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Detection of Kyuri Green Mottle Mosaic Virus from Soil by the Immunocapture Reverse Transcription Loop-mediated Isothermal Amplification Reaction

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Abstract: Kyuri green mottle mosaic virus (KGMMV) which is a major soil-borne virus, causes severe yield reduction in cucumber in Japan. We developed immunocapture reverse transcription loop-mediated isothermal amplification (Ic/RT-LAMP) method to detect KGMMV in soil. Degenerate primer set from KGMMV C strain (KGMMV-C) and Yodo strain (KGMMV-Yodo) was designed for the detection of both strains. To optimize the reaction condition, the RT-LAMP reaction using the degenerate primer set was performed at different temperature (60-65°C). The reaction at 60°C gave the best results. The result on specificity test using 7 cucumber pathogenic viral isolates indicated that the RT-LAMP assay established in this study had no cross reactions. The detection limit of the RT-LAMP assay was 10 pg of purified KGMMV. The sensitivity of RT-LAMP was 10 times higher than that of RT-PCR. Detection limit of DAS-ELISA and Ic/RT-LAMP was compared by using of the dilution of purified KGMMV-C in soil. Ic/RT-LAMP could detect 0.5 ng of KGMMV from 100 mg of soil sample and the sensitivity of Ic/RT-LAMP was 200 times higher than that of DAS-ELISA. The Ic/RT-LAMP assay could successfully detect KGMMV in soil samples from 10 fields where KGMMV had been detected in a previous cultivation. The present Ic/RT-LAMP method showed to be a rapid, simple and available detection method of KGMMV in soil.

Key words: Cucumber, RT-LAMP, RT-PCR, ELISA, diagnosis, soil-borne

INTRODUCTION

At present, five cucurbit-infecting Tobamoviruses are known: Cucumber Fruit Mottle Mosaic Virus (CFMMV), Cucumber Green Mottle Mosaic Virus (CGMMV), Kyuri Green Mottle Mosaic Virus (KGMMV), Zucchini Green Mottle Mosaic Virus (ZGMMV) and Cucumber Mottle Virus (CuMoV) (Kim *et al*., 2009; Moradi and Jafarpour, 2011; Valizadeh *et al*., 2011). KGMMV is one of the most significant pathogenic viruses of cucumber (*Cucumis sativas*) (Lee *et al*., 2000; Kubota *et al*., 2009). KGMMV was first reported as a CGMMV cucumber strain in Japan (Inoue *et al*., 1967). Then CGMMV cucumber strain was indicated to be different from the CGMMV watermelon strain in the point of serological analysis and RNA-cDNA hybridization patterns (Francki *et al*., 1986). Consequently, the cucumber strain has been examined and named KGMMV as distinctive species in the genus Tobamovirus.

KGMMV is transmitted mechanically and through seeds and soil, because it has very stable structure (Daryono *et al*., 2006; Kubota *et al*., 2009). Some diagnosis methods were reported for soilborne Tobamovirus (Fillhart *et al*., 1998; Jacobi *et al*., 1998; Varveri *et al*., 2002; Lee *et al*., 2003; Ikegashira *et al*., 2004). ELISA and RT-PCR have been widely used.

Loop-mediated isothermal amplification (LAMP) can amplify DNAs under isothermal condition ranging from 60-65°C (Notomi *et al*., 2000), because *Bst* DNA polymerase, which is used for the LAMP reaction, has displacement activity (Notomi *et al*., 2000). Four primers (F3, B3, FIP and BIP) which recognize six regions, are used for the LAMP reaction (Fig. 1). Furthermore, the LAMP reaction is accelerated by adding of two loop primers (Nagamine *et al*., 2002) (Fig. 1). LAMP has high amplification efficiency and produces a very amount of DNA. Therefore, the detection can be performed by a visual assessment of turbidity, by the use of the turbid meter (Mori *et al*., 2001), or by the addition of fluorescent reagents (Li *et al*., 2010). Several detection methods using LAMP or RT-LAMP have been developed for plant viruses and viroids (Fukuta *et al*., 2003, 2004; Nie, 2005; Boubourakas *et al*., 2009).

In this study, we developed the detection method of KGMMV in soil by immunocapture RT-LAMP method.

