMV, 374/94, 430/94 and EPMV-PN1 yielded a major band each (Fig. 2), similar in size to those observed in SDS-PAGE. The minor bands of the two latter isolates were fairly strong, whereas those of the former two were very weak. Furthermore, the CPs in the purified preparations of PVMV and 374/94 were indistinguishable in size and slightly larger than the very similar CP sizes of EPMV-PN1 and 430/94. While an extract from a non-infected plant did not give any band, each extract from the four isolates studied contained a major protein that was indistinguishable in size from that of the purified preparations. However, each extract also gave a faint fairly diffuse band migrating slightly slower than the major band, suggesting that those of the intact CP is already degraded even in the leaf extracts and the actual size of the undegraded CP is slightly larger (by about 2 kDa) than the sizes estimated in SDS-PAGE.

Table 1. Test plant reactions upon mechanical inoculation with isolates 374/94 and 430/94 as well as Ethiopian pepper mottle (EPMV-PN1), chilli veinal mottle (CVMV-1037) and pepper veinal mottle (PVMV) potyviruses used for comparison.

| Host species | Reactions[a] of | | | | |
|---|---|---|---|---|---|
| | 374/94 | 430/94 | EPMV-PN1 | CVMV-1037 | PVMV |
| *Capsicum annuum* 'Yolo Wonder' | L/M,G | L/M,Vb | L/M,Vb | L/Sn | L/Mo |
| *C. annuum* 'Bako Local' | M | Mo,St,Vb | Mo,St,Vb | Pd | Si |
| *C. annuum* 'Mareko Fana' | VC,Ld | M,Ld | M,Ld | Pd | Si |
| *Datura metel* | L/VC, M | L/VC,M | L/VC,M | - | L/VC,M |
| *D. stramonium* | L/Mo | L/Sn | L/Sn | L/Sn | L/M |
| *Lycopersicon lycopersicum* 'Linda' | L/St | - | - | - | L/M |
| *Nicandra physalodes* | L/St | L/St | - | L/M | L/M,St |
| *Nicotiana benthamiana* | L/VC,Ld | L/VC,Ld | L/VC,Ld | L/VC,Ld | L/VC,Ld |
| *N. clevelandii* | Cs/VC, M | Cs/VC, St | Cs/VC, M | Cs/VC,St | Cs/VC,St |
| *N. debneyi* | - | - | L/ M | L/St | L/Scs |
| *N. glutinosa* | Cs/VC | - | - | Cs/M | Cs/M |
| *N. hesperis* | L/Scs | L/Scs | L/Scs | n.t. | n.t. |
| *N. megalosiphon* | Cs/VC, Ld | Cs/VC,Ld | Cs/VC,Ld | Cs/VC,Ld | - |
| *N. miersii* | LL | L/Sc | L/Sc | L/Sc | L/Sc |
| *N. occidentalis* | LL | Cs/VC | Cs/VC | Cs/VC | Cs/VC |
| *N. rustica* | L/- | L/- | L/Ld | L/Ld | - |
| *N. sylvestris* | LL | LL | - | - | - |
| *N. tabaccum* 'Samsun NN' | - | - | - | L/Sc | - |
| *N. tabaccumm* 'White Burley' | - | - | - | L/Sn | L/Scs |
| *Physalis floridana* | L/Scs, D | L/Scs, D | L/Scs, D | L/Scs, D | L/Scs, D |
| *Solanum demissum* | - | - | - | St | - |

[a]*L, symptomless infection of inoculated leaves. Reactions on upper leaves were: Cs, chlorotic spot; Scs, systemic chlorotic spots; G, green spots; Pd, plant death; Ld, leaf deformation; LL, local lesion; Mo, leaf mottle; M, leaf mosaic; Vb, vein banding; si, symptomless infection; Sn, systemic necrosis; St, stunting; VC, vein clearing, -, no symptoms; and n.t., not tested.*

Table 2. Reactions of pepper and tomato breeding lines to the Ethiopian isolates 374/94 and 430/94 as well as EPMV-N1, CVMV-1037 and PVMV included as reference strains.

