Comparison of peanut stripe virus isolates using symptomatology on particular hosts and serology

Sopone WONGKAEW (1) and Michel DOLLET (2)

Summary. — Twenty four isolates of peanut stripe virus from 8 different countries were compared under controlled conditions at the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France. The isolates had their origins from Burma, India, Indonesia, Philippines, China, Thailand and USA. Four of the isolates were collected from Thailand in 1972 but maintained in Japan and sent to France for this particular study By using disease reactions on a set of groundnut genotypes and some other host species, the isolates could be grouped into 8 strains that are mild mottle, blotch, stripe, blotch-stripe, blotch-CP-N, chlorotic ring-mottle, chlorotic line-pattern and necrotic strains. Similarity was noted among isolates within the same strain grouping regardless of their origins. Serological results indicated that peanut chlorotic-ring mottle was an isolate of peanut stripe virus (PStV) but groundnut eyespot virus was a different virus. Serotyping using serological reactions of viral antigens from lesional tissue of *Chenopodium amaranticolor* to different polyclonal antibodies of different PStV isolates and PStV-related viruses, correlated well with the grouping based on host disease reactions. Similar testing using antigens from groundnuts was less effective in differentiating the strains Since all the isolates sent from Japan are identical to PStV, it could be assumed that PStV has been in Southeast Asia as early as 1972 when the samples were collected from Thailand.

INTRODUCTION

After its first report as « a virus producing mild mottle » (VPMM) in groundnut from China (Xu *et al.*, 1983), the virus was further characterized and named peanut stripe virus (PStV) in the following year (Demski *et al.*, 1984). Since then similar viruses causing stripe, blotch, green blotch, chlorotic rings, mild mottle or green mosaic in groundnuts have been reported from different countries in Southeast Asia (Wongkaew, 1986 ; Fukumoto *et al.*, 1986 ; Middleton and Saleh, 1988 ; Adalla and Natural, 1988). Because the virus isolates share most of the common properties of PStV and are serologically indistinguishable from PStV, the ad hoc committee on PStV nomenclature proposed that they should be recognized as isolates of PStV (Demski *et al.*, 1988). The committee, however, recommended that all these isolates should be tested under identical conditions to determine their relationships (ICRISAT, 1988). This precise identification is critical if a program such as multilocational testing of groundnut germplasm for PStV resistance is to be established. Third countries which can accept live infected groundnut material and are willing to provide facilities for the study were approached and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) at Montpellier, France, kindly accepted the responsibility in early 1989 when the experiment started.

In this study, the objective was to determine the relationships of PStV isolates collected from different countries by indicator host reactions and serology. Initially 28 isolates were included in the study but after prolonged storage during the transit, only 24 isolates survived. The isolates were kindly contributed by PStV coordinators from 7 different countries that are, China, India, Indonesia, Japan, Philippines, Thailand and USA.

(1) Departement of Plant Pathology, Faculty of Agriculture Khon Kaen University, Khon Kaen 400002, Thailand

(2) IRHO Virology Division, Michel Dollet, laboratoire de phytovirologie des régions chaudes. CIRAD, B.P. 5035, 34032 Montpellier Cedex, France. To whom requests for reprints should be addressed.

MATERIALS AND METHODS

Virus isolates.

The origin and description of 24 PStV isolates under study are given in table I. Except those isolates from Burma and India, the virus was preserved in infected dried leaf pieces of groundnut (*Arachis hypogaea* L.) or kintoki bean (*Phaseolus vulgaris* L.) or cowpea (*Vigna unguiculata* (L.) Walp.) and brought to France after a brief transit in Thailand. Most of the isolates were sent directly from the country of origin except those from Japan, and Burma. The isolates from Japan were collected from various sources in Thailand in 1972, maintained briefly in the greenhouse and preserved as freeze dried ever since. The isolate from Burma was recovered from infected groundnut seeds intercepted at the Plant quarantine Station of the National Bureau of Plant Genetic Resources, India. The Indian isolates were found in the same manner but the seeds were collected from native grown samples. Both Burman and Indian isolates had been subjected to one single lesion isolation in *Chenopodium amaranticolor* Coste & Reyn and preserved as dried necrotic lesions in the same species. Prior to the study, all isolates regardless of their origins, were subjected to one single lesion transfer in *C. amaranticolor*. Subsequently, the cloned isolates in Chenopodium sap were diluted 1 : 100 (w/V) with an inoculation buffer to eliminate the inhibitory effect of the sap before being inoculated and maintained in Tainan 9 groundnut.

Plant maintenance.

Plants were grown in a climatic chamber in 12 cm diameter plastic pots using Humin-Substrate Soil Mixture (Imex-Neuhaus, Italia) as a planting medium. Pokon plant nutrient (Pokon & Chrysal, Naarder-Holland), was supplemented to the plants once at 3 weeks after seeding. Light from day-light fluorescent and Groslux lamps (1 : 2 ratio) with the intensity of 10.000 lux at bench floor was given 12 hr daily. The temperatures of 21/29 °C, night/day were maintained throughout the planting cycle.

Inoculation procedure and host reaction.

Infected leaf tissue from Tainan 9 groundnut 15 days postinoculation was ground in a chilled mortar with 0.05 M

TABLE I. — **Origin of 24 isolates of PStV studied at CIRAD, Montpellier, France in 1989**

Isolate	Origin	Preserved host	Described symptom (a) on groundnut	Contributor	Remark
A_4	USA	Cowpea	Stripe	Demski	Isolated from groundnut
A_5	USA	Cowpea	Mild	Demski	Isolated from groundnut
A_6	USA	Groundnut	Blotch	Demski	Isolated from fresh groundnut leaves
B_2	Burma	Chenopodium	ND	Dollet-Reddy	Isolated from infected seed in India
C	China	Groundnut			
		Red Flower 1	ND	Xu	Isolated from infected seed
I_1	Indonesia	Groundnut	Blotch	Saleh	Isolated from fresh groundnut leaves
I_2	Indonesia	Groundnut	Stripe	Saleh	Isolated from fresh groundnut leaves
Id_1	India	Chenopodium	ND	Dollet-Reddy	From native grown groundnut
Id_2	India	Chenopodium	ND	Dollet-Reddy	2nd clone of Id_1
J_1	Japan	Kintokibean	ND	Kameya-Iwaki	Isolated from groundnut
J_2	Japan	Kintokibean	ND	Kameya-Iwaki	Collected from Thailand in 1972
J_4	Japan	Kintokibean	ND	Kameya-Iwaki	
P_4	Philippines	Groundnut	Blotch	Natural	—
P_5	Philippines	Groundnut	Oak-leaf pattern	Natural	—
P_6	Philippines	Groundnut	Chlorotic ring	Natural	—
T_1	Thailand	Groundnut, Tainan 9	Mild	Wongkaew	From Moket groundnut
T_2	Thailand	Groundnut, Tainan 9	Stripe	Wongkaew	From Tainan 9 groundnut
T_3	Thailand	Groundnut, Tainan 9	Blotch	Wongkaew	Induced necrosis in cowpea
T_4	Thailand	Groundnut, Tainan 9	Stripe	Wongkaew	Derived from T2
T_5	Thailand	Groundnut, Tainan 9	Chlorotic ring	Wongkaew	Maintained in greenhouse since 1984
T_6	Thailand	Groundnut, Tainan 9	Necrosis	Wongkaew	From Moket groundnut
T_7	Thailand	Groundnut, Tainan 9	Blotch	Wongkaew	From Tainan 9 groundnut
T_8	Thailand	Groundnut, Tainan 9	Blotch	Wongkaew	From Moket groundnut
T_9	Thailand	Groundnut, Tainan 9	Mild	Wongkaew	Induced local lesion in Topcrop

(a) ND = not described

potassium phosphate buffer pH 7.5 containing 0.1 % sodium sulfite and 1 % celite (inoculation buffer). Host plants as listed in table III were inoculated 7-10 days after seeding by rubbing the upper surface of young leaves or primary leaves with the expressed sap. Eight plants of each host were inoculated with sap from each isolate and 4 plants per host were rubbed with healthy groundnut sap as controls. Except for Tainan 9 in which seeds used in the study were collected from apparently virus free plants, none of the other hosts could be certified as virus free. However, all abnormal seedlings were discarded before starting the inoculation. Disease reactions were observed at 2 days interval from 4 to 20 days after inoculation.