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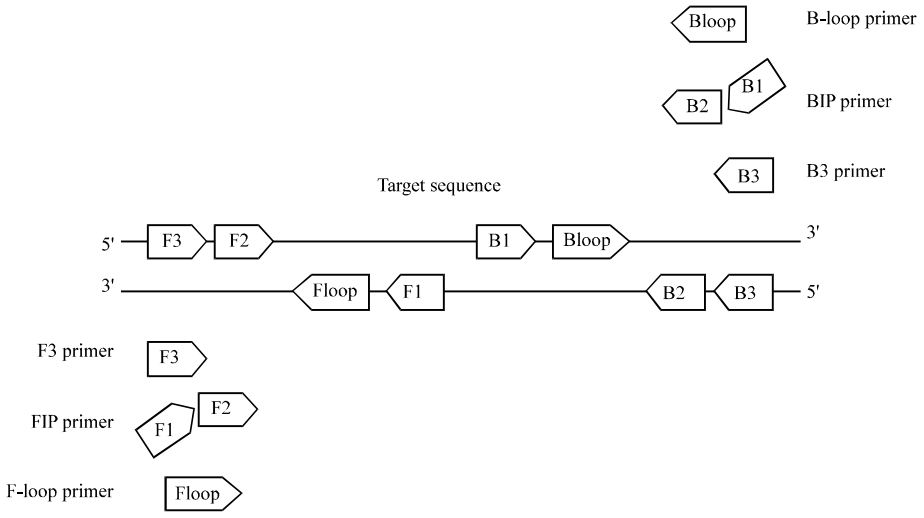

Fig. 1: Schematic diagram showing the position of the LAMP primer

MATERIALS AND METHODS

Virus isolates and antisera: Seven cucumber pathogenic viral isolates were used in this study (Table 1).

Two purified KGMMV isolates (KGMMV C strain and Yodo strain) were kindly provided by Dr. K. Kubota (Research Team for Vegetables and Ornamental Crops in Southern Japan, National Agricultural Research Center for Kyushu Okinawa Region, Fukuoka, Japan). Cucumber plants (*Cucumis sativas*) were grown in greenhouse. Carborunbum-dusted leaves on the fourth leaf stages were inoculated with purified KGMMV preparations diluted in 10 mM phosphate buffer (pH 7.0).

Zucchini Yellow Mosaic Virus (ZYMV), Cucumber Mosaic Virus (CMV), Water Melon Mosaic Virus (WMV), Melon Yellow Spot Virus (MYSV), or Beet Pseudo-Yellows Virus (BPYV) were gathered in the cucumber fields in Japan. The purified viruses and cucumber leaves infected with the viruses were kept in -80°C until use.

The 0.1-0.5 g of cucumber leaves were macerated and diluted in 100 mM tris-HCl (pH 8.0) to give a final dilution of 1:200. One microliter of the extraction was used to investigate the specificity of the RT-LAMP assay.

Anti-KGMMV IgG was purchased from Japan Plant Protection Association.

Primer design: The degenerate primers used for RT-LAMP amplification were designed from the coat protein gene of KGMMV-C and KGMMV-Yodo. The sequence of KGMMV-C and KGMMV-Yodo were retrieved from the GenBank database accession No. AB015144 and AB015145, respectively. Sequences of six primers comprising two outer (F3 and B3), two inner (FIP and BIP) and two loop primers (F-loop and B-loop) were shown in Table 2 and Fig. 2.

Table 1: Viral isolates used in this study

Virus	Designation	Origin
Kyuri green mottle mosaic virus	KGMMV-C	Cucumber, Japan
	KGMMV-Yodo	Cucumber, Japan
Zucchini yellow mosaic virus	ZYMV	Cucumber, Japan
Cucumber mosaic virus	CMV	Cucumber, Japan
Water melon mosaic virus	WMV	Cucumber, Japan
Melon yellow spot virus	MYSV	Cucumber, Japan
Beet pseudo-yellows virus	BPYV	Cucumber, Japan

Table 2: List of RT-LAMP primers for KGMMV detection

		Sequence
KGMMV degenerate primer set	F3	5'-CG**Y**GAGTC**Y**C**Y**CA**M**TGGG-3'
	B3	5'-GGGTTTTCAACTTCGATAGC-3'
	FIP	5'-GACCACACGAAA**RMY**TCA**YY**-GTTAAC**Y**TCTGTTGCGTCTC-3'
	BIP	5'-CGTGAGTCGCGCATTGC**W**GC-CCTATTTCTAGAATCCAAAGCGG-3'
	F-loop	5'-AGC**W**GGAAAACGTGAM**T**TGR-3'
	B-loop	5'-AT**W**MTCGATTC**K**TTGTTA-3'

Bold letters show degenerate sequence

RT-LAMP reaction: The RT-LAMP reaction was performed in a total volume of 25 μL. The reaction contained 20 mM tris-HCl pH 8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 0.8 M betaine (Sigma-Aldrich), 4 mM $MgSO_4$, 5 mM DTT, 1.6 mM dNTPs, 0.2 μM each of F3 and B3 primer, 1.6 μM each of FIP and BIP primer, 0.8 μM each of F-loop and B-loop primer, 8 units of *Bst* DNA polymerase (New England Biolabs), 1.25 units of AMV reveres transcriptase (Promega Corp.), 8 units of RNase inhibitor (Promega Corp.) and 1 μL of RNA solution. The mixture was incubated at 60-65°C monitoring the turbidity of the reaction solution by the real time turbid meter LA200 (Teramecs).

