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Comparative Studies on Biological and Serological Properties of Turnip Mosaic Virus Isolates

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Based on the symptoms on *Brassica rapa* L. cv. Hakatasuwari, eight isolates of ordinary strain of turnip mosaic virus (TuMV) collected from Japan could be differentiated into three groups, which were respectively designated as OA, OB and OC groups. Biological and serological properties of the virus isolates belonging to these groups were compared from each other. All isolates were almost identical in host range, electrophoretic mobility and in agar gel immunodiffusion test, however, they were much different in some of the other properties such as symptoms on several indicator plants, local lesion size on *Nicotiana tabacum* L. cv. Samsun, mode of multiplication in *B. rapa*, physical properties, and serological properties in micro-precipitin test. Some of these properties showed variation correlating with the groups.

INTRODUCTION

The biological and serological properties of turnip mosaic virus (TuMV) isolates have been reported by several workers (Tompkins, 1938; Pound, 1948; Pound *et al.*, 1962; Yoshii *et al.*, 1963; Tochihara, 1965; Provvidenti, 1978). Yoshii (1963) divided TuMV isolates into two strains, ordinary strain and cabbage strain, according to the symptoms appeared on *Nicotiana glutinosa* and cabbage *(Brassica oleracea* L. var. *capitata).* The ordinary strain which produces mild symptom on N. *glutinosa* (Yoshii *et al,,* 1963) is known to be commonly distributed on turnip and radish in Japan. By means of compliment fixation test, Tochihara (1961) serologically classified TuMV isolates into two strains, P and R. Such differences were reported to exist among the isolates but they did not carry out comparative studies on the characteristics of the isolates in any detail.

The following investigation was conducted to compare biological properties of the isolates and to set up some criteria for possible grouping of the isolates.

MATERIALS AND METHODS

Virus sources

Eight isolates of TuMV ordinary strain were used in this work. A typical

isolate of the ordinary strain, No. 67, first reported by Yoshii *et al.* (1963) was maintained in our laboratory. An isolate from rape (Tochihara, 1965) (C 10) was provided by Dr. H. Tochihara of the Institute of Plant Virus Research, Japan, and that from iris (Inoue and Mitsuhata, 1978) (Ir 16) from Dr. N. Inoue of Okayama University. The isolates T 3 and T 17 were obtained from turnip leaves collected respectively from Fukuoka and Mie Prefectures, and Rd 28, Rd 29 and Rd 34 were obtained from radish leaves in Fukuoka Prefecture. All isolates were maintained on turnip (B. *rapa* L. cv. Hakatasuwari) by mechanical transmission in air conditioned green-house.

Symptoms on host plants

All test plants were raised under the same conditions in a temperature controlled (20 – 27°C) green-house. To compare the symptoms on turnip, 25-day old plants after sowing (15-day after transplanting) were inoculated. For testing host range, six to ten plants of each species were used. The symptoms on the inoculated plants were observed periodically for a month, and then each isolate was back-inoculated to turnip to test whether they still maintain the pathogenicity to cause original symptoms or not.

The inoculum was prepared by homogenizing systemically infected turnip leaves with 2 volumes of 0.1 M phosphate buffer (pH 7.0), and inoculation was made by rubbing Carborundum (400 mesh) dusted leaves with a cotton swab impregnated with the inoculum.

Mode of multiplication

To make clear the mode of multiplication of TuMV isolates on turnip plants, 25-day old plants were inoculated and virus concentration was assayed periodically at 5, 10, 15, 20, 25 and 30 days after inoculation. For assaying virus concentration, the inoculum was prepared by homogenizing five completely expanded leaves with 5 volumes of 0.1 M phosphate buffer (pH 7.0). The relative infectivities of the isolates were determined by counting the number of local lesions appeared on 12 half-leaves (Kuhn, 1965) of *N. tabacum* L. cv. Samsun.

