Relationships Among Some Ilarviruses: Proposed Revision of Subgroup A

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ABSTRACT

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Two strains of asparagus virus II (AV II-P and AV II-S) and one isolate each of citrus leaf rugose virus (CLRV), citrus variegation virus (CVV), elm mottle virus (EMV), and Tulare apple mosaic virus (TAMV) were found to be serologically interrelated in tests with 11 antisera prepared against these viruses. Only three of the 11 antisera could detect all five viruses in either ring interface or agar gel double diffusion tests. None of the five viruses was related serologically to a group of four isolates comprising two serotypes of tobacco streak virus (TSV). The five serologically related viruses exhibited ultraviolet absorption maxima and minima at 262 and 245 nm, respectively, with $A_{260/280}$ values consistently lower than that of TSV. The results of these tests indicate that AV II, CLRV, CVV, EMV, and TAMV differ sufficiently from TSV to justify restructuring the subgroups. A revision of ilarvirus subgroup A was proposed based on these properties.

Additional key words: black raspberry latent virus, ilarvirus subgroup B.

The Plant Virus Subcommittee of the International Committee on Taxonomy of Viruses (ICTV) proposed two serological subgroups of ilarviruses based on serological relationships between members within subgroups (12). Included in subgroup A were tobacco streak virus (TSV), Tulare apple mosaic virus (TAMV), elm mottle virus (EMV), citrus leaf rugose virus (CLRV), citrus variegation virus (CVV), and black raspberry latent virus (BRLV) (12). Although these six viruses have many similar properties (1,2,4–9), the serological relationships among them are not clear. Both TSV and BRLV are known to be serologically related (8) as are CLRV and CVV (4), but no such relationship has been demonstrated between TSV and either CLRV or CVV (6). In addition, EMV was reported to be serologically unrelated to TSV, BRLV, or TAMV (7). So far as we are aware, there has been no attempt to examine the interrelationships among all the members proposed for subgroup A.

Recently we described properties of asparagus virus II (AV II), which placed it in the ilarvirus group (13). Serological tests performed with antisera from various sources (13) suggested that AV II is serologically related to CLRV, CVV, and EMV, but not to

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TABLE 1. Reciprocal titers of homologous and heterologous antisera tested against four isolates of tobacco streak virus[a]

	Antiserum				Normal
Antigen	RN-B	RN-S	Ro-A	Ro-S	serum
RN-20	245	512	32	128	0
RN-S	128	512	32	128	0
Ro-A	64	128	128	256	0
Ro-S	64	128	128	256	0
Healthy bean	0	0	0	16	0
Healthy *C. quinoa*	0	0	0	64	0

[a]Ring interface tests. Concentration of purified viral antigens was 40 μg/ml. Healthy bean and *Chenopodium quinoa* antigens were prepared by the same procedures used for the viral antigens.

TAMV, TSV, or to several viruses included in subgroup B. On the other hand, Garnsey and Gonsalves (5) reported a serological relationship between CLRV and TAMV.

The objective of the work reported here was to clarify relationships among the viruses proposed for subgroup A by obtaining each of these viruses (except BRLV) and comparing them under uniform conditions.

MATERIALS AND METHODS

Virus sources. Two strains of AV II (AV II-P and AV II-S) were obtained locally and maintained as described earlier (13). Citrus leaf rugose virus (ATCC PV195), supplied by S. M. Garnsey, USDA, ARS, Orlando, FL, was maintained and increased for purification in *Nicotiana tabacum* L. 'Havana 423.' Citrus variegation virus (ATCC PV196), also supplied by S. M. Garnsey, was passed through three single-lesion transfers on *Vigna unguiculata* (L.) Walp. 'California Blackeye,' maintained in *N. glutinosa* L., and increased for purification in *Phaseolus vulgaris* L. 'Kinghorn.' Elm mottle virus, from A. T. Jones, Scottish Horticulture Research Institute, Invergowie, Dundee, Scotland, was maintained in *N. tabacum* 'Havana 423' after three single-lesion transfers on *Chenopodium amaranticolor* Coste & Reyn. and increased for purification in *C. quinoa* Willd. Tulare apple mosaic virus was obtained from the American Type Culture Collection (ATCC PV 80) and after three local-lesion transfers on *Vicia faba* L. was maintained and increased for purification in *N. tabacum* 'Havana 423.' All isolates were monitored periodically for cross contamination on four differential hosts, *C. quinoa, C. murale* L., and *P. vulgaris* 'Bountiful' and 'Topcrop.'