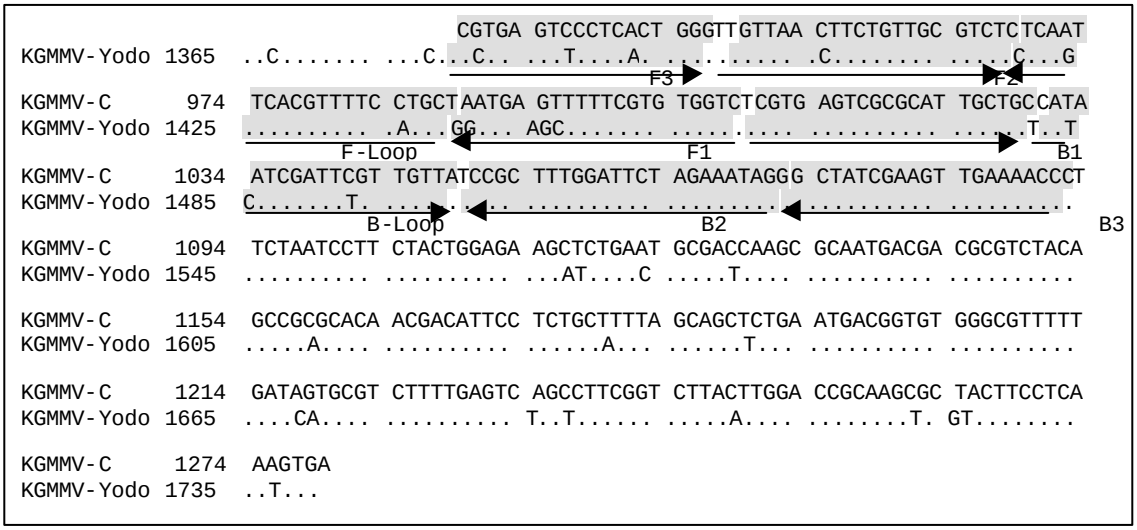

Fig. 2: Alignment of the sequences of the coat protein gene of KGMMV-C (DDBJ accession No. AB015144) and KGMMV-Yodo (DDBJ accession No. AB015145) and the location of 6 primers. Arrows indicate the direction of extension

RT-PCR reaction: RT-PCR was performed using ReverTra Dash Kit (Toyobo) according to the manufacturer's protocol. B3 primer was used for reverse transcription and B3 and F3 primer were used for the PCR amplification, respectively (Table 1). PCR was conducted in a DNA thermal cycler (Gene Amp PCR system 2700) by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 20 sec and extension at 74°C for 30 sec. Amplification products were confirmed by electrophoresis in 2% agarose. Gels were stained ethidium bromide and photographed under ultraviolet light.

Extraction of virus from soils: One hundred milligrams of soil samples were suspended in one mL of Extraction Buffer (50 mM phosphate buffer, 2% skim milk, 0.05% tween 20) in 2.0 mL centrifuge tubes. Suspensions were shaken by TissueLyser (Qiagen) in 30 Hz for 1 min. The samples were centrifuged at 14,000 xg for 2 min. One hundred microliter of supernatant were used for Ic/RT-LAMP or DAS ELISA.

Immunocapture method: Trap of the virions in the soil samples was performed according to Ikegashira *et al.* (2004) with some modification. Tubes were coated with 50 μL of 2 μg mL^{-1} of anti-KGMMV IgG in sodium carbonate coating buffer (Clark and Adams, 1977) and incubated for 2 h at 37°C. The tubes were washed three times with 500 μL of PBS-Tween (phosphate buffered saline, pH 7.4, containing 0.05% tween 20). One hundred microliter of Extraction Buffer was added to the tubes and incubated at 37°C for 1 h to prevent non-specific binding. Plates were washed three times with 500 μL of PBS-Tween. Soil sample extracts were added (100 μL) and incubated at 37°C for 2 h. After three times wash with 500 μL of PBS-Tween, RNA was extracted with 50 μL of RNase free water by incubation for 5 min at 80°C. One microliter of extraction was used for the RT-LAMP reaction.

DAS-ELISA: DAS-ELISA (Clark and Adams, 1977) was performed according to Ikegashira *et al.* (2004) with some modification. Plates were coated with 100 μL of 2 μg mL^{-1} of anti-KGMMV IgG in sodium carbonate coating buffer and incubated for 2 hr at 37°C. The plates were washed three times with 500 μL of PBS-Tween. One hundred microliter of Extraction Buffer (50 mM phosphate buffer, 2% skim milk, 0.05% tween 20) was added to the plates and incubated at 37°C for one hour to prevent non-specific binding. Plates were washed three times with 500 μL of PBS-Tween. Soil sample extracts were added (100 μL $well^{-1}$) and incubated at 37°C for two hours. Alkaline phosphatase-conjugated KGMMV antibody, diluted 2,000 times in PBST, was added to wells. After 2 h incubation, plates were washed with PBST, then substrate (1.0 mg mL^{-1} ρ-nitrophenylphosphate in 10% diethanolamine, adjusted to pH 9.8 with HCl) was added. After 30 minutes incubation at 25°C, absorbance at 405 nm (A_{405}) was determined using an ELISA plate reader (Model 550, Bio-Rad). Samples were considered positive, if the mean A_{405} of two sample wells was two times greater than the mean of two negative soil samples.

