

SEROLOGICAL RELATIONSHIP OF SOME CUCUMBER MOSAIC VIRUS STRAINS

Angela Yordanova^{1*} and Dimitrinka Hristova²

¹National Bank for Industrial Microorganisms and Cell Cultures, 1113 Sofia,
P.O.Box 239, Bulgaria; ²Plant Protection Institute, 2230 Kostinbrod, Bulgaria

Summary

The serological relationship of several strains of cucumber mosaic virus (CMV) has been studied using double immunodiffusion test and counter immunoelectrophoresis. By the application of homologous and heterologous immune sera it can be concluded that the tested strains might be referred to two serological groups - U and N. The immunoelectrophoretical analysis gives faster and more definite results.

Introduction

CMV infection is one of the most widely spread virus diseases of plants. There are numerous strains and varieties of CMV which differ in hosts range, in symptoms, in serological and physical properties, in hybridization with cDNA, etc. [2, 3, 4, 5, 13]. The CMV strains can be separated in two serological groups named by various authors as follow -

DTL and ToRS [4], C and B [10], U and N [12], corresponding to the subgroups I and II [3, 13] or WT and S [2].

The aim of the present work was to make serological grouping of some CMV strains from the collection of National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC).

Materials and methods

Viruses. 6 strains CMV, of which 3 german - CMV-G₁, CMV-G₂ and CMV-N (a referent one); 2 bulgarian - CMV-U₀ and CMV-1k-92 and 1 hungarian - CMV-H (NBIMCC 2045, 2230, 2317, 2314, 2315 and 2316) were studied. The bulgarian strains were isolated from cucumber and they caused green (CMV-U₀) and yellow (CMV-1k-92) mosaic, respectively. All viruses were propagated in *Nicotiana tabacum* cv. Xanthi nc and purified according to Lot et al. [9].

Antisera. Antiserum AS-U₀ homologous to the strain CMV-U₀ with titer 1 : 1024 in double diffusion test [1], antisera AS-G₁ and AS-

N homologous to the corresponding strains were used. AS-N as well as AS-To were obtained from Aschersleben, Germany. AS-DTL was kindly supplied from Dr. Tobias (Plant Protection Institute, Budapest, Hungary).

Immunodiffusion. A double diffusion test in 0.7% agarose gel was applied according to Ouchterlony and Nilsson [11]. The antigen had been loaded 5 h before the antiserum.

Immunoelectrophoresis. A counter immunoelectrophoresis in 0.9% agarose gel, at 150V, 10°C for 2.5 hours was carried out. The holes for the antigens were 2.5 mm, for the antiserum - 4 mm and the distance be-

tween them - 7 mm.

0.075 M veronal buffer, pH 8.6 with 5 mM EDTA [7] was used for the immunodiffusion

and immunoelectrophoresis. The plates were stained with 0.5% solution of Coomassie Brilliant Blue R - 250.

Results and Discussion

The serological relationship among the 6 studied CMV strains was determined according to 2 methods - double diffusion test and counter immunoelectrophoresis. The viruses were tested against CMV-N (referent for serogroup N) and CMV-U₀. The strain CMV-U₀ was used as a representative of serogroup U because it was placed in this group according to its host range, symptoms and serological properties [8].

The results concerning the immunodiffusion analysis of CMV using different immune sera are summarized in Table 1. AS-U₀ and AS-G₁ reacted only with strains CMV-U₀, CMV-1k-92 and CMV-G₁. A single precipitin line was formed which was weaker when AS-G₁ was used. The precipitin bands were fused and the strains were referred as identical. The application of AS-DTL and AS-To distinguished the viruses into 2 groups - first, including CMV-U₀, CMV-1k-92 and CMV-G₁

and second, combining CMV-N, CMV-H and CMV-G₂. The precipitin lines formed between the representatives of the two groups did not fuse entirely and formed a spur.

The precipitation reactions of some of the CMV strains tested with AS-To are shown in Fig. 1a. The induction of more than one line was due to the presence of "particulate" and "soluble" antigen [7] or to the not equivalent quantity of the reagents. Such a precipitation was observed using heterologous antiserum.

The investigations of the immunochemical relationship among CMV strains using immunoelectrophoresis showed that the homologous to CMV-U₀ antiserum also reacted only with CMV-U₀, CMV-1k-92 and CMV-G₁ (Table 2). A small spur was observed when comparing CMV-U₀ to CMV-G₁, as well as CMV-1k-92 to CMV-G₁. The precipitation pattern of the 6 strains with AS-N, AS-DTL and AS-To respectively was an analogous

Table 1. Double immunodiffusion test of CMV strains

CMV strains	Antisera			
	AS-U ₀	AS-G ₁	AS-DTL	AS-To
U ₀ / U ₀	I ^a	I	I	I
U ₀ / 1k-92	I	I	I	I
U ₀ / H	-	-	III ⁺⁺ - II	III ⁺⁺ - II
U ₀ / G ₁	I	I	I	I
U ₀ / G ₂	-	-	III ⁺⁺	III ⁺
U ₀ / N	-	-	III ⁺⁺	III ⁺⁺
N / 1k-92	n.t.	n.t.	III ⁺⁺	III ⁺⁺
N / H	n.t.	n.t.	I	I
N / G ₁	n.t.	n.t.	I - III ⁺	III ⁺
N / G ₂	n.t.	n.t.	I	I
N / N	n.t.	n.t.	I	I
1k-92 / H	-	n.t.	III ⁺⁺	III [±]
1k-92 / G ₁	I	I	I	III [±]
G ₁ / H	-	n.t.	I - III ⁺	n.t.

