MOLECULAR DETECTION OF NINE RICE VIRUSES BY RT-LAMP

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Abstract

In the context of rapid and unpredictable epidemic outbreaks of rice virus diseases, a system for quick and accurate identification of the causal viruses is critical for epidemiological study and monitoring the outbreaks. Such an assay system, when available, will have to be specific, sensitive and adaptable to detect newly evolved strains. To address these needs, we established the assays for molecular detection of nine major rice viruses that occur in Asia based on Reverse-Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) principles.

RT-LAMP assays were developed and evaluated for the detection of following viruses from infected rice plants: *Rice black-streaked dwarf virus* (RBSDV), *Rice dwarf virus* (RDV), *Rice gall dwarf virus* (RGDV), *Rice ragged stunt virus* (RRSV), *Rice transitory yellowing virus* (RTYV), *Rice stripe virus* (RSV), *Rice grassy stunt virus* (RGSV), *Rice tungro bacilliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV).

LAMP primers were designed based on sequences coding for structural proteins of each of the viruses. All primer sets, except for RBSDV, detected corresponding target sequences from infected rice plants at 63°C within 60 minutes. The accuracy of the assays was monitored with the use of external and internal (OsRAc1) controls. The sensitivities of the assays were either superior (for RSV, RTBV and RTYV) or similar (for RDV) to that of one-step RT-PCR. For the first time, with these RT-LAMP assays, it was possible to detect the presence of RTBV and RTSV from single viruliferous insect vectors.

The assay system in this report should facilitate studies on rice disease epidemiology, outbreak surveillance and molecular pathology.

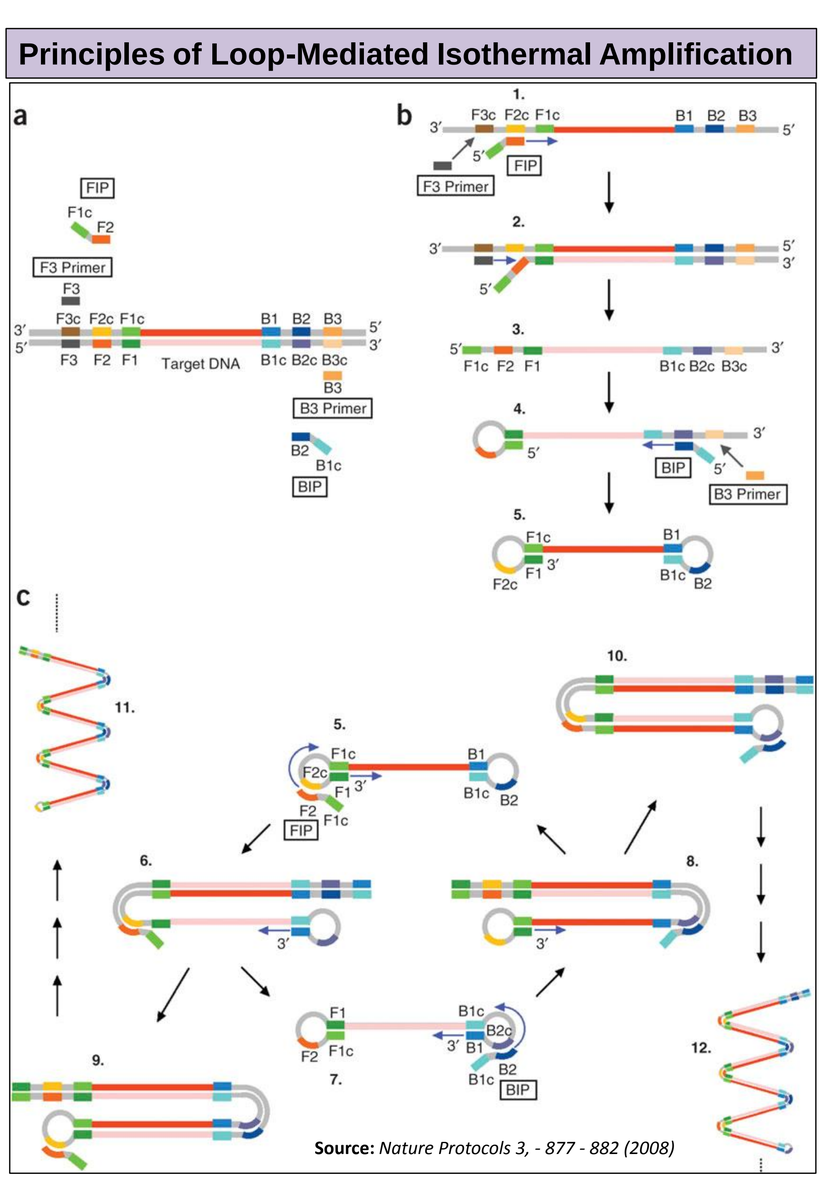
Table 1. Symptoms on rice plants caused by each of the viruses
(Source: Hibino, Annu. Rev. Phytopathol. 1996. 34:249–74)