Ic/RT-LAMP and ELISA comparison: Comparison of the sensitivity between the Ic/RT-LAMP reaction and DAS-ELISA was made in order to estimate the sensitivity of the Ic/RT-LAMP reaction. One hundred mg of soil samples seeded with 0, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 250, 500, 1,000 and 1,500 ng of purified KGMMV-C were used for Ic/RT-LAMP and DAS-ELISA.

Detection of KGMMV from soil samples derived from cucumber fields: The IC-RT-LAMP assay was tested to detect KGMMV in soil samples from cucumber fields. Sixteen fields were selected from cucumber fields in Aichi Prefecture, Japan. Each field was confirmed to have been infected (10 fields) or unifected (6 fields) in a previous cultivation. One soil sample was collected per field, from a depth of 5 to 10 cm and at a location between plants in the central part of a greenhouse in each field.

RESULTS

Optimization of RT-LAMP assay condition for KGMMV detection: The thermal conditions of the RT-LAMP reaction were investigated. RT-LAMP reaction was performed at 60, 63, 65 or 68°C for 90 min, using 100 pg of purified KGMMV-C as a template. The turbidity of the RT-LAMP reaction solutions are shown in Fig. 3. The RT-LAMP reaction at 60, 63 and 65°C could detect KGMMV both strains after 25-35 min. The RT-LAMP reaction at 60°C gave the best results and could detect KGMMV most rapidly. Consequently, the RT-LAMP reaction was performed at 60°C for further evaluation.

Specificity of the RT-LAMP assay: The specificity of the RT-LAMP reaction was tested with 7 cucumber pathogenic viral isolates (Table 1). The results are shown in Fig. 4. The turbidity of the RT-LAMP reaction solution of two KGMMV strains (KGMMV-C and KGMMV-Yodo) increased. Other viral isolates gave no amplification.

Sensitivity of RT-LAMP and RT-PCR: Comparison of the sensitivity between the RT-LAMP reaction and the RT-PCR reaction was made in order to estimate the sensitivity of the RT-LAMP reaction. The RT-LAMP reaction was performed using purified KGMMV-C in the range of 100 ng to 100 Fg. Turbidity of the RT-LAMP reaction solution increased in all samples which contained at least 10 pg purified KGMMV (Fig. 5a). The RT-PCR reaction was performed using same virus samples. Results show that the amplification products of 164 bp were observed in the samples from 100 ng to 100 pg (Fig. 5b).

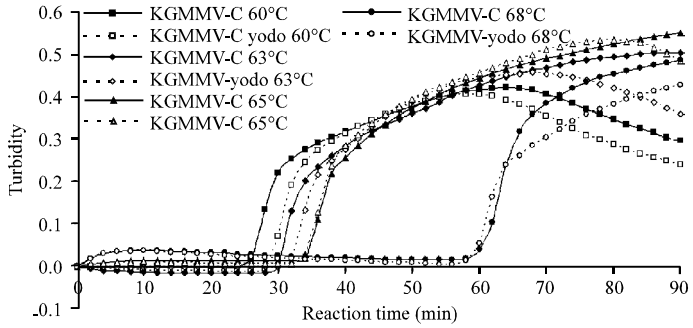

Fig. 3: Turbidity of the LAMP reaction solution for the detection of KGMMV-C and Yodo with KGMMV degenerate primer set at different temperatures

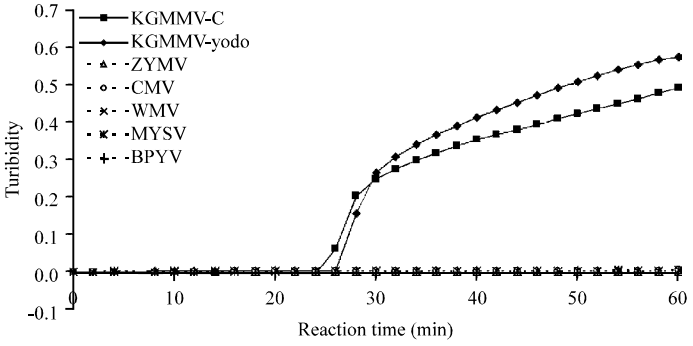

Fig. 4: The specificity of the RT-LAMP reaction with seven cucumber pathogenic viral isolates

