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Characterization of a Korean Isolate of *Dasheen mosaic virus* Isolated from Taro (*Colocasia esculenta* Schott) in Korea

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A filamentous virus was isolated from taro (*Colocasia esculenta* Schott) showing mosaic and chlorotic feathering symptoms in Chuncheon, Gangwon province in 2002. Based on ELISA, its appearance in electron microscope, serological relationships, and RT-PCR using specific primer and nucleotide sequence analysis of the CP gene, the isolated virus was identified as *Dasheen mosaic virus* (DsMV) and designated as Korean isolated (DsMV-Kr). DsMV was not serologically related to *Zantedeschia mosaic virus* (ZaMV), which has been reported to infect an *Araceae* plants. Since the coat protein revealed electrophoretic heterogeneity, about 42 kDa, 39 kDa and 31 kDa by SDS-PAGE, an improved purification method was established for the production of antisera against DsMV-Kr. The purification method used in this study may be effectively applied to the purification of other filamentous viruses.

Keywords : *Colocasia esculenta* Schott, *Dasheen mosaic virus*, *Zantedeschia mosaic virus*

Dasheen mosaic virus (DsMV), a species of the genus *Potyvirus* in the family *Potyviridae*, is one of the major virus infecting taro in Korea and other countries (Chen et al., 2001; Shimoyama et al., 1992; Zettler et al., 1987; Zettler et al., 1990). DsMV is a flexuous filamentous virion with 750 nm in length and 13 nm in width and contains about 10kb positive-sense single-stranded RNA genome, has a covalently linked protein (VPg) at the 5'-terminus and is polyadenylated at the 3'-terminus. DsMV are transmitted by aphids in a non-persistent manner (Zettler et al., 1978). DsMV is the most important viral pathogen of cultivated aroid plants worldwide, including the genera *Aglaonema, Caladium, Dieffenbachia, Epipremnum, Philodendron, Spathiphyllum*, and *Syngonium*. In addition to these foliage plants, DsMV also infects the *Araceae* plants including the genera *Cryptocoryne* (commercially grown aquarium plant), *Zantedeschia* (Calla lily), and two high-carbohydrate tropical food crops known as dasheen or taro (*Colocasia*) and malanga (*Xanthosoma*).

Taro (*Colocasia esculenta* Schott) originated from India and adjacent areas of southeast Asia is now widely cultivated in Asia and Oceania. In some Pacific Coast countries, this crop is one of the main sources of starch food. In Korea, the crop has been cultivated constantly over 4,000 tons/year. Taro leaves showing mosaic and chlorotic feathering symptoms from taro were collected in Chuncheon, Gangwon province in 2002.

Although DsMV, which was discovered in 1969 (Zettler et al., 1970), has been reported from many countries, the relationships among isolates have not yet been studied systematically. Little information is available on the relationship between the Korean isolate and other known DsMV isolates. To establish the diagnostic system for virus-free taro stock production, we conducted an experiment to identity virus disease in taro showing mosaic and chlorotic feathering symptoms, and analyzed the biological, serological, and molecular characteristics in this study.

Materials and Methods

Virus Source and Isolation. Taro (cv. Paldoo) plants showing mosaic and chlorotic feathering symptoms were collected from a field of Chuncheon city in 2002. DsMV was isolated from these taro plants by sap inoculation. After three repetitions of single lesion transfers on *Tetragonium expansa*, DsMV-kr was maintained in virus-free taro plants (Fig. 1).

Host range test and physical properties. Host range of the DsMV-Kr was determined by mechanical inoculation of purified virus onto 16 test plant species dusted with Carborundum (600 mesh). Symptomless leaves were tested for the presence of the virus by back inoculation to other plants. Physical properties of the DsMV-Kr were assayed on virus-free Calla lily (*Zantedeschia* spp.) seedling.