| Pepper and tomato breeding lines | Previous reactions at AVRDC to | | Reactions[b] to | | | | |
|---|---|---|---|---|---|---|---|
| | CVMV-1037 | PVMV | 374/94 | 430/94 | EPMV-PN1 | CVMV-1037 | PVMV |
| *Capsicum annuum* | | | | | | | |
| Florida VC23a | S | R | Mo,Gs | M,Gs | M,Gs,Ld | Pd | Mo |
| Early Carlwonder VC27a | S | S | Mo,Vb | M,Gs | M,Gs,Ld | Pd | Mo |
| HDA210 VC33a | R | | Mo,Vb | M,Vb,Gs | M,Vb,Gs | M,Ns,Pd | |
| HDA230 VC34a | R | | Mo | M,Cs,St | M,Cs | - | Mo |
| DA252 VC 37a | R | | VC,Gs | M,Gs | M,Cs | - | Cs |
| HDA295 VC41a | R | | VC,Gs | M,Gs | M,Vb,Gs | - | Cs |
| Serr. Huasteco VC 86a | R | | Mo | M,Cs | M,Cs,St | - | Si |
| Perennial HDV VC 16a | | S | - | M | M | M | - |
| HDA273 VC 40a | R | | Mo | Cs | - | M,Ns | - |
| HDA249 VC 36a | R | R | Si | Si | - | - | Cs |
| HDA248 VC 35a | R | R | Si | Si | - | - | Si |
| HDA268 VC 39a | R | | - | - | - | - | Si |
| HDA832 VC 58a | R | R | - | - | - | - | - |
| *C. frutescens* | | | | | | | |
| Tobasco VC 170a | S | S | Mo,St | M,Ld | M,Ld | M,Ld | Mo,St |
| PSP-11 VC160a | R | R | Mo | - | - | - | - |
| L. lycopersicum | | | | | | | |
| TK70 | | S | Si | - | - | - | Si |

*Symptoms recorded were Ld, leaf deformation; M, mosaic; Mo, leaf mottle; Ns, necrotic spots; Si, symptomless infection; St, stunting; Pd, plant death; Vb, vein banding; VC, vein clearing; -, no infection*

Table 3. Reactions of an international set of pepper cultivars to the Ethiopian hot pepper isolates 374/94, 430/94 and EPMV-PN1 as well as CVMV-1037 and PVMV used for comparison.

| Pepper Varieties | Reactions[1] to | | | | |
|---|---|---|---|---|---|
| | 374/94 | 430/94 | EPMV-PN1 | CVMV-1037 | PVMV |
| *Capsicum annuum* | | | | | |
| Agronomico 8 | Si | Si | - | Si | Si |
| Anaheim F-6 | Si | Mo,Vb | Mo,Vb | Pd | Si |
| Avelar | Si | Mo | M | Pd | Si |
| Early Calwonder | Si | M,Vb | M | St,Ns | - |
| New Ace | Si | Si | Mo | Pd | Si |
| PBC-276 | Si | M,Vb,St | Mo,Vb,St | Pd | - |
| PBC-535 | Si | Mo,St | VC,Mo | Mo | Si |
| PBC-581 | Si | Si | Si | Ns,St | Si |
| PBC-694 | Si | St,M | Mo | Si | - |
| Serrano Vera Cruz | - | - | - | - | - |
| Yolo Y | Si | Mo,Vb | Mo | Mo,Ns | Si |
| *C. frutescens* | | | | | |
| Tobasco | VC,Mo | St,Ld,M | M,St,Ld | St,Ld | Si |

[1]*symptoms recorded were: Ld, leaf deformation; M, mosaic; Mo, leaf mottle; Ns, necrotic spots; Si, symptomless infection; St, stunting; Pd, plant death; Vb, vein banding; VC, vein clearing; -, no infection.*

Table 4. Transmission of the hot pepper isolates 374/94 and 430/94 by *Myzus persicae* from various sources to different recipient plant species.

| Virus isolates and Recipient hosts | Virus source plant species | | |
|---|---|---|---|
| | *C. annuum* 'Mareko Fana' | *D. metel* | *D. stramonium* |
| **374/94** | | | |
| *Capsicum annuum* 'Mareko Fana' | 3/3[a] | 3/3 | 2/2 |
| *Datura metel* | 3/3 | 3/3 | 3/3 |
| *D. stramonium* | 3/3 | 2/2 | 2/2 |
| **430/94** | | | |
| *C. annuum* 'Mareko Fana' | 3/3 | 3/3 | 3/3 |
| *D. metel* | 3/3 | 2/2 | 3/3 |
| *D. stramonium* | 2/2 | 2/2 | 2/2 |

*anumber of infected and ELISA-positive plants / total number of plants tested, using 30 apterous aphids per plant.*

## Discussion

Biological properties of plant viruses such as symptomatology, host range and mode of transmission are generally considered unreliable indicators of their identity However, theses characteristics are still widely used as important means for distinguishing strains (Hollings and Brunt 1981). Moreover, information on the host range of a virus and the ways in which it is transmitted from one plant to another is usually an essential prerequisite for developing appropriate measures of virus control (Matthews 1991).