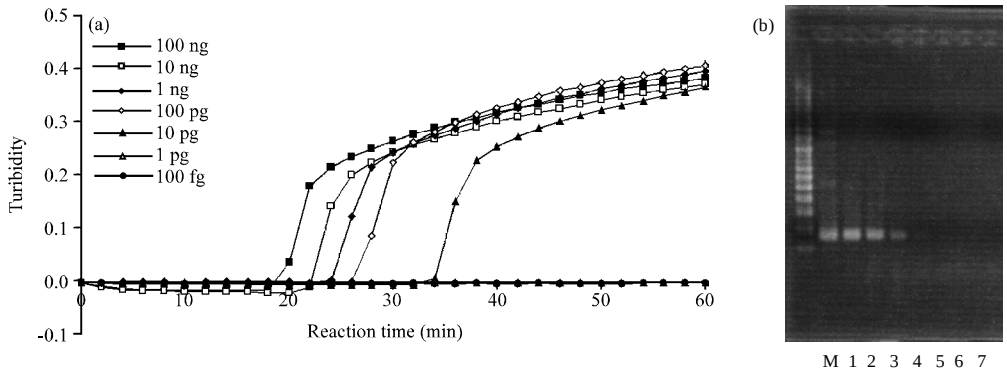

Fig. 5(a-b): Relative sensitivity of the RT-LAMP reaction (a) and RT-PCR for the detection of KGMMV. (a) The turbidity of RT-LAMP reaction solution with KGMMV in the range of 100 ng to 100 Fg. (b) Agarose gel electrophoretic analysis of the RT-PCR reaction with 100 ng (lane 1), 10 ng (lane 2), 1 ng (lane 3), 100 pg (lane 4), 10 pg (lane 5), 1 pg (lane 6) and 100 Fg (lane 7) of KGMMV

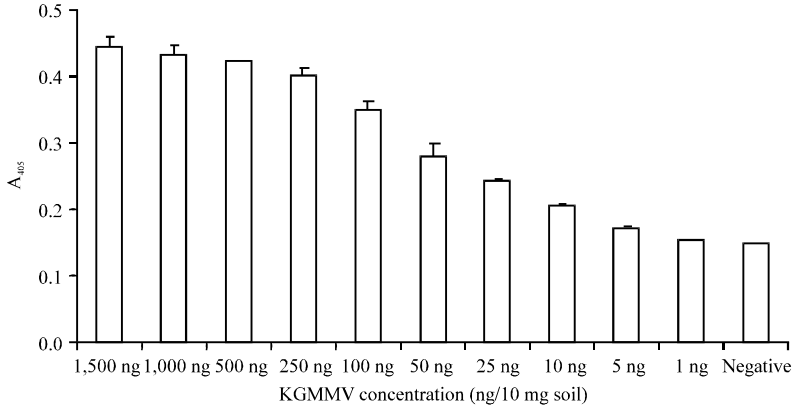

Fig. 6: Means of A_{405} values DAS-ELISA to detect purified KGMMV in soil. A_{405} values represent the mean of two wells per sample as determined by DAS-ELISA. A_{405} values above 0.3 (twice value of negative) was judged positive (dotted line)

Consequently, the sensitivity of the RT-LAMP reaction was ten times higher than that of the RT-PCR reaction.

Ic/RT-LAMP and ELISA comparison: DAS-ELISA and the Ic/RT-LAMP reaction was performed to detect KGMMV from soil containing 0, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 250, 500, 1,000 or 1,500 ng of purified KGMMV-C. The A_{405} values of DAS-ELISA are shown in Fig. 6. DAS-ELISA could detect 100 ng of KGMMV from 100 mg of soil (Fig. 6). On the other hand, the Ic/RT-LAMP reaction could detect 1,500 ng of KGMMV in 20 min, 1-1,000 ng within 35 min and 0.5 ng after 45 min (Fig. 7). As a result, Ic/RT-LAMP was proved to be 200 times as sensitive as DAS-ELISA.

Application of the Ic/RT-LAMP reaction to soil diagnosis of KGMMV in soil samples from cucumber fields: The Ic/RT-LAMP reaction was used to detect KGMMV from soil samples from 16 cucumber fields (10 infected fields and 6 uninfected fields). As shown in Fig. 8, the turbidity of the samples from infected fields increase within 60 min while those from uninfected fields did not increase. The Ic/RT-LAMP reaction tubes in soil samples from 16 cucumber fields are shown in Fig. 9. The RT-LAMP reaction yields large amount of by-product, pyrophosphate ion, leading to a white precipitate of magnesium pyrophosphate in the reaction mixture. Detection is simplified by visual judgment by the unaided eye without electrophoresis.

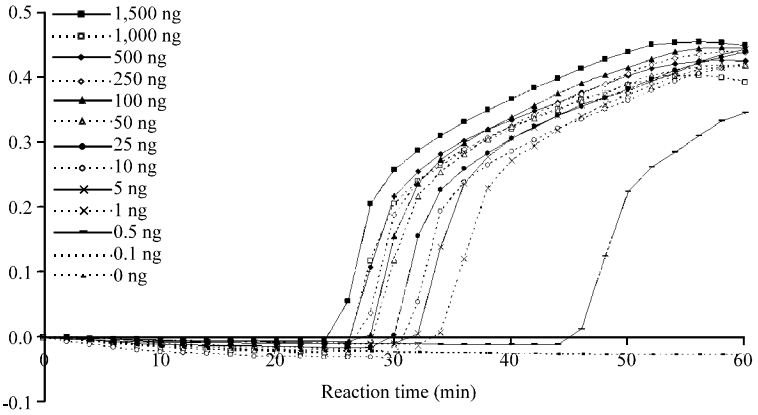

Fig. 7: The turbidity of the Ic/RT-LAMP reaction to detect purified KGMMV in soil

