Etiology

Symptomatological, Serological, and Electrophoretic Diversity of Isolates of Andean Potato Latent Virus from Different Regions of the Andes

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ABSTRACT

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The serological and electrophoretic properties of twenty-six new isolates of Andean potato latent virus (APLV) from different regions of the Andes varied considerably. Fourteen of these isolates were studied symptomatologically and they all differed. Three major serological strain groups were recognized: the Hu-group was found only in the southern Andes; the CCC-group was found only in the northern Andes; and the Col-Caj-group occurred throughout the Andean region, but it was more common in the north than in the south. Serologically, the Hu-group was related more closely to the type and Abelia latent strains of eggplant mosaic virus than to the Col-Caj and CCC-groups. The three major strain groups were easily differentiated by enzyme-linked immunosorbent assay, whereas in the latex test all were detected by the same sensitized antiserum. In the agar gel double diffusion test, different antisera differed in ability to differentiate between closely related isolates of the Col-Caj-group.

Additional key words: classification, tymoviruses.

Fribourg et al (3) and Jones and Fribourg (6,7) characterized three Peruvian isolates of Andean potato latent virus (APLV): Caj, Ay, and Hu. These differed symptomatologically and serologically from Col, the original Colombian isolate of APLV described by Gibbs et al (5). We report the serological and electrophoretic properties of 26 new isolates of APLV originating from four countries in the Andes. Fourteen of these isolates also were studied symptomatologically.

MATERIALS AND METHODS

New APLV isolates were obtained from potato germplasm collections from Colombia, Ecuador, Peru, and Bolivia brought to the International Potato Center by C. Ochoa in 1976 and 1977. All were new collections except the clones from the Central Colombian Potato Collection. Initial screening was done by testing sap from one leaf sample per clone by agar double diffusion with antiserum to APLV strain Hu. At the time of sampling, potato plants were growing in isolation in an insect-proof screenhouse and were in the first or second generations since introduction to Peru. Thus, the possibility of cross contaminations was minimized. Samples from clones that gave a positive reaction with APLV antiserum were inoculated to *Nicotiana bigelovii* Wats and *N. debneyi* Domin. In some instances the APLV isolates were present in mixed infections with potato viruses X, S, or Y. These viruses were eliminated by mixing 1 ml of infective *N. bigelovii* or *N. debneyi* sap with suitably diluted homologous antisera, incubating the mixture for 2 hr at room temperature, and centrifuging at 8,000 g for 30 min. The supernatant fluids were diluted 1/20 with distilled water before inoculation to *N. bigelovii* which was used for maintaining cultures. The contaminating viruses were eliminated in all instances by the antiserum treatment.

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Additional viruses used were APLV isolates Col (5), Caj, Hu, and Ay (3), and the type and Abelia latent strains of eggplant mosaic, EMV-t and EMV-Al (12), respectively. Antisera to EMV-t, belladonna mottle (BMV), dulcamara mottle (DMV), Ononis yellow mosaic (OYMV), Scrophularia mottle (ScrMV), plantago mottle (PLMV), Clitoria yellow vein (CYVV), and Okra mosaic (OkMV) viruses used in comparative tests were from previous studies (8).

The procedures for working with indicator hosts, purifying the viruses, immunizing rabbits, and for doing the agar double diffusion, latex, enzyme-linked immunodiffusion assay (EIA), and agarose gel electrophoresis tests were described previously (3,8–10).

RESULTS

Geographical origin of isolates. Twenty six new APLV isolates were obtained, six from the Central Colombian Potato Collection (Col-2 to Col-7), three from northern Ecuador (Ec-1 to Ec-3), one from southern Peru (Cuz) and 16 from southern Bolivia (Bo-1 to Bo-16). The original sites of collection of the different potato clones in which these isolates were detected are shown in Fig. 1. Only the location of the station is given for the clones from the Central Colombian Collection. The origins of Col, Caj, Ay, and Hu also are marked in Fig. 1. Isolate Col-2 was referred to as APLV-300 in a previous paper (9).