Virus purification. Group of flexuous filamentous viruses, which are very difficult to purify. After several modifications in the

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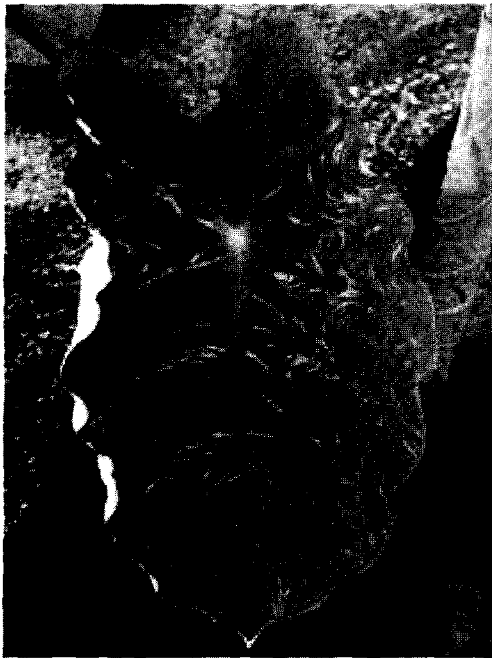

Fig. 1. Symptoms of taro leaves infected with *Dasheen mosaic virus*-Kr.

FRESH/FROZEN INFECTED TARO/CALLA LILY LEAVES
- Homogenize with blender & homogenizer in 3 vol. EB [a]
- Filtered & Squeezed through four-layers of cheesecloth

CRUDE SAP
- Centrifuge at 7,000rpm, 10min at 4℃,
- Centrifuge at 7,500rpm, 10min at 4℃

SUPERNATANT
- Add 2% (v/v) Triton X-100
- Stir for 1hr at 4℃
- Centrifuge at 32,000rpm, 90min at 4℃
- (20% sucrose cushion of 1/4 vol. of total vol.)

PELLET
- Resuspended in RB [b]
- Stir for 1hr at 4℃
- Centrifuge at 6,000rpm, 10min at 4℃

SUPERNATANT
- Centrifuge at 30,000rpm, 90min at 4℃

PELLET
- Resuspended in RB
- Stir for 1hr at 4℃
- Centrifuge at 6,000rpm, 10min at 4℃

SUPERNATANT
- Centrifuge in CsCl (1.85g/5ml)
- Density Gradient at 32,000rpm, 16hrs, at 6℃

VIRUS ZONE
- Dialyzed overnight in DB [c] at 4℃
- Centrifuge at 6,000rpm, 10min at 4℃

SUPERNATANT (Purified Virus)

Fig. 2. Procedure for purification of DsMV-Kr. [a]EB (Extraction buffer): 0.05 M K_2HPO_4, 0.01 M Na_2-EDTA, 0.1%(w/v) Na_2SO_3, 5%(v/v) 100% Et-OH, pH 7.6. [b]RB (Resuspension buffer): Extraction buffer + 0.1% urea, excluding 100% Et-OH. [c]DB (Dialysis buffer): 0.05 M NaH_2PO_4.

purification protocol (Abo El-Nil et al., 1977; Brakke and Ball, 1968; Choi et al., 1977; Damirdagh and Shepherd, 1970; Delgano-Sanchez and Grogan, 1966; Sehgal and Jean, 1970), the better results were obtained with the following steps: Fresh or frozen infected tissue homogenized with 0.01 M potassium phosphate buffer, pH 7.6 without organic solvents. The supernatant was layered on 20% sucrose cushion and centrifuged. The pellets were resuspended and layered on a CsCl density gradient 1.85 g/5 ml in potassium phosphate buffer 0.01 M, pH 7.6. Viral fractions were collected and concentrated by centrifugation and dialyzed (Fig. 2).

Electron microscopy. Purified virus particles were examined by transmission electron microscopy (Carl Zeiss, TEM 109, 80 kv) after negative staining with 2% phosphotungstic acid (pH 7.0). Particle size was determined from a total of about 100 particles by examination at ×20,000.

