Pak. J. Bot., 21(2): 323—330, 1989.

SEPARATION OF TOMATO YELLOW TOP VIRUS AND BEET WESTERN YELLOWS VIRUS FROM DOUBLY INFECTED PLANTS

SHER HASSAN AND P.E. THOMAS*

Department of Plant Pathology,
NWFP Agricultural University, Peshawar, Pakistan.

Abstract

The efficacy of double antibody sandwitch (DAS) form of ELISA and the differences in transmission behaviour of two closely related persistent phloem restricted circulatively aphid transmitted viruses, tomato yellow top virus and beet western yellows virus, was evaluated for the separation of single virion from mixed infection. Both viruses were successfully separated from doubly infected plants on account of extreme specificity, complete absence of heterologous reactivity and sensitivity of this technique.

Introduction

Tomato yellow top virus (TYTV) and Beet Western yellows virus (BWYV) are economically destructive viruses of many cultivated plants. The host range of TYTV is mainly but not entirely restricted to Solanaceous plants (Hassan *et al.*, 1985). BWYV infects plants in about 21 dicotyledonous families, and is the most important virus of sugar beet (Duffus, 1960, 1964).

TYTV and BWYV both have been reported from several countries of the world (Braithwaite & Blake, 1961, Costa & Carvahlo, 1961; Duffus, 1960, 1964, 1977, 1977; Hassan *et al.*, 1985; Lana & Wilson, 1976; Thomas, 1981; Thomas & Martin, 1973; Zitter & Tsai, 1981). The predominant symptoms of TYTV on tomato are bright yellowing of the terminal growth of the infected plants, ovate, small, upward or downward cupped leaflets, few and asymmetrically developed fruits and new growth proliferations resulting in a cushion shape appearance of the infected plants (Hassan *et al.*, 1985). BWYV causes bright yellowing symptoms, stunting and necrosis (Duffus, 1964). The transmission properties, host range, symptomatology, particle shape, size and restriction of the virus to phloem tissues confirm that both viruses are members of the luteovirus group. Both viruses are related to each other and have overlapping host range but at the same time have several dissimilarities also. TYTV is etiologically distinct from BWYV, and the epidemiology TYTV disease is not associated with BWYV (Hassan & Thomas, 1984). They also differ in the breadth of their host range (Hassan *et al.*, 1985).

TYTV did not infect sugar beet and radish, the two major hosts of BWYV (Hassan *et al.*, 1985) and the BWYV did not infect., tomato the principal host of TYTV (Duffus, 1977). Minimum acquisition and transmission access periods of TYTV are 2.5 and 2h and of BWYV are 5 and 10 mins., respectively (Duffus, 1960, 1974; Hassan *et al.*, 1985).

*Present address: Research Plant Pathologist, Irrigated Agricultural Research and Extension Centre Prosser, Washington 99350, USA.

There are frequent chances of mixed and simultaneous infections of many plants by related viruses both in natural and green house conditions. These mixed infections are of great importance in disease epidemiology, in the development of resistant cultivars and adoption of control practices. Mixed infections of plants with closely related viruses also often lead to erroneous results in the characterization of viruses.

Studies were carried out to develop procedures for the separation of TYTV and BWYV from doubly infected plants by utilizing the extreme specificity of double antibody sandwitch form of ELISA (DAS) and the differences in transmission behaviour. Both of these viruses have absolutely no cross reactivity with heterologous antisera in DAS-ELISA.

Materials and Methods

Plant culture: Seeds of *Physalis floridana* were germinated in vermiculite and young seedlings transplanted in 10cm square plastic pots containing a mixture of loam, sand and peat moss. Plants were grown in green house at 23-30°C, and liquid fertilizers added to irrigation water @ 500ppm.

Insect culture: Non viruliferous green peach aphids *(Myzus persicae)* were reared on healthy radish *(Raphanus sativus)* plants in an isolated insectory, under aphid proof nylon net covered cages and regularly tested on healthy plants to check for virus contamination.

Virus transmission: Datura tatula and *Capsella bursapastoris* plants infected with TYTV and BWYV, respectively, were used as virus source and *Physalis floridana* as test plant for both viruses. All virus acquisitions were conducted on detached leaves of virus source plants placed on moist filter paper in Petri plates. Aphids were allowed various acquisition and transmission periods. Aphids were caged on seedlings of test plants under inverted plastic tumblers which had their bottom removed and covered with nylon net for ventilation.

Virus source: TYTV isolates 79 and 82 were obtained from field infected tomato plants at Prosser, Washington in 1981. These isolates produce the most characteristic symptoms on tomato. A standard BWYV isolate R-Y-I-R and a potato derived isolate PR were supplied by J.E. Duffus (USAD-ARS, Salinas California) and maintained in *Capsella bursapastoris* in green house.