Serological spur formation tests. Three major strain groups were recognized in serological spur formation tests when the isolates were tested in adjacent wells against 34 different antisera; ie, six to Col-1, four each to Caj and Hu, and two each to Ay, Col-3, Bo-15, Ec-1, ScrMV, OYMV, DMV, CYVV, PLMV and EMV-t. Isolates Bo-1 to Bo-13 from Bolivia and Cuz from southern Peru could not be differentiated from one another and from the serologically identical isolates Hu and Ay (3). This strain group was named the Hu-group. The rest of the isolates usually formed strong spurs when tested in wells adjacent with isolates of the Hu-group. Serologically, most of these isolates were rather similar to Col, the original isolate of APLV (4), and to isolate Caj (3). However, spur formations were observed between some of these isolates (Table 1); in particular, isolates Col-2 and Col-3 usually formed strong spurs with most of the other isolates. These two isolates formed the CCC-group (CCC means Central Colombian Potato Collection). Isolates Col, Caj, Col-4 to Col-7, Bo-14 to Bo-16, and Ec-1 to Ec-3 were assigned to the Col-Caj group (Table 1).

Different antisera differed considerably in ability to differentiate between individual isolates in the Col-Caj-group. With some antisera, which usually were obtained after immunization periods

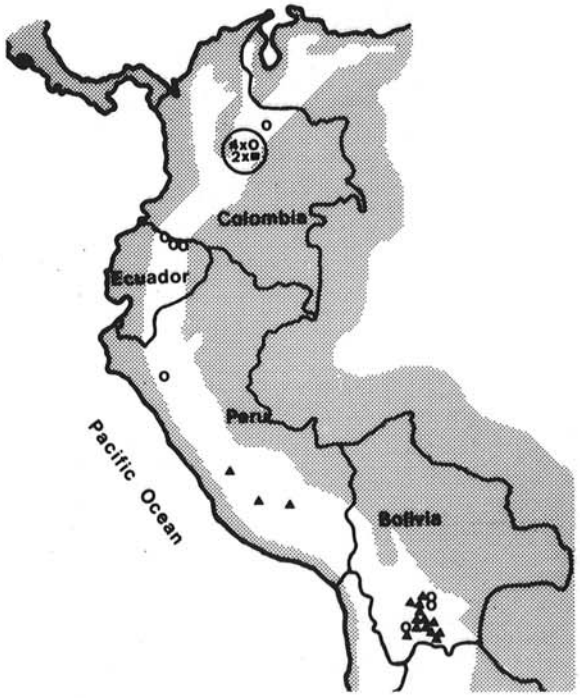

Fig. 1. Geographical origin of Andean potato latent virus isolates. Representatives of the Col-Caj-group (o), of the CCC-group (■), and of the Hu-group (▲).

TABLE 1. Differentiation of Andean potato latent virus (APLV) isolates

	APLV isolates:				
	Hu Ay Bo-1 to Bo-12	Caj Col-4 Col-5	Bo-14 Bo-15	Col Ec-1	Col-2 Col-3
1. Symptomatology	← all isolates tested could be differentiated[a] →				
2. Major strain group	← Hu[b] →	← Col-Caj[c] →			← CCC →
3. Spur formation between isolates					
a. never observed	← →				
b. rarely observed		← →	← →	← →	← →
c. weak spurs observed with most antisera		← between the three subgroups of the Col-Caj-group →			
d. strong spurs observed with most antisera	← between the three major strain groups Hu, Col-Caj, and CCC →				
4. Reactivity in EIA[d]					
a. with antiserum to Hu	← strong →	← very weak or missing →			
b. with antiserum to Caj	← very weak or missing →	← strong →	← medium →		← very weak →
c. with antiserum to Col	← very weak or missing →	← medium →		← strong →	← weak to medium →
d. with antiserum to Col-3	← very weak or missing →	← weak →		← medium →	← strong →
5. Detectability with latex-serum to Col	← →				
6. Differences in electrophoretic migration	← none observed →	← slight differences between most isolates →			

[a] Isolates Bo-15 to Bo-14, Bo-16, and Cuz were not included in the symptomatological studies.
[b] This group also includes Bo-13 and Cuz. The serological and electrophoretic properties of these isolates were not studied in detail.
[c] This group also includes Col-6, Col-7, Ec-2, Ec-3, and Bo-16. The serological and electrophoretic properties of these isolates were not studied in detail.
[d] EIA = enzyme-linked immunosorbent assay.