Reaction on *Chenopodium amaranticolor*.

Similar study to the host reaction experiment were conducted using sap prepared from one single lesion collected from each cloned isolate on *C. amaranticolor*. The lesion was triturated in 100 µl of the inoculation buffer and rubbed onto the 4th, 5th and 6th leaves of 2-months old *C amaranticolor*. Observation on disease reactions were observed everyday starting 2 days after the inoculation. The experiment was repeated twice.

Serological study.

The reactions of viral antigen of all 24 isolates in *C. amaranticolor* and Tainan 9 groundnut to 11 polyclonal potyvirus antisera were compared quantitatively in enzymed-linked immunosorbent assays (ELISA). Origin and details of the antisera are given in table II. In Chenopodium,

TABLE II — **Antisera to 11 potyviuses used in the direct antigen coating-indirect anti-Fc ELISA of 24 peanut stripe virus isolates**

Antiserum to	Source
Bean common mosaic virus (BCMV)	Silbernagel, USA
Bean yellow mosaic virus (BYMV)	Albrechtsen, Denmark
Blackeye cowpea mosaic virus (BlCMV)	Gonsalves, USA
Groundnut eyespot virus (GEV)	Through Dollet, France
Lettuce mosaic virus (LMV)	Albrechtsen, Denmark
Peanut chlorotic ring mottle virus (PCRMV)	Kameya-Iwaki, Japan
Papaya ringspot virus (PRV)	J. B. Quiot, France
Peanut mottle virus (PMV)	Reddy, India
Peanut stripe virus-stripe isolate (PStV-St)	Demski, USA
Peanut stripe virus-chlorotic ring isolate (PStV-CR)	Wongkaew, Thailand
Soybean mosaic virus (SMV)	Kittipakorn, Thailand
Anti-rabbit Fc alkaline phosphatase conjugate	Reddy, India

lesional tissue from inoculated leaves were used as antigen. The tissue was ground in 0.05 M sodium carbonate buffer pH 9.6 and adjusted to 6.7 % tissue concentration for all the isolates. The groundnut tissue was prepared similarly but 2 leaflets of the 1st fully unfold quadrifoliolate 11-days postinoculation were used instead. Three replications from 3 separate infected plants of each isolate were assayed using the direct antigen-coating-indirect anti-Fc ELISA. Briefly, the antigen samples were placed in ELISA plates and incubated for 7 h at 4 °C. After washing, the antiserum of each virus at 1 : 5,000 dilution in the conjugate buffer (Clark and Adams, 1977), were pipetted into the wells and incubated overnight at 4° C. A goat anti-rabbit Fc alkaline phosphatase conjugate at 1 : 10,000 dilution was used as a second antiserum. After 90 min of incubation at 37 °C, the reactant was washed away and replaced by the substrate (p-nitrophenyl phosphate 1 mg/ml in substrate buffer). Absorbance values (405 nm) were determined with a Titertek Multiscan ELISA Reader (Flow Laboratories) after the incubation at 22 °C for 1 hr. Absorbance values were considered positive if they exceeded the healthy control by a factor of two.

RESULTS

Reactions in groundnut.

Chlorotic flecks first appeared on some groundnut genotypes at 4 days after inoculation. The infection frequencies observed at 12 days were 75-100 % regardless of the isolates or genotypes. At 16 days the symptoms caused by each isolate became distinctively varied but were almost identical among the groundnut genotypes. The symptoms could be classified into 7 groups (Table III) ranging from mild mottle to severe necrosis and stunting. Majority of the isolates falls into mild mottle and blotch groups. Some isolates induced the combination of symptoms between blotch and stripe. Isolates T_5, A_4 and T_6 caused considerable unique symptoms

TABLE III — **Systemic disease reactions of 10 legume genotypes to 24 isolates of peanut stripe virus**

Isolate	Host and systemic disease reaction (a)									
	Arachis hypogaea				*Glycine max*		*Vigna unguiculata*		*Phaseolus vulgaris* (b)	
	Tainan 9	55-437	Florunner	RMP 12	SJ 5	45-76	KC 84 R	C 152	Topcrop	Contender
Mild mottle group										
T_1	MMt	MMt	MMt	MMt	0	0	VMMt	0	0	0
C	MMt	MMt	MMt	MMt	0	0	VMMt	0	0	0
I_1	MMt	MMt	MMt	MMt	0	0	VMMt	0	0	0
B_2	MMt	MMt	B	MMt	Mt 1/8	0	VMMt	0	0	0
Id_1	MMt	MMt	MMt	MMt	Mt 1/8	0	VMMt	0	0	0
Id_2	MMt	MMt	MMt	MMt	Mt 1/8	0	VMMt	0	0	0
P_4	MB	MB	MB	MMt	0	0	VMMt	0	0	0
J_4	MMt	MMt	MMt	MMt	M	0	VMMt	0	0	0
Blotch group										
T_7	B	B	B	B	Mt 1/8	0	VMMt	VMMt	0	0
T_8	B	B	B	B	Mt 1/8	0	VMMt	VMMt	0	0
P_6	B	B	B	B	Mt	MT	VMMt	0	0	0
A_6	MB	B	B	B	M	M	VMMt	Mt	0	0
J_1	MB	B	B	B	0	CS	VMMt	0	0	0
J_2	SB	SB	SB	SB	0	CS	VMMt	0	0	0
T_3	B	B	B	B	VN, M	VN, M	N	VMMt	0	0
A_5	MB	B	B	B	Mt	0	VMMt	0	0	0
Stripe group										
T_2	St, Stu	St, Stu	St, Stu	St, Stu	M	YVB	Ch, Stu	Mt	0	0
T_4	St, Stu	St, Stu	St, Stu	St, Stu	GVB	YVB	YVB	Mt	0	0
P_5	St, Stu	St, Stu	St, Stu	St, Stu	VC	YVB	Ch, Stu	Mt	0	0
Blotch-stripe group										
T_9	St	Mt	B	MB	0	CS	VMMt	VMMt	0-VN	0
I_2	St	OP	B	St	0	CS	VMMt	VMMt	0	0
Chlorotic ring-mottle group										
T_5	CRM	CRM	CRM	CRM	CRM	M	YVB	Mt	0	0
Chlorotic line-pattern group										
A_4	CLP	CLP	CLP	CLP	YVB	Mt	CR	CR	O-VN	0
Necrotic group										
T_6	N, Stu	N, Stu	N, Stu	N, Stu	0	0	Mt	0	0	0

(a) 0 = no systemic infection, B = blotch, Ch = chlorosis, CLP = Chlorotic line pattern, CR = chlorotic ring, CRM = Chlorotic ring mottle, CS = Chlorotic spot, GVB = green vein-banding, M = mosaic, MB = mild blotch, Mt = mottle, MMt = mild mottle, N = necrosis, OP = Oakleaf-pattern, St = stripe, SB = stripe-blotch, Stu = stunt, VC = vein clearing, VN = vein necrosis YVB = yellow vein-banding Seventy-five to 100 % of the plants became infected except those indicated by number ie 1/8 = 1 infected plant from 8 being inoculated.

(b) Local vein necrosis appeared on the leaves of Topcrop bean inoculated with T_9 and A_4

therefore, they were placed as distinctive groups. Symptoms induced by a representative member of each group on Tainan 9 groundnut are as shown in figure 1.

Reactions in other legumes.