Antisera toTYTV 79 and 82, and BWYV isolate RYIR were prepared in rabbits. The rabbits were injected intramuscularly at 3-week intervals with 0.5 to 0.7mg of freshly purified virus emulsified with Freund's incomplete adjuvant (1:1 V/V). Three weeks after the first injection, 30-45ml of blood was harvested weekly.

Double antibody sandwitch ELISA was performed by the methods of (Clark & Admas, 1977), with immunoglobulins and enzyme conjugates also prepared by their methods. Buffer, healthy plant tissue and infected plant tissues were used as control in all tests. A_{405} was measured at 60min., intervals after adding substrate.

Results

Non viruliferous green peach aphids were fed on detached leaves of *D. tatula* infected with TYTV isolates 79 and 82 and on *Capsela bursapastoris* infected with BWYV isolates RYIR and PR in Petri plates for 48 acquisition access period. Fifteen aphids from each virus sources were transferred simultaneously to 9 test seedlings of *P. floridana*, the common host of the two viruses for a 72h inoculation feeding period. After 4 wk of inoculation the doubly inoculated plants were tested by ELISA against antisera to YT79, YT82, and PYIR. Plants infected with TYTV isolates, 79, 82 and BWYV isolates YRIR and PR were used as positive controls and healthy plant tissue as negative controls. ELISA readings against each antisera at A_{405} nm (Table 1) indicated that both the viruses could not infect all the test plants. Many plants were infected either by TYTV or BWYV isolates, while several plants became infected with both the viruses. Plant with asterisks had a high concentration of both the TYTV and BWYV. These doubly infected plants were used for the separation of the two viruses in subsequent studies. Non viruliferous aphids, allowed to feed on detached leaves of the doubly infected plant after feeding on virus mixture for 2.5 and 24h were transferred to groups of 18 *P. floridana* test plants for 2 and 48h transmission access periods. All plants were assayed for the presence of virus by ELISA after 6 wk of inoculation (Table 2).

The aphids selectively transmitted both the viruses after they fed on the doubly infected plants (Table 3). In case of 2.5 and 2hr acquisition and transmission access periods either TYTV or BWYV isolates were predominantly transmitted to a high number of test plants. Although to a small number of plants both the viruses were also transmitted simultaneously but at low concentration. In case of 24 and 48h acquisition and transmission access periods both the viruses were not transmitted when simultaneously infected only with one virus. However, in case of long transmission access period both the viruses were simultaneously transmitted comparatively to a greater number of plants and in greater concentration.

Discussion

TYTV and BWYV, the two economically destructive viruses of many cultivated plants, having an over lapping host range and sharing many other properties could implicate several problems in the characterization, etiology and epidemiology of these viruses if present in mixed infection in nature. The situation further complicates the formulation of control strategies and breeding for resistance if both the viruses are not separated into

Table 1. Reaction of doubly infected plants to homologous and heterologous antisera in ELISA.

		Extinction Values	
		Antisera to	
Plant No.	Viruses	YT 79	RYIR
1	YT79+RYIR	1.752	0.634
2	"	1.508	0.496
3	"	1.436	1.041
4*	"	1.748	0.978
5	"	0.954	0.602
6*	"	1.231	0.739
7	"	0.765	1.213
8	"	1.312	0.438
9	"	0.727	0.558
10	+79	1.393	0.035
11	+RYIR	0	1.494
12	Healthy Control	0	0.071
13	YT79+PR	0.846	0.337
14*	"	1.176	1.028
15	"	1.112	0.284
16	"	1.116	1.245
17	"	0.368	0.844
18*	YT+PR	1.316	1.202
19*	"	0.666	0.842
20	"	1.208	0.348
21	"	0.992	0.456
22	+79	1.193	0.052
23	+PR	0	1.621
24	Healthy control	0	1.062
25	YT82+RYIR	*YT 82*	*YRIR*
26	"	0.941	0.288
27*	"	1.046	0.984
28	"	0.932	0.871
29	"	1.420	0.655
30*	"	1.23	1.048
31	"	0.862	0.410
32	"	1.036	0.548
33	"	0.934	0.217

34	+82	1.402	0.026
35	+RYIR	.015	2.103
36	Healthy control	.006	.042
		YT 82	*RYIR*
37	YT82+PR	0.336	0.433
38	"	0.539	0.673
39*	"	1.022	1.616
41*	"	1.325	1.148
42	"	0.866	1.045
43*	"	1.422	1.306
44	"	0.456	0.389
45	"	0.345	1.028
46-47	+82	1.510	0.042
48-49	+PR	0	1.621
50-51	Healthy control	0	0.062

single entity from the mixture. Separation of mechanically transmitted viruses having a local lesion host has widely been practiced, and such viruses have been successfully separated from mixed infection by transfer of virus from a single lesion to a propagative host. This is not the case with vector-transmitted viruses, because they cause systemic infections in which the viruses are mixed in the host. Since no local lesions develop, separation of a single virus free of the second virus is extremely difficult. Moreover the close relationship and identical symptomatology of the two viruses make their detection quite difficult if not impossible.