TABLE 2. Symptoms[a] induced in selected indicator hosts by Andean potato latent virus (APLV) isolates from different regions of the Andes

Isolate	*Nicotiana* spp.		*Chenopodium amaranticolor*	*Solanum chacoense*
	N. bigelovii	*N. clevelandii*		
Colombian region				
Col-2	MM,SCS,VB	MM	DCS	SM
Col-3	LCS,SM,SCS,SNS,NVN,Df	SM,SCS,Cr	SCS,DCS	SM,Df
Col-4	LCS,NVN,SM,SNS	MM,CVN	DCS	SM,CVN
Col-5	SM,NVN,SCS	SM,CVN,Cr	LCS,SCS	MM,DLS
Col-6	SM,CVN,SCS,Df	MM,CVN,Df	DCS	SM,Cr
Col-7	MM,CVN,SCS	CVN,Cr	DCS	MM
Ecuadorean region				
Ec-1	SM,SNS,VB	MM,SCS,Cr	SCS	MM,CVN
Ec-2	LCS,SCS,SM,Df	SM,SCS,Cr	DCS	MM
Ec-3	NVN,VB,Df	SM,SCS,Cr	DCS	SM,Df
Peruvian region				
Caj	LNS,NVN,SM,SNS	SM,CVN,Cr,SNS	LNS,SCS,SCR	CVN
Hu	LCS,NVN,Df,SM	LCS,Cr,VB	SCS,DCS	SM
Ay	LCS,NVN,Df,SM	NVN,Cr,SM	LCS,DCS	SM,Df,Cr
Bolivian region				
Bo-1	NVN,MM	NVN,MM,VB	DCS	MM
Bo-2	NVN,MM	NVN,MM,VB	DCS	MM
Bo-3	NVN,SCS,MM	NVN,MM	DCS	MM
Bo-4	NVN,MM	NVN,MM,VB	DCS,SCS	SM
Bo-15	LNS,NVN,MM,SNS	NVN,SM,SCS	SCS	MM,CVN

[a] Abbreviations: LCS, local chlorotic spots; LNS, local necrotic spots or dots; CVN, chlorotic vein netting; Cr, crinkling; DCS, diffuse chlorotic spots alongside the veins; DLS, markedly diminished leaf size; Df, leaf deformation; MM, mild mosaic; NVN, necrotic vein netting; SCS, generalized chlorotic spotting and flecking; SCR, systemic chlorotic rings; SM, strong mosaic; SNS, systemic necrotic spotting; and VB, vein banding.

of several months, all these isolates appeared identical (Fig. 2). With other sera, however, especially with those from early bleedings or with sera to viruses in the Hu-group, several isolates of the Col-Caj-group could be differentiated (Fig. 3, Table 1).

EIA and latex test. Some observations made with isolates Col, Caj, Hu, and Col-2 in EIA and on the routine detection of APLV with the latex test have been described previously (9,2,10). These studies were extended to the new isolates. The results (Fig. 4, and Table 1) confirm the high degree of strain selectivity of EIA in the case of APLV isolates (9). Thus, conjugates made to isolates in one strain group reacted only moderately, weakly, or not at all with isolates in other strain groups. Antisera to different viruses in the Col-Caj group differed somewhat in reactivity with viruses in the CCC group (eg, Table 1, lines 4b and 4c). It is not known whether this is caused by differences in the response of individual rabbits to antigens or by differences in the antigenic properties of isolates.

Latex particles sensitized with an antiserum to isolate Col were agglutinated by isolates in all other strain groups. The latex test was slightly less sensitive than EIA (9,10).

Symptomatology. Fourteen of the new isolates and Caj, Hu, and Ay were inoculated to four indicator hosts. To ensure uniform environmental conditions these tests were done simultaneously at 18–22 C, a higher temperature than used previously (3). No isolate was identical to any other (Table 2) on the four indicator species *N. bigelovii, N. clevelandii, Chenopodium amaranticolor,* and *Solanum chacoense*. The Hu-group was more uniform in its reactions than the Col-Caj group. Not enough isolates of the CCC-group were available to allow generalizations.

Electrophoresis. All isolates in the Hu-group migrated at the same rate towards the cathode in agarose gel electrophoresis with 0.025 M phosphate buffer, pH 7.0. Most isolates of the other two strain groups showed slight differences in their migration rates (Fig. 5).