Pathogen	Virus types	Symptoms on infected rice plants
RBSDV	dsRNA	Stunting; darkening of leaves; twisting of leaf tips, splitting of the leaf margin, and waxy white-to-black galls along the veins on the underside of leaf blades and the outer surface of sheaths and columns
RDV	dsRNA	Stunting; increased tillering; short leaves with darker green in color and fine chlorotic specks
RGDV	dsRNA	Stunting; reduced number of tillers; short darker green leaves; small galls along the leaf veins on the under surface of leaves and the outer surface of sheaths
RGSV	ssRNA	Stunting; short, erect, and narrow leaves with pale green or pale yellow color; leaves may show mottling symptoms
RRSV	dsRNA	Stunting; abnormal leaves with serrated edges or twisted tips; vein swelling or galls on the underside of leaf blades and outer surface of the leaf sheaths
RSV	ssRNA	Chlorotic stripes or mottling; necrotic streaks on leaves; and premature wilting
RTBV	dsDNA	Stunting; yellow or yellow-orange discoloration; and reduced tillering
RTSV	ssRNA	Mild stunting
RTYV	ssRNA	Leaf yellowing; reduced tillering; and mild stunting

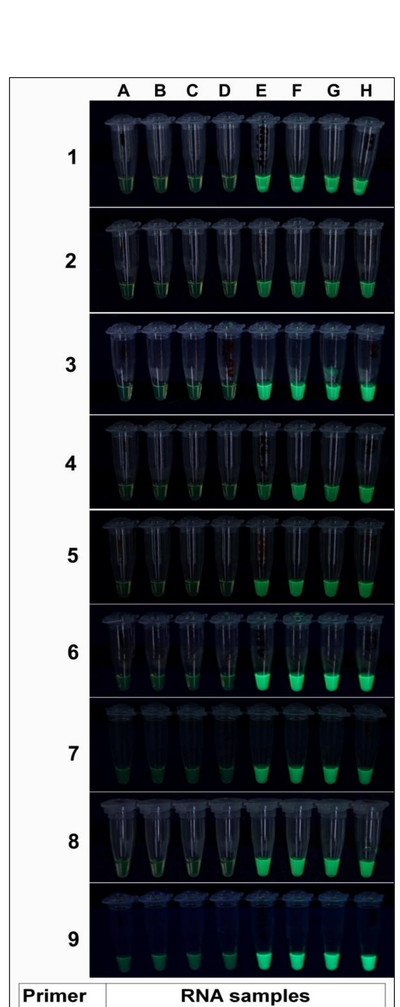
Table 2. Primer sets and assay conditions for detection of rice viruses