^a - no visible reaction; I - fusion of the precipitin lines; II - partial fusion and cross of the precipitin lines; III[±], III⁺, III⁺⁺ - partial fusion of the precipitin lines and spur formation of different size [11]; n.t. - not tested.

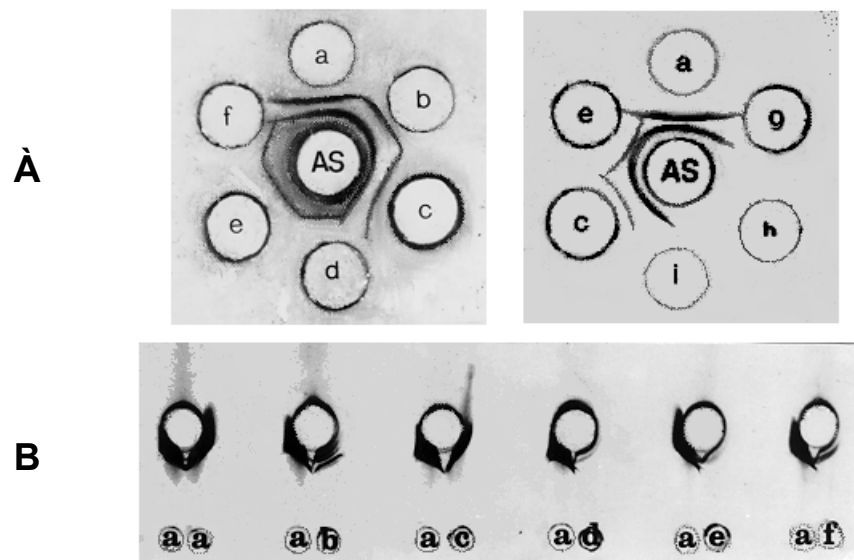


Fig.1. Serological relationship of CMV strains: A. Double immunodiffusion test - in the central hole AS-To; CMV: U₀ (a), 1k-92 (b), G₁ (c), G₂ (d), N (e), H (f); sap of healthy plant (g); buffer (h); empty hole (I); B. Counter immunoelectrophoresis - in the upper start AS-To; a - f as in A.

one. Reactions of I and II type [11] were found out and the spur formation was quite definite when AS-DTL and AS-To were used. The serological relationship of strain CMV-U₀ with the rest 5 strains CMV is illustrated in Fig. 1b. The spurs between the strain CMV-U₀ and strains CMV-H, CMV-G₂ and CMV-N were well defined which showed a presence of a partial identity. The loading of CMV-U₀ in two adjacent wells served as a control of the experiment. Several precipitin lines could be ob-

served because of the different electrophoretic mobilities of the whole virus and the capsid protein.

The summarized results of the serological investigations of the CMV strains using both methods showed that the viruses belonged to two serological groups. CMV-1k-92 and CMV-G₁ were placed in the group U and CMV-H and CMV-G₂ - to group N. The homologous antisera of CMV-U₀ and CMV-G₁

Table 2. Counter immunoelectrophoresis of CMV strains

CMV strains	Antisera			
	AS-U ₀	AS-N	AS-DTL	AS-To
U ₀ / U ₀	I ^a	I	I	I
U ₀ / 1k-92	I	I	I	I
U ₀ / H	-	III ⁺	III ⁺⁺	III ⁺⁺
U ₀ / G ₁	III [±]	I	I	I
U ₀ / G ₂	-	III ⁺	III ⁺⁺	III ⁺⁺
U ₀ / N	-	III ⁺	III ⁺⁺	III ⁺⁺
N / 1k-92	n.t.	III ⁺	III ⁺⁺	III ⁺⁺
N / H	n.t.	I	I	I
N / G ₁	n.t.	III ⁺	III ⁺⁺	III ⁺⁺
N / G ₂	n.t.	I	I	I
N / N	n.t.	I	I	I
1k-92 / G ₁	III [±]	n.t.	n.t.	n.t.

^a - the symbols are as in Table 1.

did not react with the strains of group N. In the group U serologically identical were CMV-U₀ and CMV-1k-92 while the strain CMV-G₁ partly differed from them. CMV-H was the outmost related in regards to the tested viruses of U group.

The counter immunoelectrophoresis

gave much better outlined and clear precipitin lines and the result was reported more quickly [11] as compared with the widely used in the immunochemistry of plant viruses double immunodiffusion test. The combined application of the both methods allowed better characterization of the serological relationship among the strains.

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