Aphid transmission. Vector transmission experiments were carried out using apterous aphids (4-5 day old nymphs) of *Myzus persicae*, which were starved for 1 h prior to a 3 minutes acquisition access period on infected and healthy young taro plants and Calla lily seedlings. Transparent plastic-box covered nylon gause in incubator compartment for a 16 h photoperiod at 21 ± 2°C. the test was performed three times, with a total of 200 aphids.

Viral RNA and dsRNA analysis. Viral RNA was extracted from purified virions by the method of Berger et al. (1998). RNA preparations were treated at 65°C for 15 min in the presence of 50% formamide and 10% formaldehyde, and separated in a 1.5% denatured agarose gel at 100V for 90 min in 1×TBE. Viral RNA were obtained from purified preparations of DsMv-Kr compared to those of ZYMV-Cu and PVY-Tu. Viral double-stranded RNA (dsRNA) was extracted from leaf tissues of the taro infected with DsMV-Kr by the procedure of Zhang et al. (1998). The dsRNAs of *Cucumber mosaic virus* (CMV)-Paf strain and the dsRNA of *Zucchni yellow mosaic virus* (ZYMV)-Cu were used as molecular markers. DsRNA was analyzed by electrophoresis through 6% polyacrylamide gel containing 1×TBE. The RNA and dsRNA bands were visualized by staining gels in 100 ng/ml Et-Br solution and photographed under a UV transilluminator.

Coat protein (CP) analysis. The purified virus (1 mg/ml) was mixed with an equal volume of 1× sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS and 5% β-mercaptoethanol, 0.5% bromophenol blue) and heated for 5 minutes at 100°C. The sample was separated on 3% (separating gel; acrylamide solution, 1.5 M Tris-HCl, 2% SDS, APS, TEMED) and 12.5% (stacking gel; acrylamide solution, 0.5 M Tris-HCl, 2% SDS, APS, TEMED) discontinuous polyacrylamide gel. The gel was stained with protein staining solution containing Coomassie brilliant blue R-250 (Sigma, USA) for 2 hours and destained background in the stained gel with the destaining solution (40% methanol, 7% acetic acid). The molecular weight of viral protein was determined by comparison of distances of comigrated protein markers (Bio-Rad).

RT-PCR and treatment of restriction enzyme. Extraction of total nucleic acids from leaf was done as descrived by Lee et al. (1996). A pair primers for detection of DsMV-Kr was designed based on nucleotide sequence analysis of the reported DsMV (GenBank; AF048981). The 5'-upstream primer DCP3-F (5'-GT-GTG *AATTC*GCAAGCAGATGACAC-3') and the 3'-upstream

primer DCP3-R (5'-GTGT*GAGCTC*AACACCGTGCACGAA-3') were designed to detected and amplify the CP and 3'-UTR gene of DsMV (Li et al., 1998; Pappu et al., 1993; Pappu et al., 1994a). Reverse Transcription was carried out with one cycle at 42°C for 45 minutes and 35 cycles of PCR amplification using the step program (94°C, 60 seconds; 50°C, 30 seconds; 72°C, 80 seconds) followed by a final extension at 72°C for 5 minutes. The amplified PCR products were digested with restriction enzyme *AluI* (Promega) and analyzed by electrophoresis in 1.2% agarose gel.

Cloning and sequencing of CP gene. The PCR product was analysed by 1.2% agarose gel electrophoresis, the bands were isolated, purified using the DEAE membrane and cloned in pGEM-T Easy vector (Promega, USA) by standard procedures (Sambrook et al., 1989). Determinations of sequences of cDNA recombinant clones were performed by the dideoxynucleotide chain termination method at Bioneer Co., Korea. Analysis of DsMV-Kr RNA sequences and amino acid sequences were performed by DNASTAR Program (USA) and were compared with published data using programs of the DNA sequence analysis computer package for PC (DNASTAR Program., USA).