All of the isolates could not infect Contender bean but T_9 and A_4 caused vein necrosis on the inoculated primary leaves of Topcrop bean. Sap made from the lesional tissue gave positive reaction when assayed by ELISA indicating presence of the virus in the lesions. All of the isolates could infect KC 84 R cowpea but the symptoms varied depending on the groups. Those that induced mild mottle, blotch or blotch-stripe symptoms in groundnuts mostly caused very mild mottle symptoms in KC 84 R cowpea (Table III and Fig. 2). Most of the plants with this type of symptoms had been confirmed for the presence of PStV by ELISA. Within this group, T_3 induced red local necrotic lesions which stayed discrete on the inoculated leaves, subsequently necrotic flecks appeared on young leaves and stems. The plants eventually died within 8 days after inoculation. Symptoms caused by other groups ranged from chlorosis with stunting, vein banding, chlorotic rings to mottling. Most of the isolates in the mild mottle group could not infect C 152 cowpea and Senegal 45-76 soybean. SJ 5 soybean was extremely resistant to most of the isolates in this group except J_4 which could induce mosaic symptom on 75 % of the inoculated plants. These 3 genotypes reacted similary to isolate T_6 in the necrotic group. Isolates in stripe, chlorotic ring-mottle and chlorotic line-pattern groups, all could infect C 152 cowpea, SJ 5 and 45-76 soybeans.

Reactions in *Chenopodium amaranticolor*.

Disease reactions in Chenopodium were of 4 types (Table IV and Fig. 3). Most of the isolates in mild mottle group produced an average number of chlorotic lesions (16-40) with 6-7 days latent period. In some isolates (T_1, I_1 and J_4) few chlorotic lesions developed on the uninoculated young leaves indicating a systemic infection. Reactions to isolates in blotch or blotch-stripe groups appeared as necrotic lesions with a short latent period (4 days). In general, they looked similar but the numbers produced by those in blotch group were numerous (24-93) while the blotch-stripe produced only few lesions (6-7). Reactions to isolate A_5 of the blotch group resembles those of the mild mottle group in that the lesions were chlorotic and had a longer latent period. Lesions produced by those isolates in stripe, chlorotic ring-mottle and necrotic groups were also of necrotic, short-latent period type but having a larger diameter than those in the blotch group. Reactions to isolate A_4 of the chlorotic line-pattern group were similar to those in the mild mottle group.

Serological reaction.

All PStV isolates reacted positively to antisera to PCRMV, PStV-St, PStV-CR, SMV, BlCMV and BCMV regardless of the hosts. All other antisera, (BYMV, PMV, PRV, LMV and GEV) gave negative results (data not shown). Because it was not possible to equalize the titers of all the antisera that gave positive reactions to the isolates, the absorbance (405) value of the isolate that gave the highest reading to each particular antiserum was converted into 1 unit and the readings from other isolates were adjusted accordingly.

In Chenopodium, reactions of the isolates to 6 antisera could be classified into 5 serotypes, as shown in table V. In serotype-CI the antigens gave highest A 405 with the antiserum to PCRMV while other antisera gave average equal values. Most of the isolates classifed as mild mottle and blotch fall into this serotype. Those isolates which gave stripe (T_2, T_4), chlorotic ring mottle (T_5), severe blotch (J_2) and chlorotic line-pattern belong to serotype-CII in which the reaction to anti-PCRMV and anti-BlCMV were equally strong. Average equal values were noted in the reactions with other antisera. In serotype-CIII the isolates reacted strongly with anti-PCRMV, anti-PStV-CR and anti-BlCMV but less strong with the others. In serotype-CIV, the isolates gave highest A 405 values with all antisera (T_6) while in serotype-CV the isolate (T_3) reacted most strongly with anti-PStV-CR but gave average equal values with all the others.

TABLE IV. — **Disease reactions in *Chenopodium amaranticolor* inoculated with one single lesion of different isolates of peanut stripe virus**

Isolate	Disease reaction on inoculated leaves		
	Symptom (a)	Lesion number (b)	Latent period (c)
Mild mottle group			
T_1	CL-S	18	6
C	CL	16	6
I_1	CL-S	27	7
B_2	CL	18	6
Id_1	CL	22	6
Id_2	CL	40	6
P_4	CL	18	6
J_4	NL-S	52	4
Blotch group			
T_7	NL-S	48	4
T_8	NL	44	4
P_6	NL	24	4
A_6	NL-S	93	4
J_1	NL	84	4
J_2	NL	92	4
J_3	NL	26	4
A_5	CL	30	7
Blotch-stripe group			
T_9	NL	6	4
I_2	NL	7	4
Stripe group			
T_2	LNL	39	4
T_4	LNL	52	4
P_5	LNL	71	4
Chlorotic ring-mottle groupe			
T_5	NL	16	4
Chlorotic line-pattern group			
A_4	CL	28	7
Necrotic group			
T_6	LNL-S	38	4

(a) CL = Chlorotic lesion, CL-S = chlorotic lesion subsequently turned systemic,
NL = necrotic lesion (smaller than 2.0 mm in diameter),
LNL = large necrotic lesion (larger than 2.0 mm in diameter).
(b) Number of lesion developed on 3 leaves averaged from 2 trials taken at 10 days postinoculation.
(c) The period when 50 % of lesions appeared on the inoculated leaves

In groundnut, the reactions could be divided into 6 different serotypes (Table VI) which were considerably different from those of Chenopodium. In serotype-GI the A 405 noted from the reactions to anti-PStV-St, anti-SMV and anti-BlCMV were equally high but those obtained from the others were slightly lower. Sixteen of the isolates fall into this type. In serotype-GII the reactions were equally strong with all antisera. In serotype-GIII the isolates reacted strongly with anti-PCRMV and anti-BlCMV, followed by anti-PStV-St and anti-SMV. The weakest reactions were obtained from anti-PStV-CR and anti-BCMV. In serotypes-G IV and GV the isolates reacted very strongly with anti-SMV and anti-BlCMV respectively. In serotype-GVI, the reactions to all antisera were equally high except to that of anti-PStV-CR which was exceptionally low.

DISCUSSION

Results from both the serological relationship with the 6 antisera and the ability to infect most of the groundnut cultivars tested, indicate clearly that all 24 isolates are PStV. Although isolate T_9 in groundnut sap reacted strongly with anti-SMV, its failure to infect SJ 5 soybean, and the ability to infect all groundnut genotypes suggested that it is still an isolate of PStV. Likewise, isolate A_5 which reacted strongly with anti-BlCMV failed to infect C 152 cowpea but could infect Florunner and other groundnut genotypes confirmed that it is also an isolate of PStV not BlCMV. Florunner has been reported to be resistant to BlCMV (Lima *et al.*, 1979).

From host range and disease reactions, a certain similarity was found among isolates that gave mild mottle and blotch symptoms regardless of the geographical origins. In general, mild mottle group could be separated from blotch and the others by its typical symptoms on groundnut genotypes, its inability to infect C 152 cowpea and Senegal 45-76 soybean and its inability or difficulty to infect SJ 5 soybean. Isolates in this group also gave a distinctive symptom on *C. amaranticolor*. In this view combining with the disease reactions on soybean, isolate J_4 which although gave mild symptom on groundnuts, but induced necrotic lesions typical of those in blotch group, should be placed in the blotch group. Again, isolate A_5 which gave blotch symptom on groundnuts but induced chlorotic lesions with long latent period on *C. amaranticolor* should belong to the mild mottle group. From this result, it clearly indicates that minor variations in terms of symptom expression on groundnuts do exist within the group. Isolate A_5, originally was designated as « mild ». Within the blotch group itself isolate T_3

TABLE V. — **Absorbance values (A 405) in direct antigen coating-indirect anti Fc ELISA of homologous reactions between peanut stripe virus isolates in lesional tissue of *Chenopodium amaranticolor* to 6 antisera of potyviruses** (a)