Serological comparisons of the three strain groups of APLV with other recognized strains of EMV. The original APLV isolate Col (5) has been considered to be a strain of EMV (4). Therefore, serum titer determinations were made in the agar gel double diffusion test to compare the original isolate Col and representatives of the three major strain groups of APLV with EMV-t and EMV-Al (Table 3).

TABLE 3. Differentiation of members of the three major strain groups of Andean potato latent virus (Col and Caj [Col-Caj-group], Col-2 [CCC-group] and Hu [Hu-group]), the Abelia latent (EMV-Al) and type (EMV-t) strains of eggplant mosaic virus with antisera to several tymoviruses

Antiserum to	Serological reactions[a] of antigens:					
	Col	Caj	Col-2	Hu	EMV-Al	EMV-t
Col[b]	256	256	128	32	32	32
EMV-t[c]	256	256	256	512	1,024	1,024
BMV	64	32	32	16	8	8
DMV	256	128	128	32	8	8
OYMV	32	16	32	4	2	1
ScrMV	64	16	64	4	2	2
CYVV	8	8	4	0	0	0
OkMV	1	2	1	4	0	8

[a] Reciprocal values of serum titers.
[b] Similar results were obtained with several bleedings from three rabbits immunized with Col.
[c] Similar results were obtained with several bleedings from three rabbits immunized with EMV-t.

The serological properties of Caj, Col-2, and Hu were intermediate between those of Col and EMV. The Hu resembled EMV and EMV-al more closely than did Caj or Col-2. As with other tymoviruses (1,11), antisera to more distantly related viruses (eg, DMV, OYMV, and ScrMV in Table 3) frequently allowed a better differentiation than antisera to the closely related forms (eg, EMV-t).

DISCUSSION

Our results, summarized in Table 1, show that different isolates of APLV vary in symptomatological, and to a somewhat lesser extent in serological and electrophoretic properties. Of the three major serological strain groups which were recognized, the Col-Caj-group bears the greatest resemblance to Col, the original isolate of APLV (5) and to isolate Caj (3). However, members of

this group were not uniform symptomatologically, serologically, or electrophoretically. They were found more commonly in the northern Andean region, but they also occurred in the south. The Hu-group was serologically more closely related to EMV-t and EMV-Al (Table 3). Members of this group showed some differences in symptomatology, but were serologically and electrophoretically similar. They were found in the southern Andes, from central Peru to Bolivia. Only two members of the CCC-group were found. They were serologically more similar to the Col-Caj group than to the Hu-group. However, the results of spur formation tests and EIA (Fig. 5) suggest that they form a third strain group. Both isolates were from the northern Andes (Colombia.)

The latex test (2,10) and EIA (9) have been used for the sensitive serological detection of APLV isolates in potato leaves and tubers. With APLV, EIA was about 10 times more sensitive than the latex test which was about 1,000 times more sensitive than the agar gel double diffusion test. The latex test detected all known isolates of APLV and even EMV-t and EMV-Al with the same antiserum. On the other hand, EIA proved to be rather strain specific. Antisera to a member of each of the three major strain groups were necessary for the reliable detection of all isolates. This inconvenience can be overcome by using mixed antisera and mixed conjugates. However, other strain groups which differ from the three groups may occur in the vast Andean region, and these new groups may not be detected by the conjugates currently available. However, such new groups might readily be detected by the simultaneous use of the latex test and EIA.