Antiserum production and serology. First immunization consisted purified virus (1 mg/ml) mixed with 1 ml of PBS buffer (NaCl 137 mM, KH_2PO_4 1.5 mM, Na_2HPO_4 8.0 mM, KCl 2.7 mM, pH 7.4) by intravenous boosting injection into marginal vein of rabbit ear. The second injection consisted of purified virus (0.5 mg/ml) mixed with equal volume of Freund's complete adjuvant (Sigma). The two subsequent injections consisted of purified virus (0.5 mg/0.5 ml) mixed with equal volume of Freund's incomplete adjuvant (Sigma) by intramuscular injections at fifteen days intervals. The rabbits were bled 1 week after fourth injection. Gel double diffusion test was performed in disposable petri-dish containing 0.8% Noble agar or Gelrite (Sigma) in 1xPBS (NaCl 137 mM, KH_2PO_4 1.5 mM, Na_2HPO_4 8.0 mM, KCl 2.7 mM, NaN_3 3 mM, pH 7.4) (Ohki et al., 1987). Crude sap from infected plant and purified virus preparations in 2% SDS were incubated at 40°C for 30 min prior to its application. Crude sap from healthy plant was used as a negative control.

Results

Field-grown taro plants. 300 samples (150 samples in 2002 and 2003, respectively) showing mosaic and chlorotic feathering symptoms were examined for viruses. DsMV-Kr was detected in 80-90% samples, and the 18 samples were infected with CMV, which was serologicany identified (Table 1).

Host range test and physical properties. The purified virus caused local lesion on inoculated leaves of *Tetragonia expansa*, systemic mosaic and chlorotic feathering symptoms on *Zantedeschia aethiopica* and *Colocasia esculenta*, respectively. No symptom development was observed on the other 13 tested plants (Table 2). The thermal inactivation point (TIP), dilution end point (DEP), longevity *in vitro* (LIV) of the purified virus were 55-60°C, 10^{-2}, and 3-4 days on Calla lily plant, respectively.

Virus purification and CP analysis. When partially purified preparations were subjected to CsCl equilibrium density gradient centrifugation, a single narrow band was formed at a density about 1.26 g/cm^3 (Fig. 3A). Several preparation of purified virus were spectrophotometrically

Table 2. Comparisons of host range and symptoms of DsMV-Kr isolated from taro and ZaMV-Kr from Calla lily

Indicator plants	Symptoms[a]	
	ZaMV-Kr[b]	DsMV-Kr
Tetragoniaceae	–/–	LL/–
Tetragonia expansa		
Araceae		
Zantedeschia aethiopica	–/Mo	–/Mo,Ma
Colocasia esculenta	–/–	–/Mo,Cf
Chenopodiaceae		
Chenopodium amaranticolor	–/–	–/–
C. quinoa	–/–	–/–
Leguminosae		
Glycine max	–/–	–/–
Paseolus vulgaris	–/–	–/–
Vicia faba	–/–	–/–
Vigna sinensis	–/–	–/–
Solanaceae		
Nicotiana benthamiana	–/–	–/–
N. clevelandii	–/–	–/–
N. glutinosa	–/–	–/–
N. rustica	–/–	–/–
N. tabacum cv. *Samsun*	–/–	–/–
N. tabacum cv. *White Burley*	–/–	–/–
Lycopersicon esculentum	–/–	–/–

[a]Mo: Mosaic, Ma: Malformation, LL: Local lesion, Cf: Chlorotic feathering, –: No symptom, [b]S. B. Kwon et al., Arch.Virol. (2002) 147:2281-2289.

Table 1. Detection of DsMV and CMV by DAS-ELISA from naturally infected taro plants in the field

Year	Investigated regions	Cultivar	No. of plant Tested	No. of plants infected with	
				CMV[1]	DsMV[2]
2002	Chunchon	Paldoo	150	6	120
2003	Chunchon	Paldoo	150	12	135

[1,2]tested by DAS-ELISA, which purchased from Agdia Co.