Viruses	Targets	Primer sequences	Conditions
RBSDV	GU322365 (P10)	F3: 5'-CCCCAGAGACTTTCCGATAC-3'; B3: 5'-GGTCTTTAAGTTGCGTGATGT-3' FIP: 5'-CGTGGGGTGCTTTGACAATGTTTCAACCGACCAACAATCACTC-3' BIP: 5'-TCGCAAACATTTGTTGACCCGACGGTAAAGTGCTAGTTTCTACG-3'	***T* (°C): 61** ***Time* (min): 75**
RDV	D13773.1 (P8)	F3: 5'-ATTCCAGCCGGGGCATAT-3'; B3: 5'-CCCACCACCAAGTGAGAAC-3' FIP: 5'-AACGCCAGCTATTGTCGTTCCAGGGCATCAGTGCTAAGTGT-3' BIP: 5'-CTACTGCAACTGCCGCAGACGTCCGTTTGGACAGGGAGG-3'	***T* (°C): 63** ***Time* (min): 60**
RGDV	D13410.1 (S8)	F3: 5'-AATCAGATTGCGCGCTTC-3'; B3: 5'-TTTTCGGGATGCAAATGG-3' FIP: 5'-CCTGATTAGCTGGCATATATTGCCTAATTTTTAGTCAGTCGATGAACAC-3' BIP: 5'-TTGCTTTTGTATCACCCTGGTACGCAGGGGTTGGTTAAACG-3'	***T* (°C): 63** ***Time* (min): 60**
RGSV	AB000403.1 (cP5)	F3: 5'-AAAGACCAACTCAGAGGCA-3'; B3: 5'-TCTAGAGCAGTTTCCTGTAGTC-3' FIP: 5'-CTGACTTAGTGTGGACACTGTGCTTTTGTGTTACCAAGTCTGTGTG-3' BIP: 5'-CACTGCATGGGTTTTGTCAACCTGGAGATCATCCTTCTACCAGCT-3'	***T* (°C): 63** ***Time* (min): 60**
RRSV	AF486811.1 (S8)	F3: 5'-GACTAGGGATGTGCGTTC-3'; B3: 5'-TGTAATCGACGTTCGCTC-3' FIP: 5'-TGTTATTCTGCCTTGTTCTTTCAACTTCTGATTTGATTGTTTTGAGCA-3' BIP: 5'-TCGACTTGGTTTAGCCAAGATG-TTGTTCAGTGATGATTCGC-3'	***T* (°C): 63** ***Time* (min): 60**
RSV	DQ333944.1 (S3)	F3: 5'-GTGACCTTTGCTGGTCAGAT-3'; B3: 5'-ACCGAGGACACTATCCCAT-3' FIP: 5'-GGCCAGTGTGTCACCACCTTGGCTATGATGCTGCAACTCT-3' BIP: 5'-GAGAGGCACTGGCTTTGTGAGACCAAGGTTGAAGCCTCTGTG-3'	***T* (°C): 63** ***Time* (min): 60**
RTBV	D10774.1 (ORF3-CP)	F3: 5'-ACTCTTTGATAGACTACCAGAAG-3'; B3: 5'-GGATTTTTCGTTTCTTATAATCTCC-3' FIP: 5'-GCTATTCCTATTCCTGCTTCATAGGGGAAAGGTAGTAAAAGCGGA-3' BIP: 5'-CATGGATGAGAGCAAAATGCATTAAGATCTACAGAATGCTAAGGATG-3'	***T* (°C): 63** ***Time* (min): 60**
RTSV	GU723290 (CP3)	F3: 5'-CCGTACTGTGCAAGAACAGA-3'; B3: 5'-GCTCTTGATGTCATCCGCG-3' FIB: 5'-GGCACCGCTACGCAAATCAAGTCCCAAAGGCTTATGCGTCTA-3' BIP: 5'-TTGTCTCGATCGCTGGGGGAGTCACTCACTGAGCCACATT-3'	***T* (°C): 63** ***Time* (min): 45**
RTYV	AB011257.1 (N protein)	F3: 5'-GGACGACCATCAAGACAGC-3'; B3: 5'-GCAACAGGTGTACCACTGTA-3' FIP: 5'-GCCCCTGAGGTTGCATCGCTATCACAACACTTTCAGCGAGACA-3' BIP: 5'-TGGCAGCACCCCTTTGTTGGCATCAGTTGACGGAGCGG-3'	***T* (°C): 63** ***Time* (min): 60**

Principles of Loop-Mediated Isothermal Amplification



Source: *Nature Protocols 3, - 877 - 882 (2008)*



Primer sets	RNA samples							
	A	B	C	D	E	F	G	H
1-RBSDV	0	-	5	6	1	1	1	+
2-RDV	0	-	3	6	2	2	2	+
3-RGDV	0	-	2	9	3	3	3	+
4-RGSV	0	-	5	6	4	4	4	+
5-RRSV	0	-	3	4	5	5	5	+
6-RSV	0	-	1	2	6	6	6	+
7-RTBV	0	-	-	8	7	7	7	+
8-RTSV	0	-	4	6	8	8	8	+
9-RTYV	0	-	2	6	9	9	9	+

***Figure 1.* Specificity of RT-LAMP assays for detection of virus RNA fragments from infected rice.**

Amplification was as carried out as described in Table 2 followed by 5 minutes at 80°C for inactivation of the enzyme. Photos were taken under irradiation of a bench-top UV lamp.

RNA sample codes:

0, H_2O control;
(-), Uninfected rice;
1, RBSDV-infected rice;
2, RDV-infected rice;
3, RGDV-infected rice;
4, RGSV-infected rice;
5, RRSV-infected rice;
6, RSV-infected rice;
7, RTBV-infected rice;
8, RTSV-infected rice;
9, RTYV-infected rice;
(+), plasmids carrying target cDNA sequences.