Four isolates of tobacco streak virus were used: RN-S and Ro-S obtained locally from soybean, Ro-A from asparagus, and the bean red node strain RN-B described earlier (11). All isolates were maintained in *Gomphrena globosa* L. and increased for purification

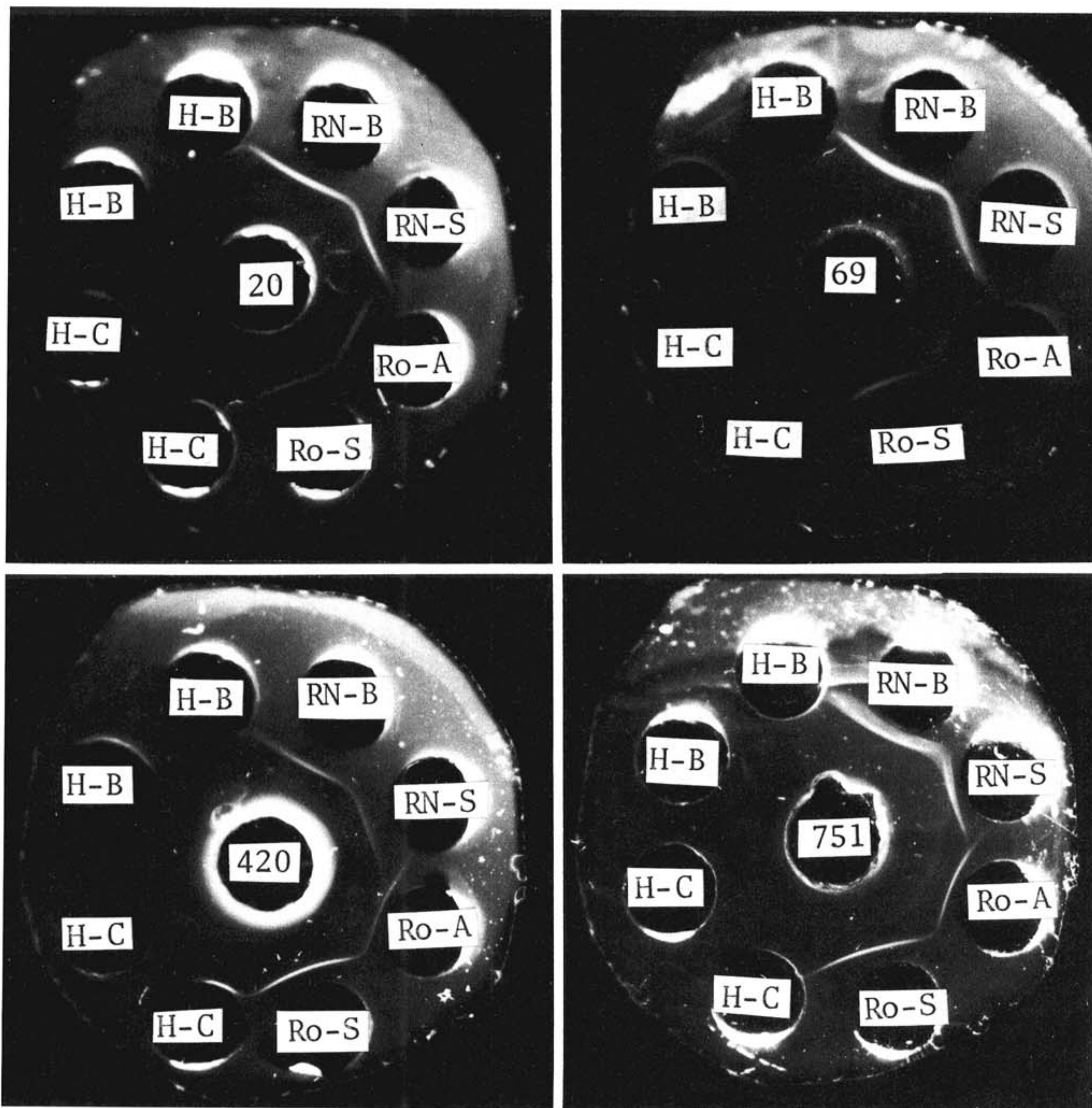

Fig. 1. Agar gel double diffusion serology tests of tobacco streak virus isolates. Center wells contained antiserum (1:1) to isolates RN-B (20), RN-S (69), Ro-A (420), and Ro-S (751). Peripheral wells contained purified virus preparations (at 0.1–0.3 mg/ml). Healthy *Chenopodium quinoa* (H-C) and bean (H-B) leaf tissues were prepared by the same procedures used for virus purification.

TABLE 2. Homologous and heterologous reciprocal titers of 11 antisera prepared against five serologically related ilaviruses[a]

Antiserum		Viral antigen						Healthy antigen[c]
Against	Code[b]	AV II-P	AV II-S	EMV	CVV	CLRV	TAMV	
AV II-P[d]	I-H[e]	512	256	4	8	4	0	0
AV II-P	II-E	256	256	4	128	64	0	0
AV II-P	III-B	128	128	8	8	4	4	0
AV II-S	I-H[e]	512	512	128	64	4	0	0
AV II-S	II-H	16	16	2	4	2	0	0
EMV	I-E[e]	1	1	256	128	1	0	0
EMV	...[f]	1	1	16	8	0	0	0
CVV	...[g]	10	10	40	80	0	0	0
CLRV	I-E[e]	64	64	2	16	512	128	0
CLRV	G[g]	40	40	80	160	1,280	640	0
TAMV	I-F[e]	0	0	0	4	16	512	0

[a]Ring interface tests. Concentration of virus antigen was 40 $\mu g/ml$.
[b]Different numbers denote different rabbits injected with the same virus; sequential bleedings are identified alphabetically with "A" taken three days after the fourth injection.