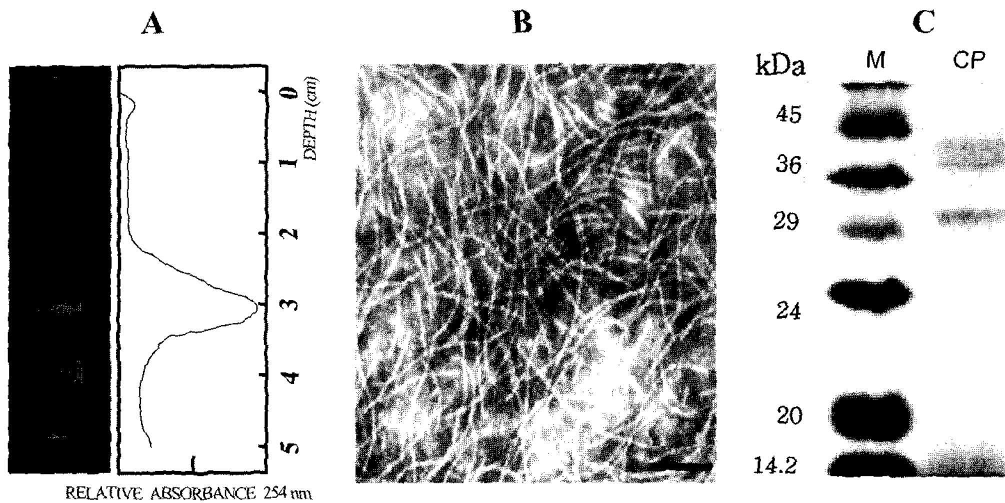

Fig. 3. Purification of DsMV-Kr, (**A**) Density gradient scanning pattern of DsMV-Kr at CsCl-density gradient centrifugation for virus purification. (**B**) Electron micrographs of virus particles from purified DsMV-Kr, Bar = 200 nm. (**C**) Electrophoretic pattern of coat protein of DsMV-Kr (CP), Molecular weight standards (M, Sigma) in 3% and 12.5% discontinuous SDS-polyacrylamide gel.

scanned and gave an A_{258}-A_{261} maximum and a A_{240}-A_{246} minimum value. The relation A_{260}/A_{280} was 1.17. The yield of virus was 0.7-3.8 mg/100 g tissues. Negative stained electron microscopy observation showed that viral particles was few degraded, aggregated and without plant host-debris or other virus particles that would suggest presence of contaminants (Fig. 3B). The CP of DsMV-Kr revealed electrophoretic heterogeneity, showing multiple bands of about 42 kDa, 39 kDa and 31 kDa in SDS-PAGE (Fig. 3C). 39 kDa and 31 kDa proteins might be the proteolytic degraded form of the CP. They might also reflect the unusual amino acid composition of threonine- and asparagine-rich at the N-terminal region of the DsMV CP (Li et al., 1999; Pappu et al., 1994b).

Fig. 4. (**A**) Electrophoretic profile of the genomic RNAs extracted from the DsMV-Kr. M: RNA marker (promega); Lane 1: genomic RNA extracted from the purified DsMV-Kr, lane 2: genomic RNA of ZYMV, lane 3: genomic RNA of PVY, lane 4: extracted from crude sap of taro infected DsMV-Kr. (**B**) DsRNA profiles of the DsMV-Kr extracted from infected taro plant. Lane 1; dsRNA of CMV-Paf, 2; dsRNA of DsMV-Kr, 3; dsRNA of ZYMV.

Aphid transmission. DsMV was experimentally transmitted by the aphid (*M. persicae*) in a non-persistent manner with a 40% on taro and 50% on Calla lily efficiencies. RT-PCR of total RNAs from aphid-transmitted plants with DsMV specific primer further confirmed virus transmissions (data not shown).