The newly developed serological technique, double antibody sandwitch form of ELISA (DAS) (Clark & Adam, 1977) has been reported to be extremely specific. Closely related and distantly related strains of viruses have been found to be serologically unrelated by DAS-method (Koenig, 1978; Bar Joseph & Salaman, 1980, Barbara *et al.*, 1978; Lister 1979) and the extent of serological cross reactivity has been widely utilized for the differentiation of strains in TMV (Regentmortal, 1975). The use of this technique has made it possible to determine the maximum serological distance between strains that are compatible with successful detection by the DAS-method. Enzyme conjugate prepared with antibodies against one virus strain do not react with related strains, which is an advantage for the separation of closely related viruses or virus strains from mixed infection.

There are possibilities of mixed infection of many economic plants by TYTV and BWYV in nature. Inspite of the close relationship of these 2 viruses, the type strains of BWYV and TYTV did not react with heterologous antisera even if used in high concentration (Unpublished). These two viruses also differed in acquisition and transmission periods. These two criteria were very successfully utilized in the separation of these 2 vi-

Table 2. Detection and separation of TYTV and BWYV isolates by ELISA in doubly infected plants.

	ANTISERA TO (Extinction values)															
	2.5/2hr		24/48hr		2.5/2hr		24/48hr		2.5/2hr		24/48hr		2.5/2hr		24/48hr	
Plant No.	YT79	RYIR	YT79	RYRI	YT79	PR	YT79	PR	YT82	RYIR	YT82	YRIR	YT82	PR	YT82	PR
1	1.676	0.189	0.509	0.834	0.002	0.28	1.048	0.986	0.028	0.328	1.224	1.036	0.326	0.222	1.048	1.616
2	0.006	0.134	0	1.185	0.008	0.102	1.342	1.426	0.104	0.042	1.328	1.235	0.036	1.218	1.112	1.230
3	0.006	0.117	1.212	0.230	0.024	0.148	0.302	1.384	0.004	1.326	0.944	0.211	0.78	1.034	0.228	1.004
4	0.740	0.044	0.892	0.111	0.781	0.842	0.066	0.985	0.635	0.116	0.846	0.020	0.235	0.448	0.416	0.876
5	0.322	1.114	0.204	1.214	0.002	0.064	1.405	0.121	0.018	0.028	1.406	1.204	0.320	0.328	1.434	0.286
6	0.003	0.080	0.121	1.034	0.042	0.947	1.348	0.027	0.856	0.960	1.201	0.864	0.022	0.236	1.434	0.286
7	0.005	0.089	1.435	0.984	1.138	0.040	0.980	1.289	0.070	1.351	0.124	1.424	0.006	0.049	0.830	1.326
8	0.010	0.095	0.008	0.114	1.245	0.032	0.070	0.684	0.004	0.877	0.625	0.980	0.022	0.877	1.234	1.248
9	0.487	1.130	0.866	0.734	0.004	1.610	1.048	1.304	0.036	1.112	0.066	0.642	0.136	0.444	1.306	0.228
10	0.507	0.150	0	.046	1.106	0.971	0.026	1.414	0.406	1.202	0.438	1.268	0.004	1.234	0.349	0.726
11	0.988	0.038	0	1.903	0.142	1.316	0.940	0.204	0.031	0.066	1.026	1.340	0.212	0.728	0.282	1.292
12	0.031	1.026	0	1.229	0.785	0.888	1.424	0.874	0.235	0.747	0.843	0.308	0.928	0.426	1.482	1.031
13	0.048	0.982	0.876	0.038	0.021	.045	1.230	1.426	0.321	1.246	0.112	1.526	0.846	0.318	0.838	1.224
14	1.002	0.036	0.328	0.568	0.100	.204	1.389	1.212	0.008	1.230	0.244	0.849	1.004	0.126	1.302	0.348
15	0.008	1.018	0.480	0.759	0.234	0.108	0.984	1.444	0.243	0.82	0.010	0.608	0.008	1.244	1.414	0.728
16	0.748	0.426	1.024	1.226	0.472	0.876	1.418	0.846	0.492	0.103	1.002	0.028	0.306	0.306	0.204	0.980
17	0.045	0.143	0.222	0.849	0.391	1.023	1.042	1.318	0.046	0.927	1.048	0.125	0.086	1.340	0.312	1.480
18	0.019	1.208	1.248	0.017	0.006	1.425	1.482	1.408	0.234	0.222	0.843	0.346	0.230	0.126	1.245	1.006
79+	1.668	0.032	1.978	0.052	—	—	—	—	—	—	—	—	—	—	—	—
82+	—	—	—	—	—	—	—	—	1.557	0.049	1.891	0.102	—	—	—	—
RYIR+	.016	1.442	.022	1.845	—	—	—	—	0.078	1.614	0.095	1.964	—	—	—	—
Healthy	.006	.042	0.018	0.054	—	—	—	—	0.030	0.060	0.051	0.105	—	—	—	—

1 = Acquisition/transmission access periods.

