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Mini-Review

Occurrence of Two Tobamovirus Diseases in Cucurbits and Control Measures in Korea

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Two Tobamoviruses, *Cucumber green mottle mosaic virus* (CGMMV) and *Zucchini green mottle mosaic virus* (ZGMMV), occurred in Korea in 463 ha in 1998, 33.9 ha in 1999, and 44.2 ha in 2000. CGMMV was detected in watermelon, cucumber, oriental melon, and melon, whereas ZGMMV was mainly detected in zucchini squash. Thirty-six CGMMV isolates were classified into three types by analysis of single strand cDNA conformational polymorphism (SSCP) of the coat protein gene. In a comparison of serological relationships among CGMMV, ZGMMV, and *Kyuri green mottle mosaic virus* (KGMMV), the three tobamoviruses specifically reacted with each homologous antibody in the double-antibody sandwich enzyme-linked immunosorbent assay and rapid immunofilter paper assay (RIPA), although ZGMMV and KGMMV were slightly biologically similar. In a survey of the three tobamoviruses in cucurbit-growing fields in Korea by RIPA, CGMMV and ZGMMV were detected but KGMMV was not found in commercially growing cucurbit crops so far. Seed contamination ratio of CGMMV in bottle gourd seeds tested was 84%, while seed transmission ratio from the virus-contaminated seeds was 2.0%. Soil transmission ratio was 0-3.5% in fields naturally infested with CGMMV or ZGMMV. Control measures of the virus diseases are roguing and sanitation. These suggest that it is important to rogue the first infected crops, which include the seed and soil, especially early in the season. This may be practicable to control the diseases because CGMMV and ZGMMV have a narrow host range restricted to cucurbitaceous crops.

Keywords **:** cucurbit, *Cucumber green mottle mosaic virus*, *Kyuri green mottle mosaic virus*, seed transmission, *Zucchini green mottle mosaic virus*.

The Cucurbitaceae family comprises 118 genera with 825 species, and cucumber, watermelon, squash, and melon are major cucurbit crops in Korea and the total cultivated area of these crops was 61,628 ha in 1999. Similarly to most other vegetables, cucurbits are affected by a large number of diseases caused by fungi, bacteria, and viruses. Diseases caused by viruses are often the most destructive and difficult to control. There are about 30 different viruses reported in cucurbits in the world (Provvidenti, 1993).

Cucumber mosaic virus (CMV) (Lee, 1981), *Watermelon mosaic virus* (WMV) (Lee and Lee, 1981), *Zucchini yellow mosaic virus* (Kim et al., 1995), *Cucumber green mottle mosaic virus* (CGMMV) (Lee et al., 1990), *Kyuri green mottle mosaic virus* (KGMMV) (Lee et al., 2000), and *Zucchini green mottle mosaic virus* (ZGMMV) (Ryu et al., 2000) have been reported to infect several cucurbit plants in Korea. CGMMV, KGMMV, and ZGMMV belong to the genus *Tobamovirus*. CGMMV was first described as cucumber virus 3 and cucumber virus 4 in England by Ainsworth (1935). Lee et al. (1990) reported the occurrence of CGMMV in watermelon in Korea in 1989. KGMMV was first found in cucumber plants in Japan and previously described as a new strain of CGMMV (CGMMV-C or CGMMV-Cu) (Inoue, 1967). However, Francki et al. (1986) renamed CGMMV-C as KGMMV based on serological and molecular hybridization analysis.

Recently, an outbreak of new virus disease occurred in zucchini squash plants showing mosaic and fruit malformation in Chonju in 1999. Lee et al. (2000) named it KGMMV-Z by comparison of coat protein gene sequences between the agent and CGMMV isolates (Lee, 1999; Ugaki et al., 1991). Ryu et al. (2000) reported that the virus isolated from zucchini squash and KGMMV (Francki et al., 1986) were on distinct species in the genus *Tobamovirus* based coat protein (CP) gene sequence analysis and comparative studies between the two viruses and other members of Tobamoviruses. They suggested that the virus was a new species and designated as ZGMMV. Thereafter, Choi et al. (2001) also proposed that KGMMV-Z (Lee et al., 2000) should be renamed as ZGMMV because of the differences in the CP gene sequence homology and serological specificity among these Tobamoviruses.