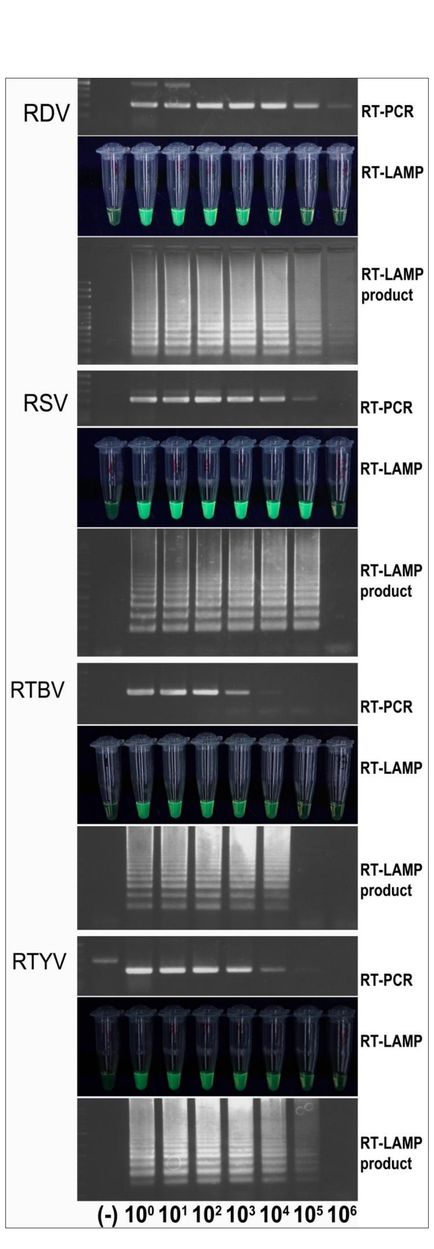


***Figure 2.* Sensitivity of RT-LAMP and RT-PCR assays for detection of the viruses from infected rice plants.**

One-step RT-PCR was carried out using outer primers of respective LAMP primer sets for RDV, RSV, RTBV and RTYV following manufacturer's instruction. Products of RT-PCR and RT-LAMP were visualized on 2% agarose gel.

Samples from left to right:

DNA ladder; (-), negative control (healthy rice RNA); 10^0 - 10^6, dilution factor - from no dilution to 1,000,000 folds.

The ladder-style gel images indicating the typical characteristic of LAMP products.

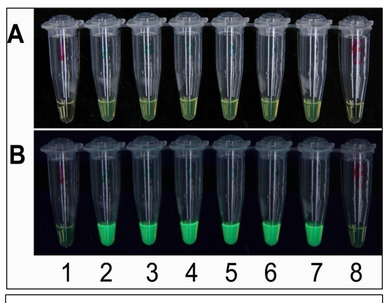


***Figure 3.* Validation of the primer set for the detection of beta-actin mRNA (OsRAc1) as an internal positive control from infected and uninfected rice plants.**
(A), image taken under white light; (B) image taken under UV light. Samples: 1, H_2O; 2, RBSDV; 3, RDV; 4, RGSV; 5, RTSV; 6, RTYV; 7, healthy rice; 8, Wheat's cDNA.

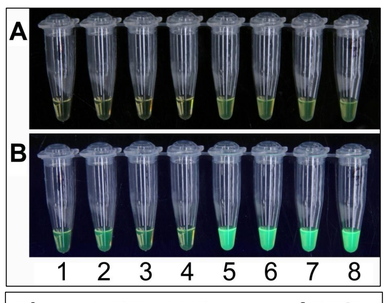


***Figure 4.* RT-LAMP assay of RTSV from RNA extracted by 0.5N NaOH.**
(A), image taken under white light; (B), image taken under UV light. Samples: 1, H_2O; 2, uninfected *nipponbare* plant; 3-4, uninfected *Taichun* (TN_1) plant; 5, RTSV-infected *nipponbare*; 6-7, RTSV-infected TN_1; 8, positive control plasmid

CONCLUSIONS

- RT-LAMP assays were established and validated for the detection of nine rice viruses from infected rice plants in which the assay conditions for eight of the viruses were identical.
- The specificity of the assay was exceptionally high as no amplification occurred in non-targeted, closely related viruses.
- The sensitivity of the assays was at least similar to or better than that of the conventional RT-PCR assays.
- The assays could be quickly adapted to detect new virus strain by adjusting primer sequences.
- The assays work consistently and reproducibly with RNA extracted by a simple method.

Simple RNA extraction for use in RT-LAMP assays

Approximately, 100mg of the plant's leaves were placed in 2-mL tubes which then included two bead beaters. 0.4mL of 0.5N NaOH was added into each tube. Tubes were then placed into the bead shaker and shaken at 2000rpm for 20 seconds ON, 10 seconds OFF for a total of 5 cycles. 10µL of the resulting solution was diluted into 490µL of 100mM Tris-Cl buffer pH8. and finally 1.0µL of the diluted samples were used directly in an RT-LAMP reaction.

References

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