Viral RNA and dsRNA analysis. A single RNA of about 10 kb was detected when viral RNA was extracted from

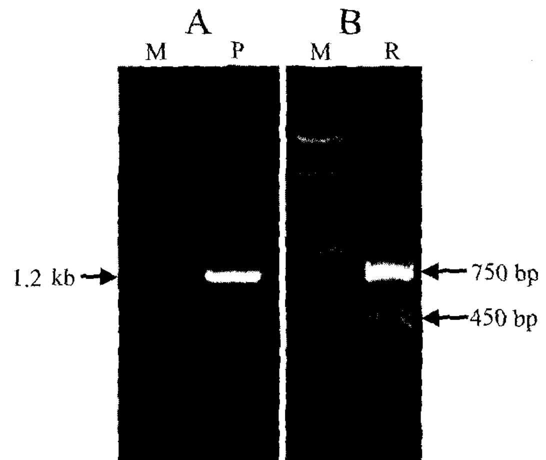

Fig. 5. RT-PCR (A) for the CP of DsMV-Kr using DCP3-F/R primer pair. *AluI* restriction analysis (B) of the RT-PCR products for DsMV-Kr CP. lane M: 1 Kb DNA ladder (promega), P: the amplified DNA product of DsMV-Kr. R: digested DNAs with *AluI*.

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   1 GCAGGCTGATGATACAGTTGATGCGGGAAACAATGATAACAAAACAAAAACAACTGAAACAAAAACTCCTGCAGCAGGTG
        A  D  D  T  V  D  A  G  N  N  D  N  K  T  K  T  T  E  T  K  T  P  A  A  G
  81 GCGGTAATAACACCAACAATACCCCACCACCACCCGCAAATAACACAACCAACAACAATCCTCCACCGCCACCACCGGCA
     G  G  N  N  T  N  N  T  P  P  P  P  A  N  N  T  T  N  N  N  P  P  P  P  P  P  A
 161 GCACCAAAGGCGACAGAGACACCAGCGAACAAGCAAGTGGTCCCCACAACAAGTGAGAAAGGGAAGGAAGTTGTTAAAGA
      A  P  K  A  T  E  T  P  A  N  K  Q  V  V  P  T  T  S  E  K  G  K  E  V  V  K  D
 241 TGTAAACGCTGGAACCAGTGGCACTTATTCTGTACCTCGATTAAATAAAATCACAAATAAGATGAACTTACCCTTAGTCA
       V  N  A  G  T  S  G  T  Y  S  V  P  R  L  N  K  I  T  N  K  M  N  L  P  L  V
 321 AGGGCAAATGCATCTTAAATTTAAACCATTTAATCGAGTACAAACCCGAACAGCGTGACATATTTAATACCAGAGCCACC
     K  G  K  C  I  L  N  L  N  H  L  I  E  Y  K  P  E  Q  R  D  I  F  N  T  R  A  T
 401 CACACGCAATTTGAAGTCTGGTACAATGCAGTCAAGAGGGAATATGAGCTGGAAGATGAACAGATGCACATTGTTATGAA
      H  T  Q  F  E  V  W  Y  N  A  V  K  R  E  Y  E  L  E  D  E  Q  M  H  I  V  M  N
 481 TGGCTTTATGGTTTGGTGCATCGACAATGGAACATCACCCGACATCAACGGGGCTTGGGTGATGATGGACGGAAACGATC
       G  F  M  V  W  C  I  D  N  G  T  S  P  D  I  N  G  A  W  V  M  M  D  G  N  D
 561 AAATTGAATACCCGTTGAAACCAATTGTGGAAAACGCGAAACCCACCTTGCGCCAGATAATGCATCACTTTTCTGACGCA
     Q  I  E  Y  P  L  K  P  I  V  E  N  A  K  P  T  L  R  Q  I  M  H  H  F  S  D  A
 641 GCAGAGGCGTACATTGAACTGAGGAATGCGGAGAAACCGTACATGCCTAGGTACGGTCTCATTCGCAACTTACGTGATGC
      A  E  A  Y  I  E  L  R  N  A  E  K  P  Y  M  P  R  Y  G  L  I  R  N  L  R  D  A
 721 AAGTCTCGCCCGGTATGCTTTTGATTTTTATGAAGTCAATTCAAAGACACCGGTACGAGCAAGAGAAGCAGTTGCACAGA
       S  L  A  R  Y  A  F  D  F  Y  E  V  N  S  K  T  P  V  R  A  R  E  A  V  A  Q
 801 TGAAGGCCGCTGCACTCTCTAACGTTACTACTAGGTTGTTTGGTTTGGATGGTAACGTTTCAACTTCAAGCGAGTACACT
     M  K  A  A  A  L  S  N  V  T  T  R  L  F  G  L  D  G  N  V  S  T  S  S  E  Y  T
 881 GAAAGGCATACTGCAAAGGACGTCACACCCAACATGCACACGTTGCTTGGTGTGGCACCTCCGCAGTGAAGGTCTGGTAA
      E  R  H  T  A  K  D  V  T  P  N  M  H  T  L  L  G  V  A  P  P  Q
 961 ACAGACTATAGTTATCGTCTCGCTGTCTGTGGTTTTATATATTTAAAGTACTGTTTGTATTCGAATAGTGTTATTTGATT
1041 ATAAACCACAGAGTGGTTTTCCACCGATGTAGAGAGGTGCCATGCACCCTACCATCTACGTCCTTTAAATATAAGAAAAC
1121 TGCTGAACTACTGCACCTACATCAGACCGTAAGTCGGCCATGGGCGCGGTAGGCGAGATGCTTCGTGCACGGTGTTCAA
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Fig. 6. Nucleotides and derived amino acids sequences of the DsMV-Kr CP gene.