Tobamoviruses are easily transmitted by foliage contact, soil contamination, and through seed (Antignus et al., 1990; Hollings et al., 1975; Tan et al., 2000). The leaves and fruits of cucurbits infected with Tobamoviruses appear to be dis-

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torted and reduced in size, resulting in poor quality in commercial products. CGMMV can induce internal discoloration and decomposition of watermelon fruits. CGMMV and ZGMMV severely affected the economic value of the fruit products of watermelon and zucchini in some vegetable-growing regions in Korea. This report discussed recent incidences, some properties, and control measures of CGMMV and ZGMMV in cucurbits in Korea.

Incidence of Tobamoviral diseases

CGMMV occurred in 463 ha of watermelon growing regions in Korea in 1998. The major cause of the disease was transmission through virus-contaminated bottle gourd seeds imported from China. Bottle gourd is used for watermelon rootstock. Farmers and seed companies suffered economically significant losses due to the disease. CGMMV occurred in 17.3 ha in 1999 and 27.2 ha in 2000. Cucurbits naturally infected with CGMMV include watermelon, cucumber, oriental melon, and melon. ZGMMV also occurred in 16.6 ha of zucchini-growing regions of Chonju in Chonbuk and Andong in Kyungbuk in 1999 (data not shown). The initial occurrence of ZGMMV was thought to be brought about by zucchini seeds from China, which carried the virus. In 2000, ZGMMV re-occurred in 17.0 ha of the same crop-growing region, which was soil-infested with the virus in the previous year (Table 1).

Some properties of CGMMV and ZGMMV

Thirty-six CGMMV isolates were collected from cucurbit plants in Korea. For the analysis of single strand conformational polymorphism (SSCP) (Kerr et al., 1995) on the CGMMV isolates, RT-PCR was performed to produce PCR products corresponding to a region of the CP gene of the virus RNA amplified expected size, approximately 630 bp cDNA long. SSCP analysis of the CGMMV isolates based on the electrophoretic mobility patterns obtained after denaturating the RT-PCR products was done and the thirty-six CGMMV isolates were regrouped into three types; type I, type II and type III (Fig. 1). CGMMV type I was the major subgroup and consisted of 27 isolates, and tpes II and III of 6 and 3 CGMMV isolates, respectively. The three types reacted positively without forming a spur in the agar gel double diffusion test against the antisera (data not shown). The results of host reactions against CGMMV types based on SSCP are shown in Table 2. All of the types caused local lesions to inoculated leaves of *Chenopodium amaranticolor* and *Nicotiana tabacum*. CGMMV produced local chlorotic lesions on *N. tabacum* inoculated only during the summer season. All the types induced systemic symptoms in *N. benthamiana*, and type III showed severe mosaic symptoms. CGMMV belonging to type I did not infect *Cucumis sativus* cv. Baegbong, while types II and III did (Table 2). All of the types did not infect *Petunia hybrida* and *Datura stramonium*. Choi et al. (1998b) suggested that

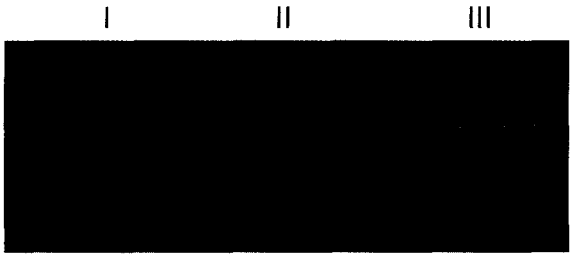

Fig. 1. Single-strand cDNA conformational polynorphism analysis of the RT-PCR products for region of coat protein gene from CGMMV isolates. I, II, and III represent *Cucumber gree mottle mosaic virus* types. Downstream primer: 5'-ACCCTCGAAAAC-TAAGCTTTC-3' and upstream primer: GAAGAGTCCAGTT-CTGTTTC, which correspond to a region of the coat protein gene. Electrophoresis was carried out at 125 V for 4 h on 6% polyacrylamide gel.