Physical properties

To test the physical properties of TuMV isolates, inoculum was prepared by homogenizing systemically infected turnip leaves with 5 volumes of the phosphate buffer. Six turnip plants were inoculated with each virus isolate in every experiments. In thermal inactivation test, undiluted inocula were heated in a water bath at different temperatures of 5°C intervals ranging from 50°C to 70°C for 10 min, cooled immediately with ice, and infectivities were assayed. To determine the longevity in vitro, the inocula stored for different periods (1, 2, 4 and 6 days) at room temperature (about 20°C) were inoculated. To determine the dilution end point, each preparation was diluted with 0.1 M phosphate buffer (pH 7.0) to make concentrations from 10^{-1} to 10^{-5} and their infectivities were assayed.

Electrophoretic mobility

Electrophoretic mobility of intact virus of each isolate was tested by the method of Makkouk and Gumpf (1976). The crude sap from turnip leaves infected with each isolate was clarified by one cycle of differential centrifugation. The specimen thus obtained was electrophoresed in the gel composed of 1 % polyacrylamide and 0.5 % agarose. After running at 5 mA/gel for 6 hr, thus gels were stained with 0.5 % aniline black in 7 % acetic acid and destained with 7 % acetic acid.

Serological relationship

Antisera of the isolates 67 (Choi *et al., 1978)* and C 10 (Tochihara, 1965) were prepared by injecting purified viruses to rabbits. Agar gel double immunodiffusion test was performed for each isolate as previously described (Choi et *al., 1978).* The immunoplates incubated in a moist chamber at 20°C for 24-48 hr were washed with 0.1 M NaCl for 10 min, dried and stained with 0.5 % Coomassie Brilliant blue R 250 in the solution of 96 % ethanol: acetic acid : water = 4.5 : 1 : 4.5. Antigen preparations to be tested were treated by ulfrasonication for 10 min. The end point of serological reaction was detected by micro-precipitin test (Noordam, 1973) in Petri dishes with serially diluted antiserum and virus antigen.

RESULTS

Symptoms on turnip

All isolates caused typical mosaic symptoms on turnip (B. *rapa* L. cv. Ha-

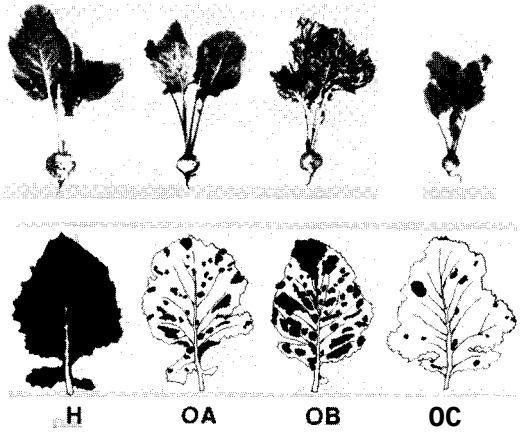

Fig. **1.** Symptoms on turnip incited by different isolates of TuMV (○ light green, and ● dark green). H, healthy plant and its leaf; OA, plant and leaf infected with isolate 67; OB, those infected with Ir 16; and OC. those infected with C 10.

katasuwari) leaves. The symptoms were classified into three types depending upon the proportion of light green area to dark green area. The isolates C 10, T 3 and Rd 28 caused stunting with greater proportion of light green area in mosaic symptom than the other isolates (Fig. 1 OC), and were designated as OC group. The isolate Ir 16 did not cause any stunting but mosaic on leaves with high proportion of dark green area (Fig. 1 OS), and was designated as OB group. The other isolates, 67, T 17, Rd 29 and Rd 34, caused a mosaic type of intermediate (Fig. 1 OA) between OB and OC, and were designated as OA group. These three different symptoms could be distinguished about 20 days after inoculation and more clearly after 25 - 30days.