Differences in host range and symptomatology were observed between isolates 430/94 and EPMV-PN1 on the one hand and isolate 374/94 on the other, although all of them infected solanaceous species only. Isolate 373/94 also infected tomato and some *Nicotiana* species (Table 1), that were not infected with EPMV-PN1 and 430/94. Even though isolate 374/94 was able to infect tomato under experimental conditions, no natural infection with this isolate was reported in Ethiopia (Yaynu *et al.* 1999). The isolate was similar to PVMV from Ghana in host range, but caused severe symptoms in some host species and most pepper breeding lines on which it induced systemic necrotic spots and vein clearing, respectively, not usually infected with PVMV (Tables 1 and 2). These results indicated that isolate 374/94 is a strain of PVMV different from those previously described from west Africa (Brunt and Kenten 1972). The isolates EPMV-PN1 and 430/94 were also similar, but different not only from PVMV, but also from CVMV-1037 in host range and symptoms. In addition, many pepper breeding lines resistant to CVMV-1037 and PVMV at the Asian Vegetable Research and Development Centre (AVRDC) were severely infected with the isolates from Ethiopia. On the other hand, a few contradictory results were obtained in this study, i.e., some breeding lines earlier regarded as resistant to CVMV-1037 and/or PVMV were susceptible to the same isolates in the present study (Table 2). This could be due to differences in environmental conditions and procedures used. More importantly, however, the pepper cv. Serrano Vera Cruz and the breeding lines 'HDA 268 VC39a' and 'HDA 832 VC 58a' were immune or tolerant to all the Ethiopian isolates, indicating their usefulness in future breeding work in Ethiopia.

The formation of CIs also referred to as pinwheels, has been regarded as a major criterion for the assignment of a new isolate to the Potyviridae (Edwardson and Christie 1996). These authors used different structures of CI to assign members of potyviruses to four groups. However, this kind of grouping has been contentious. It has been shown that strains of the virus can belong to different inclusion body subgroups, while distinctly non-related viruses were assigned to the same subgroup (Francki *et al.* 1985). Based on the observed CI structures, there was a clear distinction between isolates 430/94 and EPMV-PN1 on the one hand, and isolate 374/94 on the other. The former two were confidently assigned to the CI type III, while the latter induced CI

which places this isolate in the type IV category.

Based on SDS-PAGE analysis of purified preparation, the capsid protein sizes of the isolates of EPMV-PN1, 430/94, PVMV and 374/94 were 33.5, 33.5, 34.5 and 34.5 kDa. EBIA (Western-blot) experiments comparing the CP sizes in purified preparations and extracts from plants infected with the three aforementioned isolates and PVMV showed that PVMV and 374/94 on the one hand and 430/94 and EPMV-PN1 on the other have indistinguishable CP sizes of 34.5 and 33.5 kDa, respectively. However, the reason why the CPs of the latter two was severely degraded than the former two may be due to their differential sensitivity to partial proteolysis (Fig. 2). This indicates that molecular biological method may be useful to determine the exact size of the CPs of the potyvirus isolates and strains.