[c]Healthy *Chenopodium quinoa*, tobacco, and bean tissues were prepared in the same way as the viral antigens and tested separately.
[d]AV II = asparagus virus II, EMV = elm mottle virus, CVV = citrus variegation virus, CLRV = citrus leaf rugose virus, and TAMV = Tulare apple mosaic virus.
[e]Antiserum was made against virus fixed with 0.2% formaldehyde.
[f]Provided by A. T. Jones.
[g]Provided by S. M. Garnsey.

in *C. quinoa* or *P. vulgaris* 'Bountiful.'

Purification. All viruses were purified according to the procedure described earlier for AV II (13), except that Triton X-100 was included only for purification of AV II. After sucrose density gradient ultracentrifugation, all viral components (13) associated with a particular virus were pooled and concentrated into 0.01 M potassium phosphate buffer, pH 7.2–7.4, by one cycle of differential ultracentrifugation. Ultraviolet light absorption spectra of the component mixtures were determined with a Beckman model DB-G recording spectrophotometer at 20 C.

Antisera. One or more antisera were produced against each virus (except CVV) by injecting 0.2–0.4 mg of purified virus (in some cases fixed with 0.2% formaldehyde) intravenously into rabbits four times at 2- to 3-day intervals. Two additional injections were made at weekly intervals after the fourth injection. Bleedings were made at 2- to 3-day intervals after the fourth injection. Additional antisera against CLRV and CVV were provided by S. M. Garnsey. Antiserum against EMV was provided by A. T. Jones.