Table 1. Occurrence of *Cucumber green mottle mosaic virus* (CGMMV) and *Zucchini green mottle mosaic virus* (ZGMMV) on cucurbits in Korea, 1998-2000

Viruses	Cucurbits	Virus disease incidence (ha)[a]		
		1998	1999	2000
CGMMV	Watermelon	463	12.0	16.2
	Cucumber	–	5.3	9.8
	Oriental melon	–	–	0.6
	Melon	–	–	0.6
ZGMMV	Zucchini	–	16.6	17.0
Total		463	33.9	44.2

[a] Incidence of the virus diseases was surveyed by the Rural Development Administration (RDA) in 1998-2000. (–) notes that occurrence of the diseases was not surveyed in that year.

Table 2. Reactions of *Cucumber green mottle mosaic virus* (CGMMV) types on different indicator plants

Indicator plant	Host reactions[a] of CGMMV types		
	I	II	III
Chenopodium amaranticolor	LL/–	LL/–	LL/–
Nicotiana tabacum cv. Samsun NN	LL/–	LL/–	LL/–
N. tabacum cv. White Burley	LL/–	LL/–	LL/–
N. benthamiana	*l*/M	*l*/M	*l*/SM
Cucumis sativus cv. Baegbong	–/–	*l*/M	*l*/M
Datura stramonium	–/–	–/–	–/–
Petunia hybrida	–/–	–/–	–/–

[a] Inoculated leaves/upper leaves. LL: local lesion, M: mosaic, SM: severe mosaic, *l* : symptomless, —: not infected.

Table 3. Reactions of *Cucumber green mottle mosaic virus* (CGMMV), *Zucchini green mottle mosaic virus* (ZGMMV), and *Kyuri green mottle mosaic virus* (KGMMV) on selected indicator plants

Indicator plant	Host reactions[a] of three Tobamoviruses		
	CGMMV	ZGMMV	KGMMV
Chenopodium amaranticolor	L[b]	L	–
Citrullus lanatus	S	S	S
Cucumis sativus	S	S	S
Cucurbita moschata	–	S	S
C. pepo	–	S	S
Datura stramonium	–	L	L
Gomphrena globosa	–	S	–
Nicotiana benthamiana	S	S	S

[a] CGMMV type 1 and ZGMMV were isolated from watermelon and zucchini, respectively. CGMMV-C renamed as KGMMV (Francki *et al.*, 1986) was provided by Dr. Ryu, K. H. (Plant Virus GenBank, Seoul Women's University, Korea).
[b] L: local lesion, S: systemic infection, –: no infection.

Table 4. Detection of *Cucumber green mottle mosaic virus* (CGMMV), *Zucchini green mottle mosaic virus* (ZGMMV), and *Kyuri green mottle mosaic virus* (KGMMV) from cultivated cucurbit crops by rapid immunofilter paper assay (RIPA), 1999-2001

Cucurbit	No. of samples[a]	No. of plants infected with the viruses			
		CGMMV	ZGMMV	KGMMV	Mixed infection
Cucumber	256	64	0	0	0
Watermelon	345	125	0	0	0
Oriental melon	35	6	0	0	0
Melon	13	12	0	0	0
Zucchini	152	0	103	0	0

[a] Plants showing virus-like symptoms were collected and examined using the RIPA.

SSCP should be generally used for large-scale comparative studies of the genomes of other viruses, especially for the differentiation of serologically indistinguishable strains.

The symptoms induced by CGMMV, ZGMMV, and KGMMV on a range of indicator plants are summarized in Table 3. CGMMV and ZGMMV were isolated from watermelon and zucchini, respectively. KGMMV (Francki et al., 1986) was kindly provided by Dr. Ryu, K. H. (Plant Virus Gene Bank, Seoul Women's University, Korea) and used for comparative studies with CGMMV and ZGMMV. The three Tobamoviruses caused very similar symptoms on *C. lanatus*, *C. sativus*, and *N. bentamiana*, although some differences in symptom severity were noted on the host plants. ZGMMV and KGMMV infected *Cucurbita* spp. but CGMMV did not (Table 3). ZGMMV and KGMMV exhibited similar symptoms, however, they were distinct from ZGMMV, which induced local lesions on *C. amaranticolor* and systemic symptom on *Gomphrena globosa*. Very recently, a new Tobamovirus isolated in Israel from greenhouse cucumber plants, exhibiting severe mottling or mosaic on cucumber fruits was designated as cucumber fruit mottle mosaic virus (CFMMV) (Antignus et al., 2001). ZGMMV, like KGMMV, CGMMV-Y (Kitani et al.,