Isolate	Antiserum to PCRMV	PStV-St	PStV-CR	SMV	BlCMV	BCMV
Serotype-CI						
T_1	0.42 ± 0.19	0 27 ± 0.12	0.29 ± 0.17	0.24 ± 0.15	0.36 ± 0.13	0.29 ± 0.13
C	0.78 ± 0 16	0.68 ± 0.17	0.65 ± 0.23	0.57 ± 0.14	0.65 ± 0.14	0 52 ± 0.11
I_1	0.62 ± 0 15	0.49 ± 0.12	0.45 ± 0.08	0.40 ± 0.05	0 54 ± 0.11	0.43 ± 0.06
B_2	0.81 ± 0.23	0.76 ± 0.28	0.74 ± 0.31	0.77 ± 0 27	0 79 ± 0.25	0.80 ± 0.25
Id_1	0.83 ± 0.17	0.78 ± 0.25	0.66 ± 0.21	0.73 ± 0 25	0 76 ± 0.14	0 75 ± 0.20
Id_2	0.77 ± 0.16	0.72 ± 0.23	0.64 ± 0 20	0.68 ± 0 21	0.71 ± 0.13	0 70 ± 0.14
J_4	0.63 ± 0.15	0.45 ± 0.17	0.44 ± 0 22	0.44 ± 0 17	0.52 ± 0.10	0 43 ± 0.10
T_7	0.83 ± 0.07	0.69 ± 0.07	0.69 ± 0 03	0.65 ± 0.02	0 61 ± 0 02	0.58 ± 0 06
T_8	0.81 ± 0.21	0.60 ± 0.28	0 65 ± 0 30	0.62 ± 0.28	0 69 ± 0 23	0.60 ± 0.24
P_6	0 86 ± 0.17	0.64 ± 0.24	0 71 ± 0.24	0.68 ± 0.23	0 74 ± 0 21	0.70 ± 0.15
J_1	0 80 ± 0.12	0.56 ± 0.15	0 59 ± 0.19	0.59 ± 0.16	0 66 ± 0 13	0.58 ± 0.15
I_2	0 93 ± 0.15	0.77 ± 0.31	0 77 ± 0.28	0.76 ± 0.26	0 78 ± 0 16	0.75 ± 0 19
A_5	0.72 ± 0.14	0.67 ± 0.17	0 61 ± 0.21	0.64 ± 0.18	0 69 ± 0.14	0.63 ± 0 13
A_6	0.75 ± 0.18	0 64 ± 0.02	0 61 ± 0.24	0.60 ± 0 21	0 64 ± 0.16	0.56 ± 0.17
Serotype-CII						
T_2	0.58 ± 0.13	0 44 ± 0.15	0.46 ± 0.18	0 47 ± 0 16	0.53 ± 0.11	0 45 ± 0 10
T_4	0.48 ± 0.03	0 43 ± 0.09	0 41 ± 0 04	0.37 ± 0.10	0 51 ± 0 05	0.41 ± 0 05
T_5	0.49 ± 0.10	0.39 ± 0.13	0 40 ± 0 13	0.39 ± 0.17	0 48 ± 0 09	0.39 ± 0 09
J_2	0 62 ± 0 07	0.52 ± 0.08	0 56 ± 0 09	0 55 ± 0.08	0.65 ± 0 09	0.57 ± 0.09
A_4	0 74 ± 0.15	0.66 ± 0.22	0 67 ± 0 25	0 64 ± 0.23	0.72 ± 0 14	0.67 ± 0.16
Serotype-CIII						
T_9	0.83 ± 0.19	0 73 ± 0 22	0 89 ± 0.29	0.75 ± 0.20	0.81 ± 0.20	0.76 ± 0 20
P_4	0.94 ± 0.21	0 82 ± 0 31	0.99 ± 0.40	0.86 ± 0 23	0.92 ± 0.27	0.86 ± 0.24
P_5	0.84 ± 0.12	0 75 ± 0 13	0.83 ± 0.20	0.81 ± 0 13	0.84 ± 0.12	0 76 ± 0.10
Serotype-CIV						
T_6	1.00 ± 0.11	1.00 ± 0.24	1.00 ± 0 24	1.00 ± 0.22	1 00 ± 0.13	1.00 ± 0.17
Serotype-CV						
T_3	0.63 ± 0.12	0.51 ± 0 15	0.83 ± 0.23	0.53 ± 0.20	0.64 ± 0 13	0.52 ± 0.12
Healthy tissue	0.03	0 01	0.08	0.02	0.07	0 08

(a) Details described in the text.

TABLE VI. — **Absorbance values (A 405) in direct antigen coating-indirect anti Fc ELISA of homologous reactions between peanut stripe virus isolates in groundnut tissue to 6 antisera of potyviruses** (a)

Isolate	Antiserum to PCRMV	PStV-St	PStV-CR	SMV	BlCMV	BCMV
Serotype-GI						
T_1	0.69 ± 0.30	0.76 ± 0.03	0.61 ± 0.08	0.81 ± 0.03	0.78 ± 0 04	0.70 ± 0.08
C	0.70 ± 0.01	0.77 ± 0.06	0.62 ± 0.07	0.69 ± 0.04	0.75 ± 0 02	0.64 ± 0.02
B_2	0.88 ± 0.01	0.92 ± 0.04	0.81 ± 0.04	0 90 ± 0.02	0.91 ± 0.08	0.88 ± 0.08
Id_1	0.85 ± 0.07	0.89 ± 0.06	0.73 ± 0.12	0 89 ± 0.04	0.93 ± 0 10	0.80 ± 0.05
Id_2	0.80 ± 0.04	0.85 ± 0.07	0.78 ± 0 07	0 81 ± 0 16	0.88 ± 0 14	0 76 ± 0.10
T_7	0.75 ± 0.08	0.90 ± 0.06	0 69 ± 0 06	0 81 ± 0 03	0 82 ± 0.08	0 78 ± 0.02
T_8	0.81 ± 0.02	0.90 ± 0.72	0.76 ± 0 04	0 93 ± 0 02	0 90 ± 0.08	0 86 ± 0.12
J_1	0 78 ± 0.05	0.82 ± 0.02	0.76 ± 0 06	0.84 ± 0 04	0.86 ± 0.07	0.77 ± 0.06
J_2	0 84 ± 0.08	0 94 ± 0.13	0 75 ± 0 04	0 93 ± 0 06	0 92 ± 0.05	0.86 ± 0.05
P_4	0 86 ± 0.13	0.89 ± 0.05	0.78 ± 0.08	0.86 ± 0.02	0.91 ± 0.05	0.78 ± 0.07
P_6	0 76 ± 0.07	0.75 ± 0.07	0.65 ± 0.07	0.72 ± 0.05	0.76 ± 0.01	0.70 ± 0.09
T_2	0 63 ± 0.03	0.76 ± 0 05	0.62 ± 0.04	0.73 ± 0 04	0.73 ± 0.07	0.65 ± 0.06
T_3	0 56 ± 0.04	0.62 ± 0 05	0.55 ± 0.03	0.64 ± 0.09	0.65 ± 0.05	0.58 ± 0.09
T_4	0.76 ± 0.04	0.81 ± 0.06	0.74 ± 0.08	0.78 ± 00.6	0 87 ± 0.08	0 72 ± 0 10
T_5	0.70 ± 0.05	0.78 ± 0.09	0.68 ± 0.07	0.78 ± 0 05	0 82 ± 0.04	0 77 ± 0 07
P_5	0.67 ± 0.04	0.71 ± 0.01	0.65 ± 0.07	0.74 ± 0 06	0 77 ± 0 03	0 68 ± 0 04
Serotype-GII						
I_1	0.88 ± 0.11	0.85 ± 0 01	0 82 ± 0.10	0.86 ± 0.06	0.82 ± 0.05	0.88 ± 0.06
I_2	1.00 ± 0.13	1.00 ± 0.10	1.00 ± 0.05	1.00 ± 0.08	1.00± 0.12	1 00 ± 0 03
A_6	0 68 ± 0 04	0.69 ± 0.02	0.61 ± 0.11	0.64 ± 0.01	0.69 ± 0.08	0.68 ± 0.04
Serotype-GIII						
A_4	0.84 ± 0 13	0 79 ± 0 05	0 66 ± 0.04	0 78 ± 0.07	0.82 ± 0.02	0.66 ± 0.06
J_4	0.84 ± 0 09	0 79 ± 0 02	0 72 ± 0.03	0.73 ± 0.04	0.86 ± 0.05	0.71 ± 0.02
Serotype-GIV						
T_9	0 79 ± 0.03	0.82 ± 0.30	0.70 ± 0.06	0.95 ± 0.02	0.89 ± 0.03	0.82 ± 0.09
Serotype-GV						
A_5	0.89 ± 0.05	0 89 ± 0 16	0 75 ± 0.09	0 82 ± 0.04	0.98 ± 0.16	0.79 ± 0.05
Serotype-GVI						
T_6	0.85 ± 0.09	0 90 ± 0 04	0 70 ± 0 04	0 94 ± 0.03	0.94 ± 0.03	0.88 ± 0.08
Healthy tissue	0.24	0.16	0 25	0.28	0.20	0.24