RESULTS

Serological relationships. Antisera against all four TSV isolates reacted with homologous and heterologous TSV antigens in ring interface tests (Table 1). Results of agar gel double diffusion tests indicated that the four TSV isolates represented two serotypes with isolates RN-B and RN-S from one serotype and isolates Ro-A and Ro-S from the other (Fig. 1). However, none of the TSV antisera reacted in either test with purified antigens of AV II, CLRV, CVV, EMV, or TAMV.

Ring interface and agar gel double diffusion tests (Table 2, Fig. 2) demonstrated that AV II, CLRV, CVV, EMV, and TAMV are serologically interrelated. The degree of serological relatedness among these viruses ranged from very close to not detectable, depending upon the antiserum used. Only three of the 11 antisera listed in Table 2 detected all five viruses. One of these was prepared against AV II-P, the other two were prepared against CLRV. At the other extreme, our TAMV antiserum produced heterologous reactions with CLRV and CVV, but not with AV II or EMV. These results agree with Jones and Mayo (7) who found no direct serological relationship between EMV and TAMV. None of the antisera listed in Table 2 reacted with purified TSV antigens.

Ultraviolet absorption spectra. During this study we noted, as have others (2,3,7,13), that purified preparations of AV II, CLRV, CVV, EMV, and TAMV consistently produced ultraviolet absorption spectra that differed somewhat from that produced by TSV. Maximum and minimum absorbance for each of the five serologically related viruses consistently occurred at 262 and 245 nm, respectively, while maximum and minimum absorbance for all TSV isolates was 260 and 242 nm, respectively (Table 3). In addition, $A_{260/280}$ ratios (uncorrected for light scattering) for the five serologically related viruses ranged between 1.28 and 1.39, whereas values for TSV varied between 1.56 and 1.58.

TABLE 3. Characteristics of the ultraviolet absorption spectra of six ilarviruses

Virus[a]	A_{max}	A_{min}	$A_{260/280}$
AV II (both strains)	262	245	1.32–1.36
CVV	262	245	1.36
EMV	262	245	1.28–1.38
CLRV	262	245	1.35–1.39
TAMV	262	245	1.35–1.36
TSV (four isolates)	260	242	1.56–1.58

[a]AV II = asparagus virus II (strains P and S), CVV = citrus variegation virus, EMV = elm mottle virus, CLRV = citrus leaf rugose virus, TAMV = Tulare apple mosaic virus, and TSV = tobacco streak virus.

DISCUSSION

Our results indicate that AV II, CLRV, CVV, EMV, and TAMV comprise a subgroup of ilarviruses, all members of which are serologically interrelated. However, each of the five viruses appeared to be serologically unrelated to TSV. This confirms reports of nonrelationship between TSV and the two citrus viruses (6) and between TSV and EMV (7). If ilarvirus subgroups are to be defined on the basis of serological relationships between members within subgroups as proposed by the Plant Virus Subcommittee of ICTV (12) we propose that subgroup A be revised to reflect the experimental data as follows:

Subgroup I: Tobacco streak virus
Black raspberry latent virus

Subgroup II: Asparagus virus II
Citrus leaf rugose virus
Citrus variegation virus
Elm mottle virus
Tulare apple mosaic virus

No attempt is made at this time to classify viruses originally proposed for subgroup B, which includes Prunus necrotic ringspot, cherry rugose mosaic, rose mosaic, apple mosaic, and Danish plum line pattern viruses as well as hop viruses A and C (12). However, studies now in progress with members of this subgroup and the