Table 3. Percentage of sequence identities between the CP gene, encoded amino acid sequence and 3'-UTR of DsMV-Kr and other *Potyviruses*

Virus	Acesstion No.	Petcent similrity to DsMV-Kr (%)		
		CP		3'-UTR
		nt	aa	nt
DsMV-Cal	AF048981	88.3	92.1	86.5
DsMV-Col	U00122	83.5	93.0	90.2
DsMV-M13	AJ298033	89.9	93.3	89.3
DsMV-s	AF511485	91.5	93.6	89.3
DsMV-DK	AJ298035	80.1	85.4	90.8
DsMV-ND	AJ298034	89.4	93.9	91.8
BCMNV	U37076	56.9	61.1	52.8
BCMV	L15332	61.6	60.8	82.7
SMV-G2	S43380	58.7	62.3	53.8
ZYMV-Cal	L31350	57.1	62.0	41.8
TEV	M15239	53.0	56.7	52.0
PVY	AF345650	52.6	55.1	29.5
LMV	X65652	55.8	52.0	26.0
ZaMV-BG	AY026463	51.0	49.9	24.1
ZaMV-DB	AY026464	51.4	49.9	24.1
ZaMV-ZAN	AF332872	51.4	49.9	24.1
ZaMV-Kr	AB081519	51.5	49.9	24.5
PPV	M21847	55.6	49.9	23.2
TuMV	D10927	55.3	51.6	54.3
CLYVV	D86044	51.0	49.1	28.5

purified virions (Fig. 4A). The sizes and numbers of the dsRNA produced during infection of the DsMV-Kr in *C. esculenta* are shown in Fig. 4B and compared with those of CMV-Paf and ZYMV-Cu. DsMV-Kr revealed dsRNA with estimated molecular size about 10 kb and the mobility of the dsRNA was similar to that of ZYMV-Cu.

RT-PCR amplification and sequence analysis of the 3'-terminal region of DsMV-kr. Oligonucleotide primers DCP3-F and DCP3-R specifically amplified DNA product of 1.2 kbp corresponding to the 3'-UTR and CP gene of DsMV (Fig. 5A). When the DNA product was digested with *Alu*I, two fragments in DNA product of 750 bp and

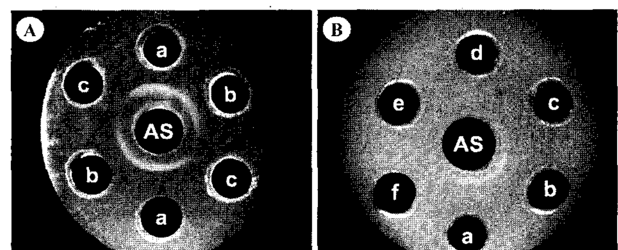

Fig. 7. Agar gel double diffusion test. Panel (**A**). central well (AS) was filled with DsMV-Kr antiserum and peripheral wells filled with purified DsMV-Kr (a), crude sap of taro infected DsMV-Kr (b), purified ZaMV-Kr from Calla lily (c). Panel (**B**). plate titer test. central well (AS) was filled with DsMV-Kr antiserum (x2 diluted) a; not diluted (approx. 0.3 mg/ml purified virus), b; x2 diluted, c; x4, d; x8, e; x16, f; x32.