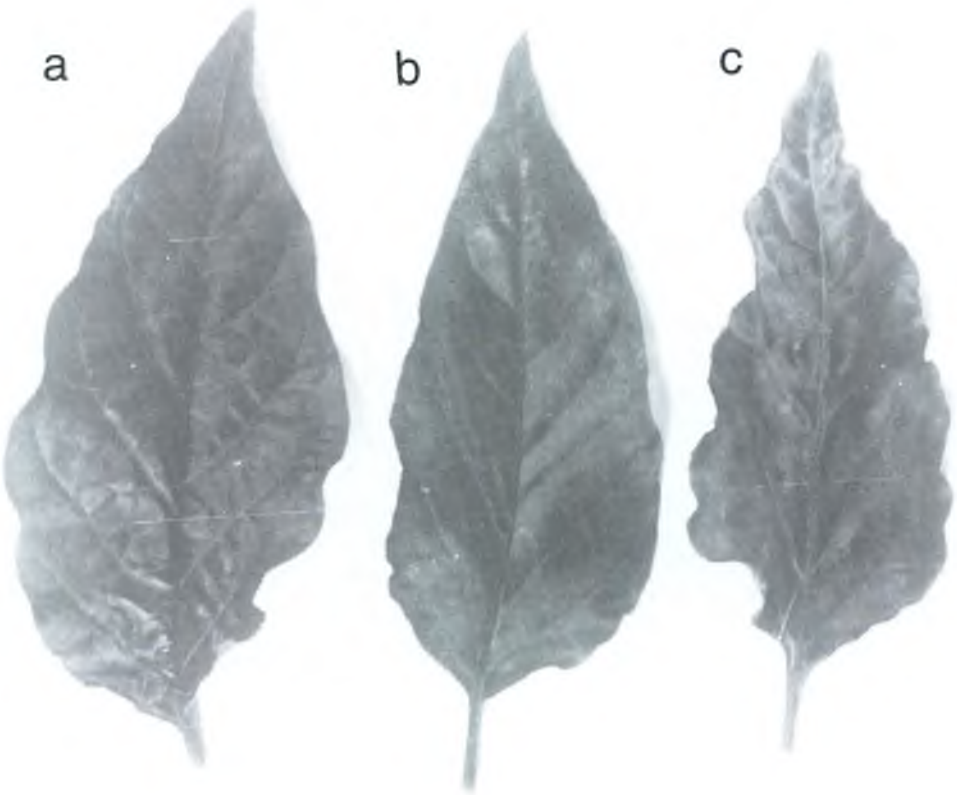


Fig. 1. Systemic infection caused by isolates 374/94 (b) and 430/94 (c) on leaves of hot pepper cv. Mareko Fana. A leaf of a non-inoculated plant (a) is shown on the left.

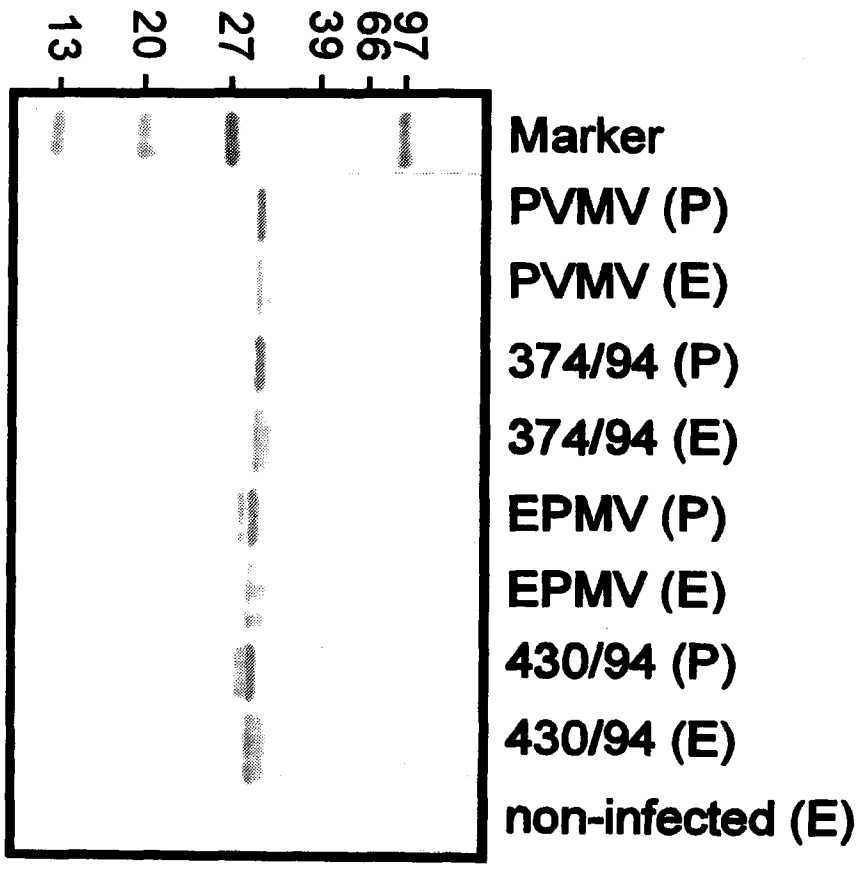


Fig. 2. Electroblot analysis of the capsid protein sizes of PVMV, 374/94, EPMV-PN1 and 430/94 in purified virus preparations (P) and extracts (E) from virus-infected and non- infected leaves using broad spectrum MAb, P-3-3H8 for detection. The molecular weights (in kDa) of the biotinylated marker proteins are indicated on the left.

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