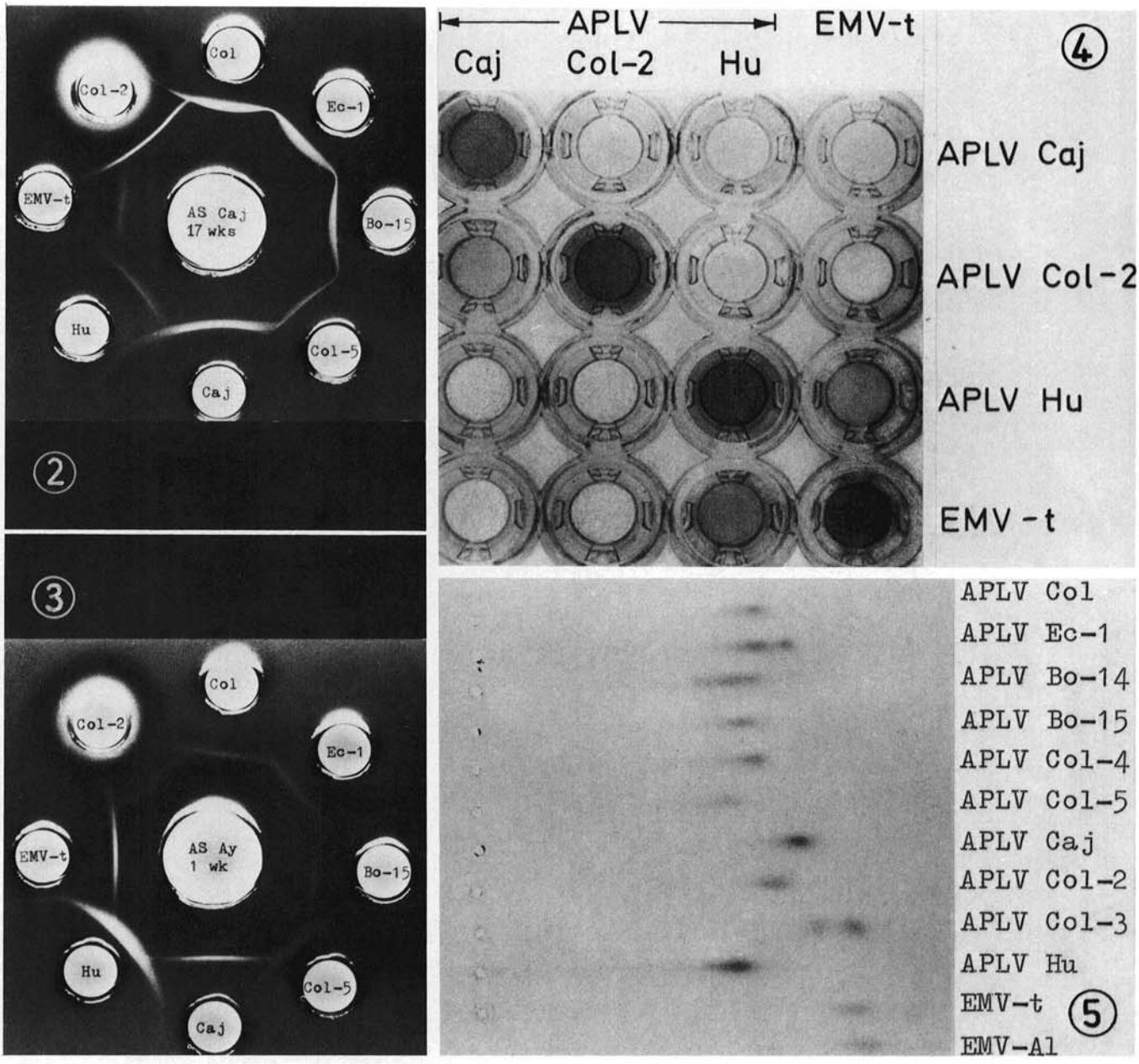

Fig. 2–5. Differentiation of Andean potato latent virus (APLV) isolates. **2,** Agar gel double diffusion test with antiserum (central well) to isolate Caj obtained after 17 wk of immunization. With this serum no serological differences were observed between five different isolates of the Col-Caj-group; ie, Col, Ec-1, Bo-15, Col-5, and Caj. Spur formations were observed with Col-2 (CCC-group), Hu (Hu-group) and EMV-t. **3,** As Fig. 2 but with antiserum to isolate Ay obtained after 1 wk of immunization. This antiserum differentiates not only between Col-2, EMV-t, and Hu, but also between the different isolates of the Col-Caj-group. **4,** EIA with representatives of the three major strain groups of APLV, ie, Caj (Col-Caj-group), Col-2 (CCC-group), Hu (Hu-group), and with EMV-t. Antigens in the vertical, antisera in the horizontal rows. A strong color is produced only in the homologous combinations. **5,** Agar gel electrophoresis with 0.025 M phosphate buffer, pH 7.0, of seven isolates of the Col-Caj-group (Col through Caj), two isolates of the CCC-group (Col-2 and Col-3), Hu, EMV-t, and EMV-Al.

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