(a) Details described in the text.

although gave a typical blotch symptom, its wider host range and necrotic reaction on KC 84 R cowpea makes it a distinct isolate. The unique serological reactions also support this view This is in contrast with isolate J_2 which gave a severe blotch or dark green-mosaic symptoms on groundnut but still shared most of the common characteristics of blotch group. In the stripe group, the isolates produced typical stripe symptoms and stunted the groundnuts considerably. They could infect most of the tested plants except beans. Although T_2 and P_5 came from different countries, their reactions on host plants especially on KC 84 R cowpea were almost identical. This is in contrast, to isolate T_4 which originally derived from T_2, this isolate consistently induced yellow vein-banding on KC 84 R while T_2 always gave chlorosis symptom. This perhaps indicates the versatility of this virus in terms of symptom expression. Necrotic lesions produced on *C. amaranticolor* by stripe isolates were similar to those produced by the blotch isolates but they were much larger in size. And by the reaction on *C. amaranticolor*, the I2 isolate which originally designated as « stripe » was put into blotch-stripe group. This isolate was very similar to T_9 isolate. Both of them do not stunt the groundnuts and could not infect SJ 5 soybean. Besides they only gave few lesions of blotch type on *C. amaranticolor*. Since these 2 isolates shared both characteristics of blotch and stripe isolates, as a consequence they were put in botch-stripe group Disease reactions in other 3 groups were considerable unique, therefore, the isolates were treated as distinctive. As based on the disease reactions and host range we proposed the isolates should be classified into 8 strains as summarized in table-VII. The similarity of some of the isolates regardless of their geographical difference may indicate their common origin. In view of vast variations of symptoms found among the Thai isolates, it could be hypothesized that PStV may have originated in this region. In fact the symptoms expressed by J_2 isolate fit the description given by Ting *et al.* (1972) from Malaysia. It should be noted that all the Japanese isolates were collected from Thailand in 1972. The J_2 isolate was collected from Mahasarakam, northeast of Thailand. This implies that PStV may have been in this area as early as 1972, almost 11 years before it was officially reported (Xu *et al.*, 1983).

The reaction given by isolates T_9 and A_4 on Topcrop bean is rather surprising since this host is normally used to

TABLE VII. — **Proposed strains of 24 isolates of peanut stripe virus as based on disease reactions on specific host genotypes**

Strain	Descriptive characteristic	Isolate
Mild mottle	Induce mild mottle on groundnuts, fail or difficult to infect SJ 5 soybean, fail to infect C 152 cowpea and Senegal 45-76 soybean, produce chlorotic lesions on *C. amaranticolor* after 6 days and have no effect on groundnut growth	**T_1, C, I_1, B_2, Id_1, Id_2, P_4 and A_5**
Blotch	Induce blotch on groundnuts, may fail to infect SJ 5 soybean and C 152 cowpea, produce necrotic lesions on *C. amaranticolor* after 4 days and have no effect on groundnut growth.	**T_7, T_8, P_6, A_6, J_1, J_2 and J_4**
Blotch-CP-N	Similar to blotch but can induce systemic necrosis on KC 84 R cowpea.	**T_3**
Stripe	Induce stripe and stunting on groundnuts, infect most of the species tested except bean, and produce large necrotic lesions on *C amaranticolor* after 4 days.	**T_2, T_4 and P_5**
Blotch-stripe	Induce combination of blotch and stripe symptoms on groundnut, fail to infect SJ 5 soybean and produce blotch type of lesions on *C. amaranticolor* but fewer in number.	**T_9 and I_2**
Chlorotic ring-mottle	Similar to stripe isolates but produce typical chlorotic ring symptom on groundnuts and have less number of stripe type of lesions on *C. amaranticolor*	**T_5**
Chlorotic line-pattern	Induce chlorotic line-patterns or rings on groundnuts, have similar host range to that of stripe, and produce necrotic lesions of mild mottle type. Do not reduce growth in groundnuts	**A_4**
Necrotic	Induce systemic necrosis and severly stunt groundnuts. Fail to infect SJ 5 and Senegal 45-76 soybeans and C 152 cowpea.	**T_6**

differentiate PMV from PStV. Topcrop has been reported to be immune to PStV (Demski *et al.*, 1984). When the symptom first appeared on Topcrop we anticipated the contamination by PMV but after the ELISA test, it was confirmed that the lesions were caused by PStV When examined closely the lesions were considerably different from those induced by PMV. They were much fewer in number and limited themselves to the lateral veins Since this happened to only 2 out of 16 leaves inoculated, we beleived it could be a chance infection. However, caution should be taken when Topcrop bean is the only index plant used in the differentiation of the two viruses. We offer the same explanation to the systemic infection in *C. amaranticolor* by some of the isolates. It occurred only on few plants and few systemic lesions developed.

With the serological experiment, we have eventually proved that PCRWV is an isolate of PStV and GEV has no relationship with PStV. As expected, anti-BCMV also reacted positively with the isolates while anti-PRV and LMV did not. Attempt has been made to arrange the isolates into serotypes using the reaction profiles to the 6 antisera that gave positive reactions to the isolates. In a certain sense the 6 antisera can behave like a set of monoclonal antibodies because they were produced specifically for particular PStV isolates or viruses. This is clearly shown when saps from infected Chenopodium was assayed. Although the profiles can not be used to differentiate the isolates in the mild mottle from that in the blotch group (serotype-CI) it can sort out those in stripe, chlorotic ring-mottle, severe blotch and chlorotic line-pattern into one type (serotype-CII). Isolates T_9 and P_5 (blotch-stripe and stripe) were placed into serotype-CIII. Isolates T_6 and T_3 which gave a distinctive symptom on the particular hosts can be clearly differentiated by the reaction profiles (serotype-CIV and serotype-CV). In groundnut saps the antigens appeared to react differently from that observed in Chenopodium. The isolates in mild mottle, blotch and stripe all fall into serotype-GI while no correlation can be established between members of the other serotypes. By comparing the reaction profiles of the 2 sources of antigens to be used for serotyping it is suggested that those from Chenopodium should be the one of choice because of its high correlation with the isolates grouping by host range and reaction. At this stage it is still unknown why the antigesn behave differently when they are produced in different hosts. In our study, inclusion bodies were never found in the necrotic tissue of PStV infected *C. amaranticolor* while the same type of examination revealed numerous numbers of the inclusion bodies in infected groundnuts. Potyvirus particles are known to attach to the inclusion arms or membrane (Kim and Fulton, 1969) and there is no exception in PStV (Rechcigl *et al.*, 1989). The attachment of the particles to the inclusion arms or membrane might interfere with the reaction between antigens and antibodies, therefore makes it more erratic. In Chenopodium by not having the inclusion bodies such an effect may not exist, as a consequence the whole particles can react with the antibodies.