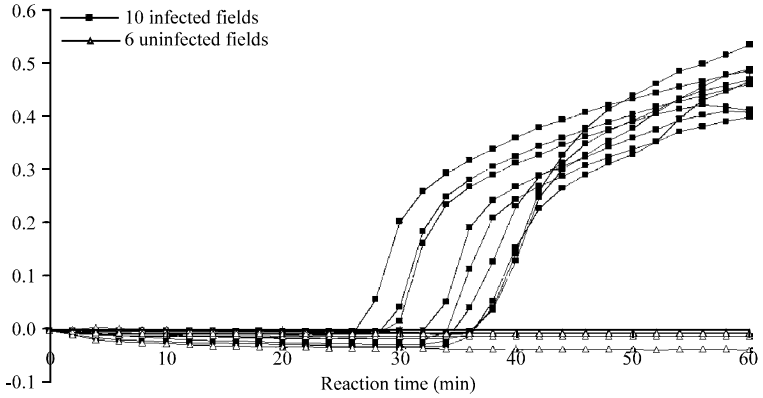

Fig. 8: The Ic/RT-LAMP reaction to detect KGMMV in soil samples from cucumber fields

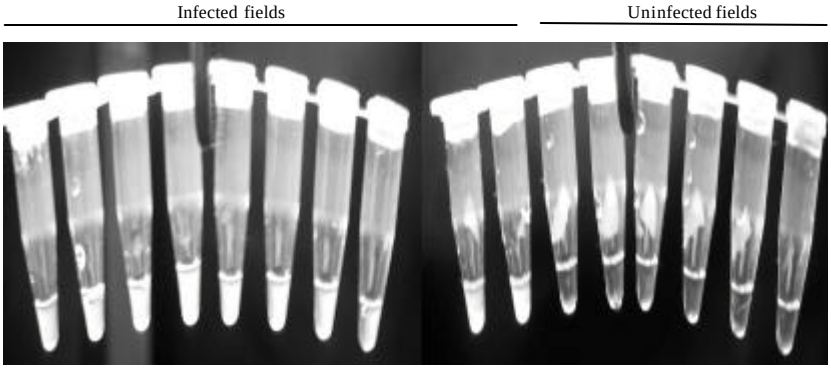

Fig. 9: The Ic/LAMP reaction tubes after 60 min reaction. The reaction tubes with soil samples from 10 infected fields and from 6 uninfected fields. Allows show the cloudiness of the reaction solution

DISCUSSION

KGMMV is the causative agent of viral mosaic disease of cucumber and has emerged as a major constraint on the culture of cucumber. KGMMV is soilborne virus. Methyl bromide has been used for the sterilization of the field polluted by many soilborne pests including KGMMV (Gilreath *et al.* 2004; Kubota *et al.*, 2009). However, methyl bromide manufacturing will completely stop in 2013 for soil use in Japan. Therefore,

development of alternative technologies to control soil transmitted KGMMV are required immediately. Especially the diagnosis method is basic tool of managing the alternative control technologies of KGMMV. In this report, we described the successful development of the Ic/RT-LAMP method for the detection of KGMMV from soil samples.

The LAMP method was highly sensitive, because six targets of template DNA were detectable in the LAMP reaction. In this study, a set of degenerate LAMP primers which specifically amplified the target sequences derived from coat protein gene of KGMMV-C and KGMMV-Yodo was designed. The degenerate LAMP primer set did work effectively to detect both strains. RT-PCR and RT-LAMP assays using degenerate primers for many plant pathogenic viruses have been developed (Ahmad and Fath-Allah, 2012; Narayana *et al*., 2007; Fukuta *et al*., 2004; Govindappa *et al*., 2011; Mansoor *et al*., 1998; Shoman and Othman, 2005). Fukuta *et al*. (2004) reported that Japanese Yam Mosaic Virus (JYMV) in the fields contain some varieties which have a few differences of sequences and degenerate primer set was effective to detect a group of JYMV simultaneously. It is considered that degenerate primers are useful to redress the over specificity of the LAMP reaction for the detection of a group including genetic varieties.

The optimum temperature condition for the RT-LAMP with the degenerate primer set was 60°C. At 60°C, two KGMMV strains were detected within 30 min. In addition, the RT-LAMP assay was proved to have high specificity, as only KGMMV was detected out of 7 cucumber pathogenic viruses.

RT-LAMP method has high sensitivity. The RT-LAMP method could detect 10 pg of purified KGMMV which was 10 times higher than the sensitivity of RT-PCR. Furthermore, Ic/RT-LAMP could detect 0.5 ng of KGMMV in soil samples. Ic/RT-LAMP was 200 times more sensitive than DAS-ELISA. The high sensitivity of RT-LAMP was also reported by other researchers, where RT-LAMP is 100 times more sensitive than RT-PCR (Fukuta *et al*., 2004; Boubourakas *et al*., 2009), ten times more sensitive than the nested RT-PCR system (Mekata *et al*., 2009).