Symptoms on different hosts

Table 1 outlines the contrast in susceptibility and symptom expression on possible indicator hosts. On *B. napus* L. cv. Norin 14 and *B. pekinensis* Rupr. cv. Nozaki 2, the isolates C 10, Ir 16 and Rd 28 induced systemic symptoms. The symptoms of Ir 16 and C 10 were more severe than that of Rd 28. The isolate T 3 caused mild symptom very rarely on *B. pekinensis,* however, isolates 67, T 17, Rd 29 ,and Rd 34 did not incite any symptoms. On *Trifolium incarnatum* L., C 10 and T 3 caused systemic infection, while other isolates did not show any symptoms. All isolates caused necrotic local lesions on the leaves of *Vicia faba* L. cv. Sanuki-nagasaya and 6 isolates with exceptions of T 17 and Rd 34 caused also necrotic spots on *Vigna sesquipedelis* L. cv. Misawa. Stem necrosis was caused by C 10, T 3 and Rd 28 on *V. faba.* All isolates induced local lesion on N. *tabacum* L. cv. Samsun, however, the size of lesions (0.23 ± 0.08 cm diameter) caused by Ir 16 was larger than those by others (0.14 ± 0.06 cm diameter) (Fig. 2). The plants *Calendula officinalis* L., *Chenopodium amaranticolar* L., *Gomphrena* globosa L., N. *rustica* L. and *Phaseolus vulgaris* L. were locally infected by all isolates, while *Raphanus sativus* L. and *Spinacia oleracea* L. systemically. *B. oleracea* L. var. *capitata* did not show symptoms by any isolates.

Mode of multiplication

With regard to multiplication of the isolates in turnip leaves, two patterns

Table **1.** Symptoms on indicator plants incited by 8 isolates of TuMV.

Host	TuMV isolate							
	67	T17	Rd29	Rd34	Ir16	C10	T3	Rd28
Brassica napus L. Norin 14					s	s	—	s
B. pekinensis Rupr. Nozaki 2	—		—	—	s. ns	s	[s]	s
B. rapa L. Hakatasuwari	s (M)	s (M)	s (M)	s (M)	s (DG)	s (LG)	s (LG)	s (LG)
Trifolium incarnatum L.						s	s	
Vicia faba L. Sanuki-nagasaya	ns	ns	ns	ns	ns	ns. cs. sn	ns. sn	ns. sn
Vigna sesquipedelis L. Misawa	ns		ns	—	ns	ns	ns	ns

s: Systemic infection. ns: Necrotic local lesion. cs: Chlorotic local lesion. sn: Stem necrosis. —: No infection. [s]: Occasionally appear. (DG): High proportion of dark green area in mosaic symptom. (LG): High proportion of light green area in mosaic symptom. (M): Intermediate type between the DG and LG types.

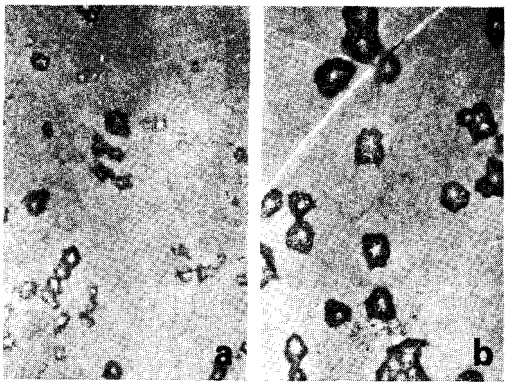

Fig. 2. Local lesions on N. *tabacum* L. cv. Samsun caused by inoculation with 67 (a) and Ir 16 (b).