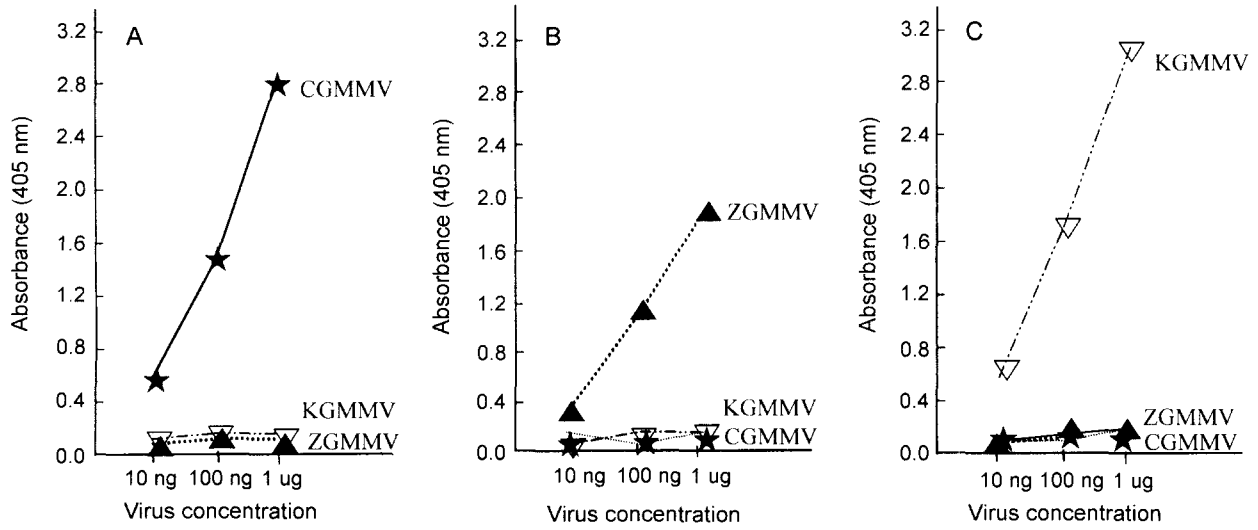

Fig. 2. Comparative absorbances of *Cucumber green mottle mosaic virus* (CGMMV), *Zucchini green mottle mosaic virus* (ZGMMV), and *Kyuri green mottle mosaic virus* (KGMMV) and cultures in double-antibody sandwich enzyme-linked immunosorbent assay with the polyclonal antibody against CGMMV (**A**), the polyclonal antibody against ZGMMV (**B**), and the polyclonal antibody against KGMMV (**C**).

Table 5. Seed transmission ratio in bottle gourd and zucchini seeds contaminated with *Cucumber green mottle mosaic virus* (CGMMV) and *Zucchni green mottle mosaic virus* (ZGMMV), respectively

Virus	Samples investigated[a]	No. of samples	No. of samples detected with virus[b]	Percentage of transmission
CGMMV	Bottle gourd seeds	150	126	84.0
	Seedlings	100	2	2.0
ZGMMV	Zucchini seeds	100	6	6.0
	Seedlings	182	2	1.1

[a] Seeds tested in the experiment are commercial bottle gourd, and the zucchini seeds are from damaged ones during cultivation in 1998 and 1999, respectively.
[b] Detection of CGMMV and ZGMMV was conducted with DAS-ELISA. The seedlings were tested 40 days after germination.

1970), and CFMMV, induced local lesion in *D. stramonium* in contrast with other CGMMV isolates (Brunt et al., 1996; Komura et al., 1968) that failed to infect this host plant. Unlike KGMMV, however, ZGMMV induced local lesions on the inoculated leaves of *C. amaranticolor*. The biological properties of ZGMMV were similar to those of CFMMV reported by Antignus et al. (2001). Further detailed characteristics such as serological relationships and genomic RNA sequences are required in order to define classification of ZGMMV and CFMMV.