The RT-LAMP method has high sensitivity and rapidity without the need for expensive equipments to detect KGMMV. RNA extraction from soil samples is still the step which is essential and requires well-trained personnel. In this study we used the Immunocapture method for extraction of RNA from soil samples. As a result, the immunocapture method captures successfully KGMMV particles from soil samples and the RNAs could be used for the RT-LAMP assay as templates. The immunocapture can shorten the time needed for extraction of virus RNA and has simple procedure (Fukuta *et al*., 2004; Soliman and El-Matbouli, 2009). This method is useful for detection of viruses which are present either at low concentrations or in samples containing interfering substances like soil since the technique cleans and concentrates the target viruses which resulted in highly sensitive amplification assay (Rampersad, 2005; Mahmoud *et al*., 2008; Naghavi *et al*., 2008; El-Araby *et al*., 2009; Soliman and El-Matbouli, 2009).

The RT-LAMP assay is considered superior because it have a higher sensitivity than ELISA and is a comparatively simple technique which can be carried out in most situations where rapid diagnosis is required, e. g., in the field condition. A water bath or block heater is sufficient for RT-LAMP since the method requires isothermal conditions (Boubourakas *et al*., 2009). This technique has considerable potential for routine diagnosis in well-equipped laboratories as well as under field conditions.

CONCLUSION

The RT-LAMP method was developed to degenerate primer set which recognize coat protein genes of two KGMMV strains. The RT-LAMP has high specificity and 10 times higher sensitivity than RT-PCR. Ic/RT-LAMP can 0.5 ng of purified KGMMV in soil and detect KGMMV in soil samples from the cucumber fields.

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REFERENCES

Ahmad, A.A. and M.M. Fath-Allah, 2012. Double infections with cucumber mosaic virus and plum pox virus (sharka) in apricot trees. Int. J. Virol., 8: 50-60.

Boubourakas, I.N., S. Fukuta and P.E. Kyriakopoulou, 2009. Sensitive and rapid detection of peach latent mosaic viroid by the reverse transcription loop-mediated isothermal amplification. J. Virol. Methods, 160: 63-68.

Clark, M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-483.

Daryono, B.S., S. Somowiyarjo and K.T. Natsuaki, 2006. Biological characterization and complete nucleotide sequence of coat protein on Kyuri green mottle mosaic virus isolated from angled loofah in Indonesia. J. Agri. Sci., Tokyo Univ. Agric., 51: 45-52.

El-Araby, W.S., I.A. Ibrahim, A.A. Hemeida, A. Mahmoud, A.M. Soliman, A.K. El-Attar and H.M. Mazyad, 2009. Biological, serological and molecular diagnosis of three major potato viruses in Egypt. Int. J. Virol., 5: 77-88.

Fillhart, R.C., G.D. Bachand and J.D. Castello, 1998. Detection of infectious Tobamoviruses in forest soils. Appl. Environ. Microbiol., 64: 1430-1435.

Francki, R.I.B., J. Hu and P. Palukaitis, 1986. Taxonomy of cucubit-infecting tobamoviruses as determined by serological and molecular hybridization analyses. Intervirology, 26: 156-163.

Fukuta, S., K. Ohishi, K. Yoshida, Y. Mizukami, A. Ishida and M. Kanbe, 2004. Development of immunocapture reverse transcription loop-mediated isothermal amplification for the detection of tomato spotted wilt virus from chrysanthemum. J. Virol. Methods, 121: 49-55.

Fukuta, S., S. Kato, K. Yoshida, Y. Mizukami and A. Ishida *et al*., 2003. Detection of tomato yellow leaf curl virus by loop-mediated isothermal amplification reaction. J. Virol. Methods, 112: 35-40.

Gilreath, J.P., B.M. Santos, J.P. Jones and J.W. Noling, 2004. Efficacy of mulches and methyl bromide alternatives on soilborne pests and weeds in spring tomato. Asian J. Plant Sci., 3: 670-674.

Govindappa, M.R., I. Shankergoud, K.S. Shankarappa, W.A.R.T. Wickramaarachchi, B.A. Reddy and K.T. Rangaswamy, 2011. Molecular detection and partial characterization of begomovirus associated with leaf curl disease of sunflower (*Helianthus annuus*) in Southern India. Plant Pathol. J., 10: 29-35.

Ikegashira, Y., T. Ohki, U.T. Ichiki, T. Higashi, K. Hagiwara, T. Omura, Y. Honda and S. Tsuda, 2004. An immunological system for the detection of pepper mild mottle virus in soil from green pepper fields. Plant Dis., 88: 650-656.

Inoue, T., N. Inoue, M. Asatani and K. Mitsuhata, 1967. Studies on cucumber green mottle mosaic virus in Japan. Nogaku Kenkyu, 51: 175-186.