It should be stated that our prime objective of using polyclonal antibodies in this study is not to differentiate the strains but to confirm the relationship of the isolates to a particular member of legume potyviruses. However with a good correlation found between the reaction profiles of the isolates to particular antisera and the host reaction, this type of comparison may be useful and could be used as an additive information in the classification. It will be most desirable to repeat this experiment employing the novel technique proposed by Shukla *et al.* (1989) in potyvirus taxonomy

Acknowledgements. *— We would like to thank the Laboratoire de phytovirologie des régions chaudes CIRAD/ ORSTOM and Institut de Recherche pour les Huiles et Oléagineux hosting the experiment. The financial supports from the International Development Research Centre (IDRC) of Canada and Peanut Collaborative Research Support Program (Peanut CRSP) of the United States are gratefully appreciated. This experiment would have been impossible without the excellent cooperation from all the colleages, France, J. W. Demski of USA, DVR Reddy of ICRISAT, India, M. Kameya-Iwaki of Japan, Xu Zeyong of China, M. Natural of the Philippines and N. Saleh of Indonesia. The assistance from ICRISAT in making the arrangement with CIRAD is fully acknowledged.*

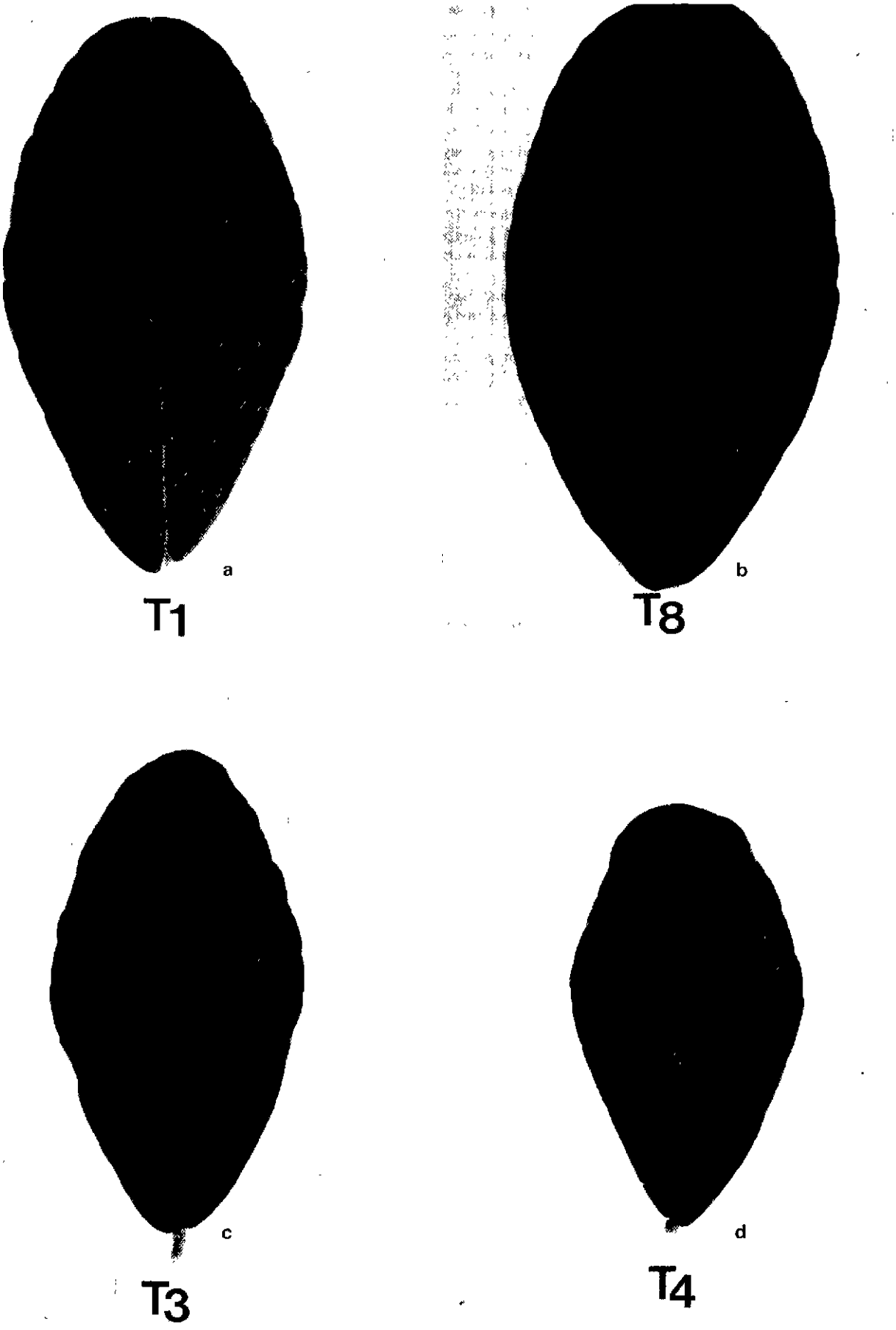
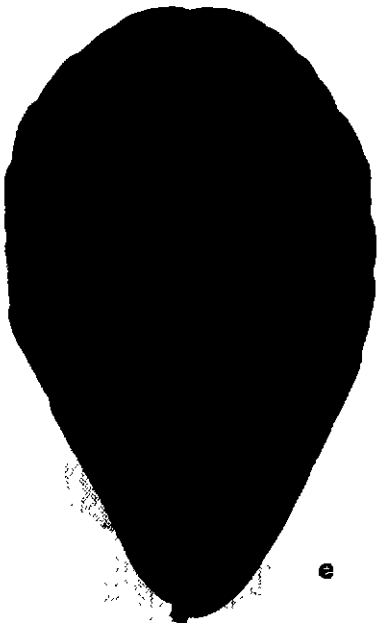
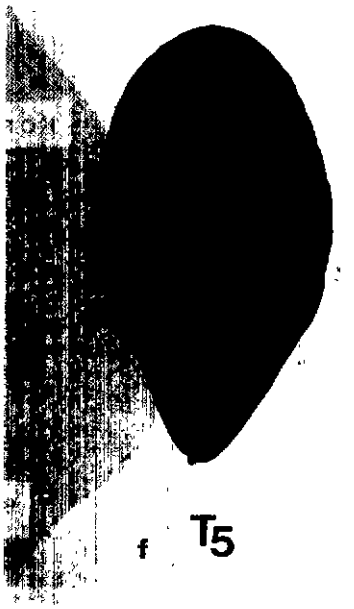


FIG 1 — Symptoms on Tainan 9 groundnut induced by a representative isolate of peanut stripe virus from 8 groups as described in Table 7. The photographs were taken at 20 days postinoculation. **a.** Mild mottle (T_1). **b.** Blotch (T_8). **c.** Blotch-CP-N (T_3) **d.** Stripe (T_4). **e.** Blotch stripe (I_2). **f.** Chlorotic ring-mottle (T_5) **g.** Chlorotic line-pattern (A_4) **h.** Necrotic (T_6)