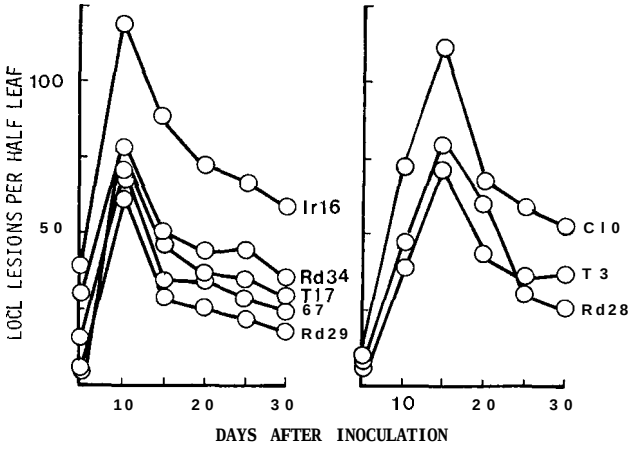

Fig. 3. Multiplication of TuMV isolates in turnip leaves. Local lesion number indicates the average number of lesions developed on 12 harf-leaves of Samsun.

different each other depending upon the isolates were observed (Fig. 3). OA and OB group isolates showed the multiplication peak at 10 days after inoculation, but OC group isolates reached peak at about 15 days.

Physical properties

Physical properties of the TuMV isolates were as shown in Figure 4. The isolates 67 and Rd 34 lost their infectivities by heating at a temperature higher than 60°C for 10 min, while C 10 and T 3 did not even at 65°C for 10 min, and lost completely at 70°C. The isolates, 67, T 17, Rd 28 and Rd 29, maintained their activities for 2 days in crude sap, which was shorter than those of the other isolates. The isolate C 10 was most stable and maintained infectivity for 6 days. Variation in dilution end point was not so large among

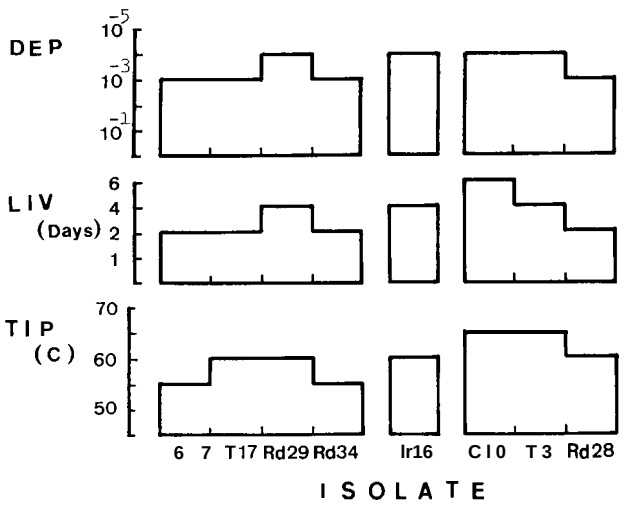

Fig. 4. Comparison of the 8 TuMV isolates in physical properties. DEP, dilution end point; LIV, longevity in vitro; and TIP, thermal inactivation point.

isolates, showing 10^{-3} to 10^{-4}.

Electrophoretic mobility

All isolates migrated toward the anode side at pH 8.2 and gave a single band at the same *Ef* value. The distance of migration was similar to that of potato virus Y (PVY) described by Makkouk and Gumpf (1976).

Serology

All ultrasonicated isolates showed a homologous reaction when subjected to agar gel immunodiffusion test against anti-67 (Fig. 5)-or anti-C 10-serum, indicating serological identity among the isolates. However, when the isolates were tested against these two antisera for detecting reaction end point

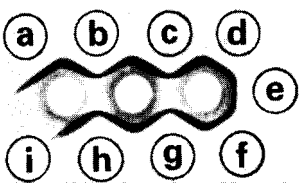

Fig. 5. Serological reaction of TuMV isolates sonicated for 10 min. Peripheral wells labeled a, b, c, d, e, f, g and h contained the sap from the plants infected with TuMV 67, T 17, Rd 29, Rd 34, Ir 16. C 10, T 3 and Rd 28. The well i contained the sap from healthy plant. Three center wells unlabeled contained anti-67-serum.