Jacobi, V., G.D. Bachand, R.C. Hamelin and J.D. Castello, 1998. Development of a multiplex immunocapture RT-PCR assay for detection and differentiation of tomato and tobacco mosaic tobamoviruses. J. Virol. Methods, 74: 167-178.

Kim, O.K., K.W. Lee and K.T. Natsuaki, 2009. Occurrence and molecular characterization on *Kyuri green mottle mosaic virus* isolated from oriental melon in Korea. J. Agric. Sci., Tokyo Univ. Agric., 54: 71-78.

Kubota, K., S. Oda, M. Nagaine, M. Inada and S. Yamasaki *et al*., 2009. Genetic diversity of *Kyuri green mottle mosaic virus* isolates in Kyushu. Kyushu Plant Prot. Res., 55: 45-51.

Lee, G.P., B.E. Min, C.S. Kim, S.H. Choi, C.H. Harn, S.U. Kim and K.H. Ryu, 2003. Plant virus cDNA chip hybridization for detection and differentiation of four cucurbit-infecting Tobamoviruses. J. Virol. Methods, 110: 19-24.

Lee, S.H., Y.K. Lee, J.W. Park, H.S. Choi, Y.T. Kim, J.K. Cheon and K.W. Lee, 2000. Nucleotide sequence of coat protein gene of Kyuri green mottle mosaic virus (KGMMV) isolated from zucchini (*Cucurbita pepo*) in Korea. Plant Pathol. J., 16: 118-124.

Li, Q., Z. Yue, H. Liu, C. Liang and X. Zheng *et al*., 2010. Development and evaluation of loop-mediated isothermal amplification assay for rapid detection of lymphocystis desease virus. J. Virol. Methods, 163: 378-384.

Mahmoud, S.Y.M., S.A. Abo-El Maaty, A.M. El-Borollosy and M.H. Abdel-Ghaffar, 2008. Identification of onion yellow dwarf potyvirus as one of the major viruses infecting garlic in Egypt. Int. J. Virol., 4: 1-13.

Mansoor, S., M. Hussain, S.H. Khan, A. Bashir and A.B. Leghari *et al*., 1998. Polymerase chain reaction-based detection of cotton leaf curl and other whitefly-transmitted geminiviruses from Sindh. Pak. J. Biol. Sci., 1: 39-43.

Mekata, T., R. Sudhakaran, T. Kono, K. U-taynapun and K. Supamattaya *et al*., 2009. Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of yellow head virus in shrimp. J. Virol. Methods, 162: 81-87.

Moradi, Z. and B. Jafarpour, 2011. First report of coat protein sequence of *Cucumber green mottle virus* in cucumber isolated from Khorasan in Iran. Int. J. Virol., 7: 1-12.

Mori, Y., K. Nagamine, N. Tomita and T. Notomi, 2001. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochem. Biophys. Res. Commun., 289: 150-154.

Nagamine, K., T. Hase and T. Notomoi, 2002. Accelerated reaction by loop mediated isothermal amplification using loop primers. Mol. Cell. Probes, 16: 223-229.

Naghavi, A., M.K. Habibi and F.N. Firouzabadi, 2008. Detection and identification of some soybean viral mosaic viruses, using molecular techniques in lorestan province, South West of Iran. Asian J. Plant Sci., 7: 557-562.

Narayana, D.S.A., K.T. Rangaswamy, K.S. Shankarappa, M.N. Maruthi, C.N.L. Reddy, A.R. Rekha and K.V.K. Murthy, 2007. Distinct begomoviruses closely related to cassava mosaic viruses cause Indian jatropha mosaic disease. Int. J. Virol., 3: 1-11.

Nie, X., 2005. Reverse transcription loop-mediated isothermal amplification of DNA for detection of *Potato virus Y*. Plant Dis., 89: 605-610.

Notomi, T., H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino and T. Hase, 2000. Loop-mediated isothermal amplification of DNA. Nucl. Acids Res., 28: e63-e63.

Rampersad, S.N., 2005. A study of dicotyledonous weed species as Hosts of *Potato yellow mosaic trinidad virus* (PYMTV). Plant Pathol. J., 4: 157-160.

Shoman, S.A. and B.A. Othman, 2005. Reverse transcription-polymerase Chain reaction mediated detection and identification of tobacco mosaic virus-Egyptian strain. Int. J. Virol., 1: 14-14.

Soliman, H. and M. El-Matbouli, 2009. Immunocapture and direct binding loop mediated isothermal amplification simply molecular diagnosis of Cyprinid herpesvirus-3. J. Virol. Methods, 162: 91-95.

Valizadeh, M., J. Valizadeh and M. Jafari, 2011. Identification, distribution and incidence of important tomato and cucurbits viruses in Southeast of Iran. Am. J. Plant Physiol., 6: 242-251.

Varveri, C., N. Vassilakos and F. Bem, 2002. Characterization and detection of cucumber green mottle mosaic virus in Greece. Phytoparasitica, 30: 493-501.