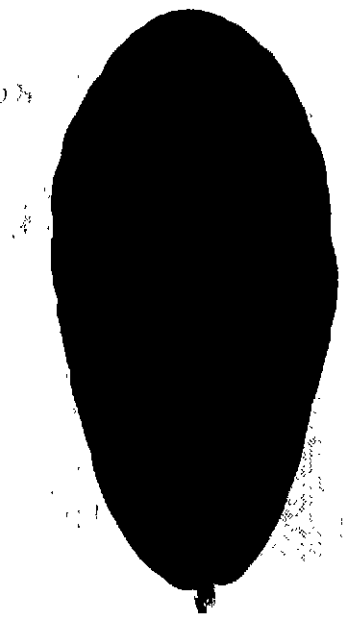


e

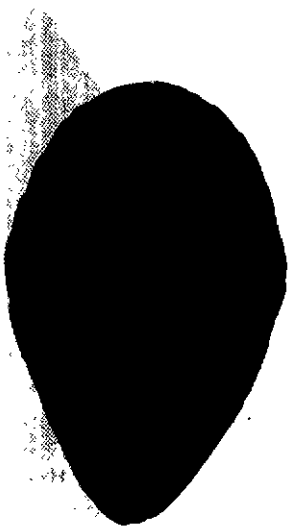
I2

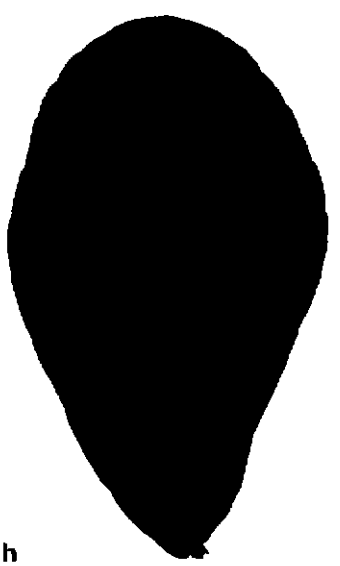


f T5



A4 g





T6 h

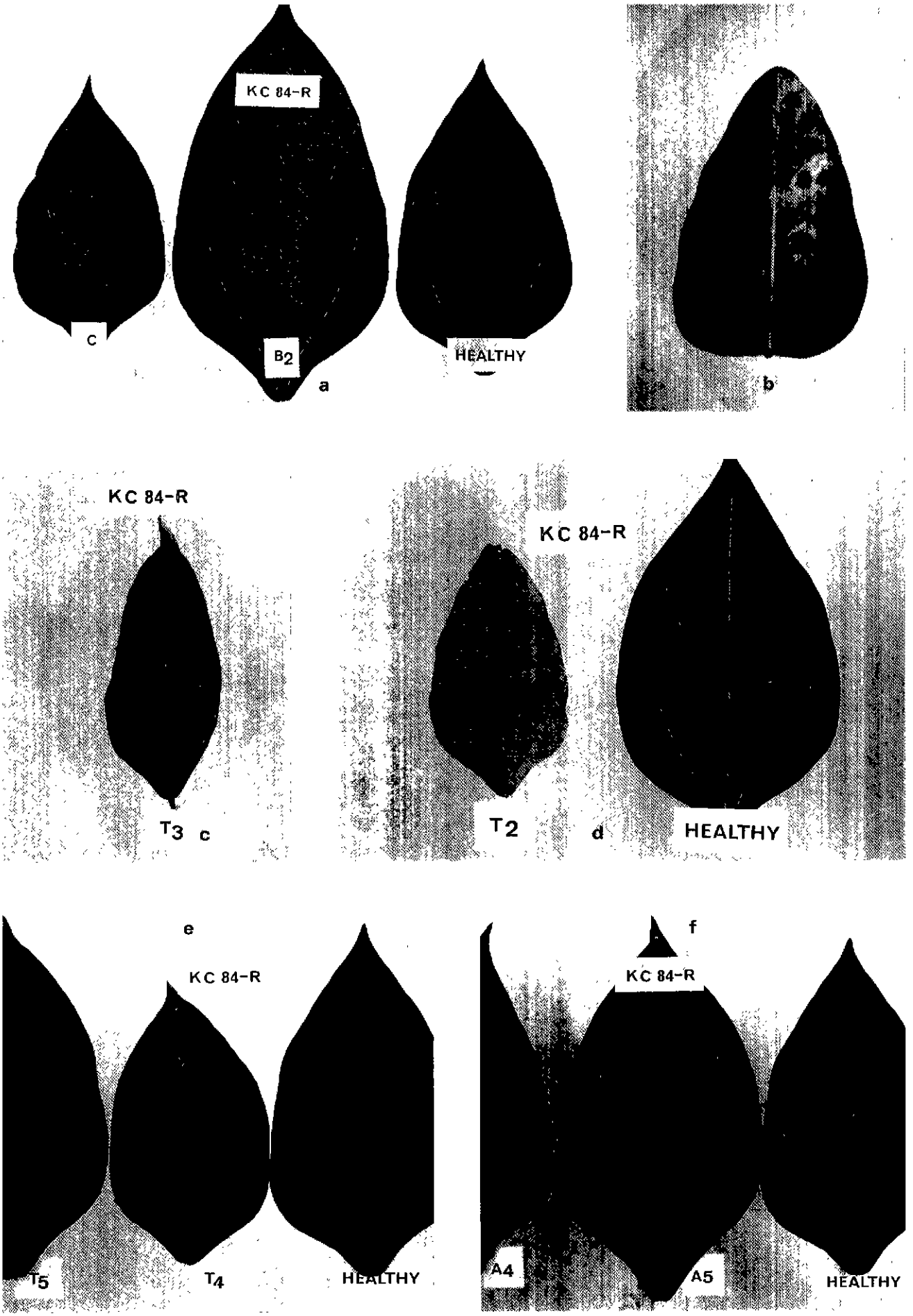


FIG 2 — Symptoms on KC 84 R cowpea inoculated with some peanut stripe virus isolates. All pictures were taken at 20 days postinoculation except Figure b. in which the picture was taken at 8 days **a.** Systemic mild mottle. **b.** Necrotic lesions on inoculated primary leaf **c.** Systemic necrotic flecks **d.** Systemic chlorosis **e.** Systemic chlorotic midrib. **f.** Systemic chlorotic rings

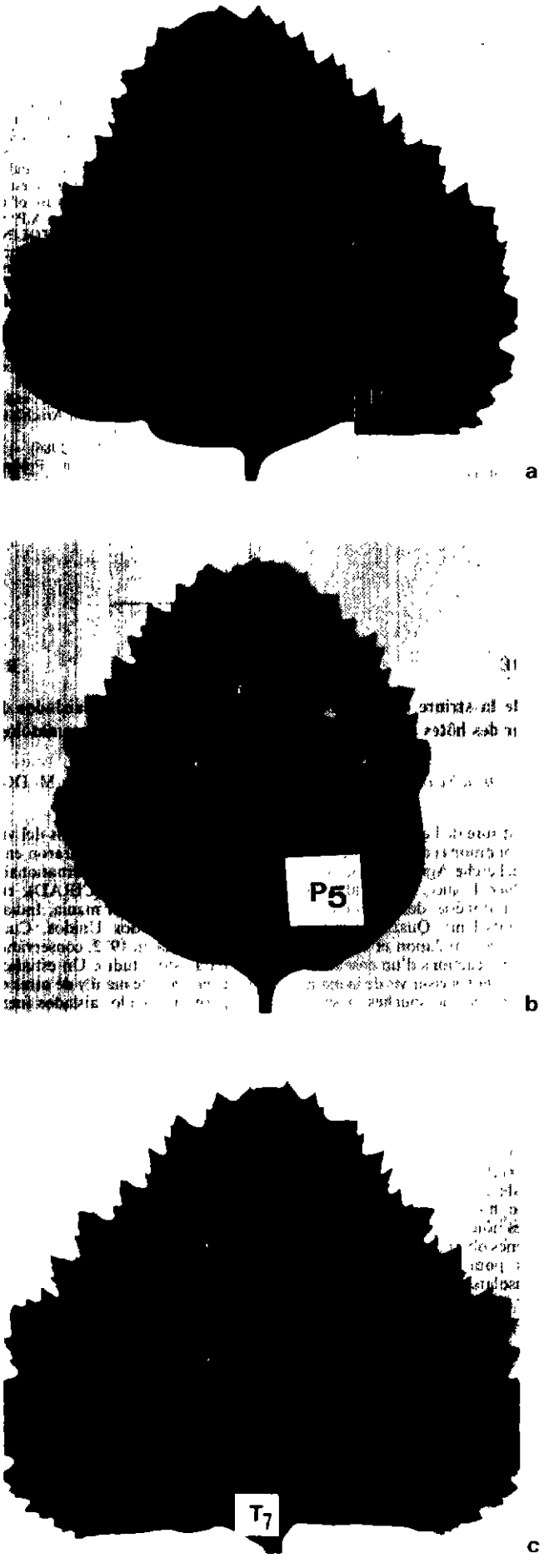


FIG. 3. — Types of reaction of *Chenopodium amaranticolor* to infection by different peanut stripe isolates at 13 days postinoculation. **a.** Chlorotic lesions **b.** Large necrotic lesions **c.** Necrotic lesions (Details are described in the text and Table 4)

REFERENCES

[1] ADELLA C. B. and NATURAL M. P. (1988). — Peanut stripe virus disease in the Philippines Page 9 in Summary proceedings of the First Meeting to Coordinate Research on Peanut Stripe Virus Disease of Groundnut 9-12 June 1987 Malang, Indonesia Patancheru, A P 502 324, India : ICRISAT.