450 bp were observed, respectively (Fig. 5B). DsMV-Kr CP gene consisted of 942 nucleotides encoding 314 amino acid residues (Fig. 6). The sequence identity of DsMV-Kr CP gene ranged from 80.1% to 91.5% in the nucleotide level and ranged from 85.4% to 93.9% in the nucleotide level compared with other DsMV isolates were previously reported. The identity of DsMV-Kr 3'-UTR between DsMV-Kr and 7 isolates of DsMV ranged from 86.5% to 93.1% in the amino acid level (Table 3).

Antiserum production and serology. Antiserum against DsMV-Kr had titer of 1/1024 with the purified virus preparation in microprecipitin test and 1/32 with the purified virus preparation in agar gel double diffusion test. SDS-denatured virus formed specific single precipitin line (Fig. 7). The DsMV-Kr antiserum did not show reaction against ZaMV-Kr and healthy plants. This result indicates that DsMV and ZaMV are not serologically related close enough to form precipitin line (Kwon et al., 2002).

Discussion

In this study, we isolated DsMV-Kr causing mosaic and chlorotic feathering symptoms from taro, and characterized its properties. In an electron microscope, flexuous filamentous shaped DsMV particles were observed under EM. With about 750 nm in length and 13 nm in width symptom developments on tested plants. The symptoms induced by DsMV-Kr on indicator plants the were very similar to those of *Zantedeschia mosaic virus* (ZaMV) which has been reported an *Araceae* plants-infecting potyvirus. Gel double diffusion test, however, revealed that DsMV was not serologically related to ZaMV. The CP gene of DsMV-Kr was cloned and their nucleotide sequences was determined. The sequence of the 3'-terminal 1,199 nucleotides, which consisted of the 3'-terminus of the partial nuclear inclusion body (NIb), coat protein (CP) genes and 3'-UTR. Similarity at the nucleotide and amino acid levels of CP and 3'-UTR were from 51.0 to 91.5% and from 49.1 to 93.9%, respectively, when compared with those of other *Potyviruses*. Phylogenetic analysis of selected potyviral CP sequences indicates that the virus is an isolate of DsMV and distinguishable from the other potyviruses. Altogether, these results indicate that new isolate of DsMV designated at the Korean isolate of DsMV (DsMV-kr) is present in taro plant.

Flexuous filamentous viruses are very difficult to isolate and purify. Those difficulties are related with the presence of mucilage in plant and the tendency of the viruses to aggregate and form insoluble complexes. Initial attempts of purification were done with protocols that used organic solvents, PEG and NaCl, Triton X-100 and many cycles of high and low centrifugation involving 4 to 5 days of work. Unfortunately, final viral suspensions contained few virions or associated with normal plant proteins and thus antiserum raised showed strong nonspecific reactions on serological tests (data not shown). After several modifications in some purification protocol, the better results were obtained with the following key steps. Fresh or frozen infected tissue were homogenized with 0.05 M potassium phosphate buffer, pH 7.6 without organic solvents. Clarification by various kinds of organic solvents used to the loss of virus particles during purification. To solve this problem, we used one or two cycles of differential centrifugation on a sucrose cushion and the CsCl equilibrium density gradient centrifugation as a final step. This method provided successful separation of virus particles from infected tissues. An improved purification method for antisera production used in this study might be effective for the purification of DsMV as well as the other filamentous viruses similar to DsMV, for example, ZYMV, ZaMV, WMV2, PRSV, PVY O/N strains, LSV, LMoV, TEV, TBV, PepMoV, TuMV and PVX.

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