A comparison of serological relationships among CGMMV, KGMMV, and ZGMMV were conducted in double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977; Flegg et al., 1979) with each polyclonal antibody to the viruses. The three viruses specifically reacted with each antibody in the DAS-ELISA (Fig. 2), although ZGMMV and KGMMV were biologically similar. DAS-ELISA analysis confirmed the lack of serological cross-reaction among the viruses. Therefore, polyclonal antisera successfully produced could be used for specific diagnosis of the three tobamoviruses. Also, a rapid immunofilter paper assay (RIPA) (Choi et al., 1998a; Choi et al., 2001; Tanaka et al., 1997) was prepared for diagnosis of CGMMV, ZGMMV, and KGMMV. Each of these viruses was specifically detected by the RIPA from virus-infected cucurbit plants. In a recent survey of the three viruses in cucurbits growing in commercial fields by RIPA, CGMMV was detected in watermelon, oriental melon, melon, and cucumber. ZGMMV was detected in only zucchini. However, KGMMV was not found in the commercially growing cucurbit crops in the survey (Table 4). The RIPA could be a simple, rapid, specific, and convenient tool to detect and differentiate these cucurbit-infecting Tobamoviruses.

Transmission of CGMMV and ZGMMV

CGMMV and ZGMMV can be transmitted through seed, soil contamination, and sap of the plants infected with the viruses. Seed and soil transmissions are recognized as the primary source in epidemic development of Tobamovirus diseases (Broadbent, 1963; Brunt et al., 1996; Matthews, 1991). Transmissions of CGMMV and ZGMMV through bottle gourd and zucchini seeds are shown in Table 5. Seed contamination ratio of CGMMV in bottle gourd seeds was 84%, while seed transmission ratio in the seeds was 2.0%. In zucchini seeds, seed contamination ratio and seed transmission ratio were 6.0% and 1.1%, respectively. These

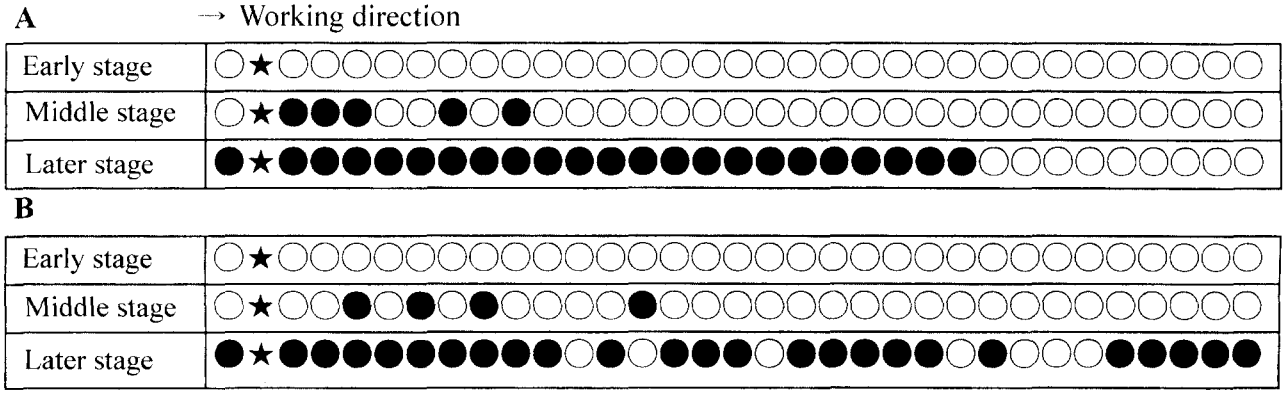

Fig. 3. Difference in spread patterns of *Cucumber green mottle mosaic virus* in the plots of watermelon (**A**) and cucumber (**B**). ★ : First infected plant, ● : secondary infected plant, ○ : healthy plant.

results suggest that even viruses transmitted to a very limited extent by seeds may initiate the disease cycle.