[2] CLARK M F and ADAMS A. N. (1977). — Characteristics of the microplate method of enzyme-immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, **34**, 475-483

[3] DEMSKI J. W., REDDY D V R WONGKAEW S , KAMEYA-IWAKI M , SALEH N. and XU Z. (1988) — Naming of peanut stripe virus. *Phytopathology*, **78**, 631-632.

[4] DEMSKI J. W , REDDY D V R , SORWELL G., Jr., and BAY D (1984). — Peanut Stripe virus-a new seed-borne potyvirus from China infecting groundnut (Arachis Hypogaea) *Ann. Appl. Biol.*, **105**, 495-501.

[5] FUKOMOTO F., THONGMEEARKOM P IWAKI M., CHOOPA-NYA D , SARINDU N , DEEMA N. and TSUCHIZAKI T (1986). Peanut Chlorotic ring mottle virus occuring on peanut in Thailand. *Tech. Bull Trop. Agri. Res Center, Japan*, **21**, 150-157.

[6] ICRISAT (1988) — Coordination of research on peanut stripe virus : summary proceedings of the First Meeting to Coordinate Research on Peanut Stripe Virus Disease of Groundnut, 9-12 June 1987. Malang, Indonesia Patancheru, A.P. 502 324, India : ICRISAT.

[7] KIM K S. and FULTON J P. (1969) Electron microscopy of pokeweed leaf cells infected with pokeneed mosaic virus. *Virology*, **37**, 297-302

[8] LIMA J. A A , PURCIFULL D E. and HIEBERT E (1979). Purification, partial characterization and serology of blackeye cowpea mosaic virus. *Phytopathology*, **69**, 1252-1258

[9] MIDDLETON K J and SALEH N (1988) — Peanut stripe virus disease in Indonesia and the ACIAR Project Pages 4-6 In Summary proceedings of the First Meeting to Coordinate Research on Peanut Stripe Virus Disease of Groundnut, 9-12 June 1987, Malang, Indonesia. Patancheru, A.P 502 324, India . ICRISAT.

[10] RECHCIGL N. A., TOLIN S A., GRAYSON R. L. and HOOPER G. R (1989). — Ultrastructural comparison of peanut infected with stripe and blotch variants of peanut stripe virus *Phytopathology*, **79**, 156-161.

[11] SHUKLA D. D., TOSIC M JILKA J , FORD R E , TOLER R. W and LANGHAM M A C. (1989) — Taxonomy of potyviruses infecting maize, sorghum, and surgarcane in Australia and the United States as determined by reactivities of polyclonal antibodies directed towards virus-specific N-termini of coat protein *Phytopathology*, **79**, 223-229.

[12] TING W P., GEH S. L. and LIM Y. C (1972). — Studies on groundnut mosaic virus of Arachis hypogaea L in West Malaysia *Exp Agric* , **8**, 355-368

[13] WONGKAEW S. (1986) — Peanut stripe and other viruses in Thailand Pages 86-90 in : Proceedings of Peanut CRSP Workshop, 19-21 August 1986 Khon Kaen University, Khon Kaen, Thailand

[14] XU Z Y , YU Z. L , LIU J. L. and BARNETT O W (1983). — A virus causing peanut mild mottle in Hubei Province, China. *Plant Dis* , **67**, 1029-1032

RÉSUMÉ

Comparaison d'isolats du virus de la striure de l'arachide à l'aide de la symptomatologie sur des hôtes spécifiques et de la sérologie.

S WONGKAEW et M. DOLLET, *Oléagineux*, 1990, **45**, N° 6, p. 267-278

Vingt-quatre isolats du virus de la striure de l'arachide provenant de 8 pays ont été comparés dans des conditions contrôlées au Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) à Montpellier, France. Les isolats provenaient de la Birmanie, de l'Inde, de l'Indonésie, des Philippines, de la Chine, de la Thaïlande et des Etats-Unis. Quatre avaient été prélevés en Thaïlande en 1972, conservés au Japon et envoyés en France pour cette étude. Une étude des réactions d'un ensemble de génotypes d'arachide et d'autres espèces hôtes vis-à-vis de la maladie a permis de classer les isolats en 8 groupes de souches, à savoir marbrure légère, blotch, striure, blotch-striure, blotch-CP-N, marbrure annulaire chlorotique, arabesques chlorotiques et nécrotiques. Des similarités ont été observées parmi les isolats d'un même groupe quelle que soit leur provenance. Les résultats sérologiques indiquent que la marbrure annulaire de l'arachide est un isolat du virus de la striure de l'arachide (PStV), tandis que le virus des taches ocellées de l'arachide est un virus différent. Le classement par sérotype selon les réactions sérologiques des antigènes viraux provenant des tissus lésionnaires de *Chenopodium amaranticolor* vis-à-vis des différents anticorps polyclonaux de différents isolats PStV et de virus du type PStV, présentait une bonne corrélation avec les classements basés sur les réactions des hôtes vis-à-vis de la maladie. Des essais comparables sur des antigènes obtenus à partir de variétés d'arachides étaient moins efficaces pour la différenciation des souches. Etant donné que tous les isolats envoyés du Japon sont identiques au PStV, on peut supposer que le PStV existait en Asie du Sud-Est dès 1972, au moment où les échantillons ont été recueillis en Thaïlande.

RESUMEN

Comparación de aislados del virus del estriado del maní, por medio de la sintomatología sobre hospederos específicos, y de la serología.

S. WONGKAEW y M. DOLLET, *Oléagineux*, 1990, **45**, N° 6, p. 267-278

Veinticuatro aislados del virus del estriado del maní procedentes de 8 países se compararon en condiciones controladas en el Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), en Montpellier, Francia. Los aislados procedían de Birmania, India, Indonesia, Filipinas, China, Tailandia y Estados Unidos. Cuatro de los mismos se tomaron en Tailandia en 1972, conservándose en Japón y enviándose a Francia para este estudio Un estudio de las reacciones de un conjunto de genotipos de maní y de otras especies hospederas con la enfermedad permitió que los aislados fueran clasificados dentro de 8 grupos de cepas, o sea : jaspeado leve, blotch, estriado, blotch-estriado, blotch-CP-N, jaspeado anular clorótico, arabescos cloróticos y necrótico. Se observaron semejanzas entre los aislados de un mismo grupo, cualquiera que sea su procedencia. Los resultados serológicos muestran que el jaspeado anular del maní es un aislado del virus del estriado del maní (PStV), mientras que el virus de las manchas anulares del maní es un virus distinto. La clasificación por serotipo según las reacciones serológicas de antígenos virales procedentes de tejidos de lesiones de *Chenopodium amaranticolor* frente a varios anticuerpos policlonales de diversos aislados de PStV y de virus de tipo PStV, mostraba una buena correlación con las clasificaciones basadas en las reacciones de los hospederos frente a la enfermedad. Unas pruebas comparables con antígenos obtenidos de variedades de maní eran menos eficaces para diferenciar cepas. Dado que todos los aislados enviados desde Japón son idénticos al PStV, puede suponerse que el PStV ya existía en el Sudeste de Asia desde 1972, que es cuando las muestras se recogieron en Tailandia.