Table 1. Reaction of doubly infected plants to homologous and heterologous antisera in ELISA.

Plant No.	Viruses	Extinction Values — Antisera to YT 79	Extinction Values — Antisera to RYIR
1	YT79+RYIR	1.752	0.634
2	"	1.508	0.496
3	"	1.436	1.041
4*	"	1.748	0.978
5	"	0.954	0.602
6*	"	1.231	0.739
7	"	0.765	1.213
8	"	1.312	0.438
9	"	0.727	0.558
10	+79	1.393	0.035
11	+RYIR	0	1.494
12	Healthy Control	0	0.071
13	YT79+PR	0.846	0.337
14*	"	1.176	1.028

ruses from doubly infected plants. Many plants reacted only to antiserum prepared either against one or the other virus. These studies also revealed that these two viruses probably occupy different infection and replication nitches and then particles accumulate in different parts and tissues of the plant. Another hypothesis which could be postulated is that when the aphids are once charged completely with one virus, subsequently second virus could not either be acquired, or if acquired it could not circulate through entire system of the vector so that it could again be reinjected in the plants.

Many etiological confusions of plant diseases are existing due to mixed infection by two or more closely related viruses. BWYV has been implicated as a major causative component of the potato leaf roll syndrome which is still an enigma (Duffus, 1981). The present report could answer and clarify many cases of complex and complicated etiology.

References

Barbara, D.J., M.F. Clark and J.M. Thresh. 1978. Rapid detection and serotyping of prunus necrotic ring spot virus in perennial crops by ELISA. *Ann. App. Biol.*, 90: 395-397.

Bar Joseph, M. and R. Salaman. 1980. Heterologous reactivity of Tobacco mosaic virus strains by ELISA. *Jour. Gen. Virol.*, 47: 509-512.

Braithwaite, B.M. and C.D. Blake. 1961. Tomato yellow top virus, its distribution, characteristics and transmission by the aphid *Macrosiphum euphorbiae*. *Aust. J. Agric. Res.*, 12: 1100-1107.

Clark, M.P. and A.M. Adams. 1977. Characteristics of the microplate method of enzyme linked imunosorbent assays. *Jour. Gen. Virol.*, 47: 509-512.

Costa, A.S. and A.M.B. Carvalho. 1961. Estudos sobre o topo amarelo do tomateiro. *Biologica*, 28: 71-83.

Duffus, J.E. 1960. Radish yellow: a virus disease of raddish, sugar beets and other crops. *Phytopathology*, 50: 389-394.

Duffs, J.E. 1964. Host relationship of beet western yellows virus strains. *Phytopathology*, 254: 736-736.

Duffs, J.E. 1977. Serological relationships among beet western yellow, barley yellow dwarf and soybean dwarf viruses. *Phytopathology*, 67: 1197-1201.

Duffus, J.E. 1977. *Aphids, viruses and the yellow plague*. (Ed.) K.F. Harris and K. Maramorosche, Academic Press, New York. pp.

Duffus, J.E. 1981. Beet Western yellows virus. A major component of some potato leaf roll affected plants. *Phytopathology*, 71: 193-196.

Hassan, H. and P.E. Thomas. 1984. Etiological distinction between TYTV, BWYV and PLRV. *Pl. Dis.*, 68: 684-685.

Hassan, H., P.E. Thomas, and G.I. Mink. 1985. Tomato yellow top virus host range, symptomatology transmission properties and variability. *Phytopathology*, 75: 287-291.

Koenig, R. 1978. ELISA in the study of homologous and heterologous reactions of plant viruses. *Jour. Gen. Virol.*, 10: 309-318.

Lana, A.F. and G.F. Wilson. 1976. *A similar virus disease in Nigeria. Pl. Dis. Reptr.*, 60: 296-298.

Lister, R.M. and W.F. Rochow. 1979. Detection of barley yellow dwarf virus by enzyme linked imunosorbent assay. *Phytopathology*, 69: 649-654.

Thomas, J.E. 1981. Tomato yellow top virus – a probable luteo-virus from Australia. *Aust. Pl. Pathol.*, 10: 33-34.

Zitter, T.E. and J.H. Tsai. 1981. *Viruses infecting tomato in southern Florida. Pl. Dis.*, 65: 787-791.

Van-Regentmortal, M.H.V. 1975. Antegenic relationship between strains of TMV. *Virology*, 64: 415-420.

(Received for publication 20 June 1989)