Tobamoviruses are spread through soil and soil water without the need for a vector (Hollings et al., 1975). Almost all viruses reported to be transmitted this way reach high concentration in plants, are released from plant roots and debris, and are readily transmitted to plants by mechanical inoculation (Matthews, 1991). Soil transmission ratio was 0-3.5% in the fields naturally infested with CGMMV or ZGMMV (data not shown). Conditions in the soils can influence the incidence of the virus diseases in various ways. CGMMV and ZGMMV are physically very stable and accumulate high concentrations in the infected tissue of host crops, are easily transmitted by the sap. In watermelon and cucumber growing fields, two different patterns of spread of CGMMV are noted (Fig. 3). The cluster of watermelon plants infected with CGMMV shows that plant-to-plant spread within the crop took place, with the virus cutting the lateral branch. However, the early spread pattern in cucumber, which is different in the fruit-harvesting stage, shows that infected plants were scattered through the field. A dispersal pattern of ZGMMV in zucchini plants was similar to that of CGMMV in cucumber plants.

Control measures

The use of chemical pesticides, which protect the plants from infection or minimize invasion, is an important method for the control of many diseases. Such direct method for the control of virus diseases is not available yet. Most of the procedures that can be used effectively involve measures designed to reduce sources of infection inside and outside the crop, to limit the spread of vectors, and to minimize the effect of infection on yield.

CGMMV and ZGMMV are transmitted through seed (Table 5). Seed transmission is an important source of infection due to early introduction of the disease and to the spread to other plants. Cucurbit seeds from CGMMV-infected crops carry the virus on the outer and inter surface of the seed coat. Avgelis et al. (1992) reported that cucumber seed contaminated with CGMMV could be eliminated by dry heat-treatment of the seeds for 3 days at 72°C without impairing seed germination.

Plants that remain in the soil may harbor mechanically transmitted viruses and act as a source of infection for the next crops. With a very stable virus like Tobacco mosaic virus (TMV), general sanitation is very important for control, particularly where the susceptible crops are grown in the same area every year (Broadbent et al., 1963). CGMMV in watermelon and ZGMMV in zucchini transmit through the infested soil. The viruses may be difficult to eliminate completely from the infested soil. In cucumber plants grafted with a rootstock, *Cucurbita maxima* × C. *moschata* 'Hukjong Squash', CGMMV may not be transmitted through the infested soil because the rootstock appears to be immune to the virus. The apparent immunity of several squash cultivars to CGMMV is proven by the fact that they could not be infected by mechanical or graft inoculation. This results were consistent with Harvath's report (1995) that CGMMV did not infect *Cucurbita pepo*. Squash cultivars were susceptible host plants to ZGMMV. However, ZGMMV was not found in the naturally growing cucurbit crops except zucchini in Korea so far.

During the growing seasons, control measures such as roguing and sanitation should be observed. It is important to rogue the first infected crops, including the seed and soil, especially early in the season. This may be practicable to control the diseases because CGMMV and ZGMMV have a narrow host range. Correct identification of the virus is essential for applying control measures such as roguing the infected plants. For mechanically transmitted CGMMV and ZGMMV, worker activities during cultivation and tending of cucurbit crops are a major means of virus spread. Once CGMMV or ZGMMV infects a crop like watermelon or zucchini, it is very difficult to prevent its spread during cultivation, particularly during such processe as fruit harvesting. Hence, workers should wash his hands as well as the cutting knives in 10% solution of skim milk. Broadbent (1963) reported that 3% solution of trisodium orthophosphate is an effective decontaminating agent against TMV. Many substances isolated from plants and other organisms have been tested for activity against plant viruses (Dawson and Boyd, 1987; Kopp et al., 1989). However, no antiviral chemicals have been developed on a commercial scale up to this time for the control of Tobamovisures.

Conclusion

Two Tobamoviruses, CGMMV and ZGMMV, occurred in cucurbit growing fields in Korea. CGMMV, ZGMMV, and KGMMV showed differences in host ranges, serological relationships, and the coat protein gene analyses. The viruses are easily transmitted by foliage contact, soil contamination, and through seed without the need for a vector. The viruses transmitted in seeds and soils to a very limited extent may become prime sources of inoculum to initiate the disease cycle. Control of the virus diseases involves precaution measures designed to reduce sources of infection inside and outside the crops and to minimize the effect of infection on yield. During the growing seasons, control measures are roguing and sanitation. For the proper application of control measures, correct identification of virus is essential. There will be no virus problem if the crops are free of virus when planted and when there is no source of

infection in the fields.

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