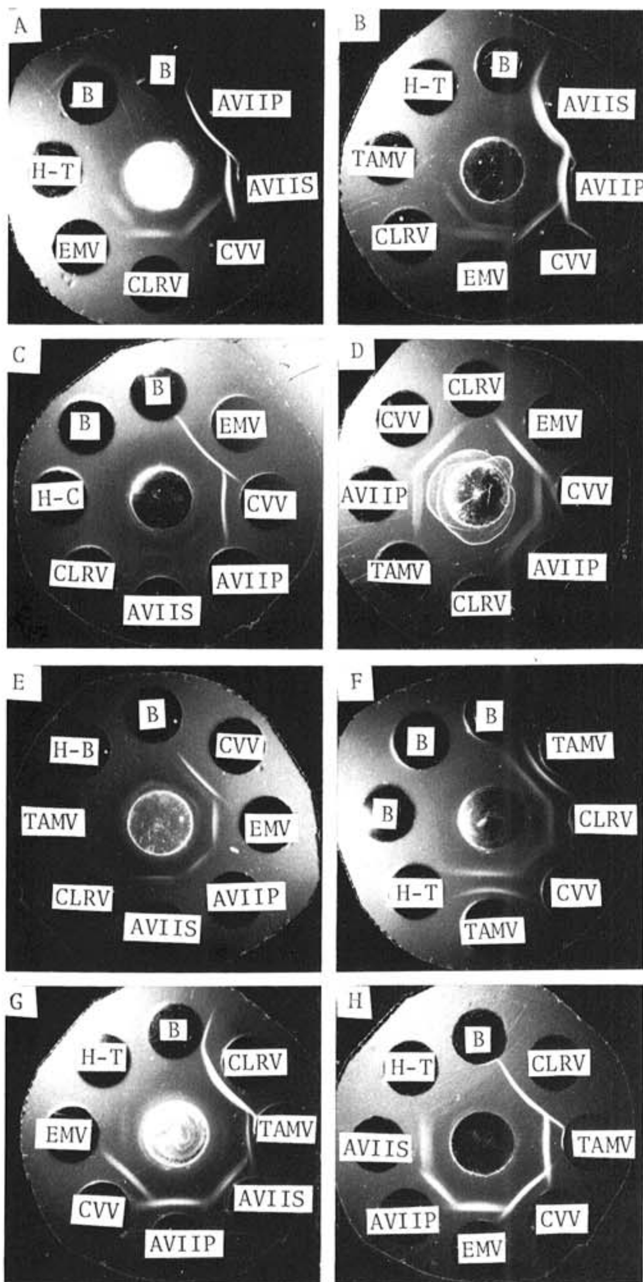

Fig. 2. Agar gel double diffusion serology tests using purified antigens (0.1–0.3 mg/ml) of asparagus virus II (AV II-P and AV II-S), citrus leaf rugose virus (CLRV), citrus variegation virus (CVV), elm mottle virus (EMV), and Tulare apple mosaic virus (TAMV) suspended in 0.01 M potassium phosphate buffer, pH 7.2 (well B). Preparations of healthy tobacco (HT), *Chenopodium quinoa* (H-C), and bean (H-B) leaf tissue were included as controls. Center wells contained the following undiluted antisera used in Table 2: **A,** AV II-P antiserum I-H; **B,** AV II-S antiserum I-H; **C,** EMV antiserum I-E; **D,** EMV antiserum from A. T. Jones; **E,** CVV antiserum G from S. M. Garnsey; **F,** TAMV antiserum I-F; **G,** CLRV antiserum I-E; and **H,** CLRV antiserum II-G from S. M. Garnsey.

preliminary results of McMorran and Cameron (10) suggest that some viruses now included in subgroup B or listed as possible members (prune dwarf and American plum line pattern viruses) will eventually be further subdivided on the basis of serological relationships. To avoid confusion in designating future subgroups, we propose elimination of the terms subgroup A and B and replace them with numerical designations to be assigned as new subgroups are defined.

It is interesting that members of the newly proposed subgroups I and II not only appeared to be serologically distinct, but they also exhibited different ultraviolet absorption characteristics. These differences were found consistently with highly purified preparations after density gradient ultracentrifugation and suggest that members of the two subgroups may differ in such characteristics as nucleic acid content or aromatic amino acid type or content. Although no attempt was made to determine the reason for these differences, the fact that they occur consistently may provide an additional characteristic for assigning new members to these subgroups.

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