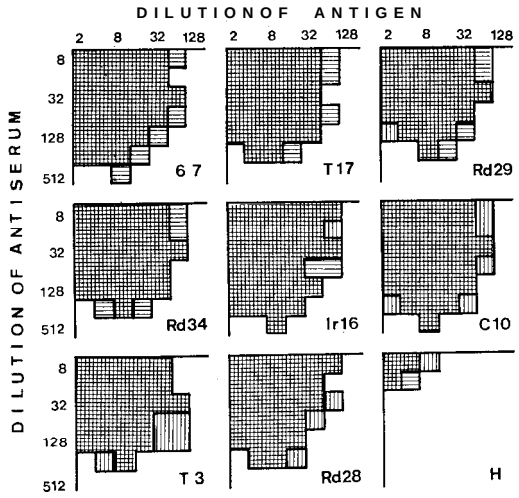

Fig. 6. End points of the reaction between two fold dilution series of antisera and TuMV isolates. ▤, precipitation range of each isolate with anti-67-serum ; ▥, those of each isolate with anti-C lo-serum.

by micro-precipitin test, slight differences were observed among the isolates (Fig. 6). The results suggest that the isolates could be divided into three groups. The first group including 67, T 17, Rd 29 and Rd 34 strongly reacted with anti-67-serum, and the second group, C 10, T 3 and Rd 28, did with anti-C lo-serum. The third group, Ir 16, reacted similarly with both antisera.

DISCUSSION AND CONCLUSION

TuMV isolates were reported to be classified into two strains, viz. ordinary strain and cabbage strain (Yoshii, 1963). Almost all isolates from Japan are said to belong to the former strain (Yoshii, 1963). Furthermore, TuMV ordinary strain was divided into three groups on the basis of symptoms on N. *glutinosa* (Yoshii *et al.*, 1963). In this cases, however, other characteristics such as physical properties or host range did not correlate with the grouping.

Depending upon the symptoms on turnip, eight TuMV isolates used in this work were divided into three groups, viz. OA (isolates 67, T 17, Rd 29 and Rd 34), OB (isolate Ir 16) and OC (isolates C 10, T 3 and Rd 28). Among other biological and serological properties of the isolates, host ranges or symptoms on some indicator plants, and serological properties shown by micro-precipitin test partially supported the grouping mentioned above. The isolates belonging to the groups OA and OC produced smaller lesions on N. *tabacum* L. cv. Samsun as compared to OB group isolate. According to the mode of multiplication in the tissue of turnip leaves, both groups OA and OB fell into the same group showing rapid multiplication, while OC group was quite different and multiplied slowly. Physical properties of the isolates such as thermal in-

activation point, longevity in *vitro* and dilution end point did not show any meaningful difference among the grouping. These results suggest that the isolates belonging to the groups, OA, OB and OC are distinguishable from each other in some biological or serological properties, however, all isolates were almost identical in electrophoretic mobility and in serological properties shown by agar gel immunodiffusion method.

The other twelve isolates collected from various localities of Japan were also divided into three groups depending upon symptoms on turnip. Among them, 8 isolates were known to belong to OA group and 3 isolates to OC group. Only one isolate was known to belong to OB group. From these results it will be concluded that the group OA seems to be most common, OC follows, and the isolate belonging to the group OB is very rarely distributed in Japan. This is also supported by the fact that the host ranges and symptoms of TuMV reported up to the present (Pound, 1948; Yoshii *et al.*, 1963; Tochihara, 1965; Provvidenti, 1978) almost coincided with those of OA group.

Among serological techniques, the micro-precipitin test was reported (Noordam, 1973) to be useful for differentiating the isolates closely related from each other. Tochihara (1961) distinguished two antigenic variants in TuMV, radish P and R, by compliment fixation test. The three groups differentiated with precipitin test in this study, however, did not show any correlations with Tochihara's two types.

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