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SHORT RESEARCH ARTICLE

Evidence for association of southern rice black-streaked dwarf virus with the recently emerged stunting disease of rice in North-West India

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Abstract

The concurrent reports on emergence of stunting disease of rice across the North-west Indian rice growing areas attracted attention for elucidating its etiology. Surveys of different rice fields recorded an incidence of stunting disease in the range of 1–20% in the affected fields. A systematic investigation employing three independent methods was undertaken. Under electron microscope, icosahedral virions of ~65-75 nm were observed. Based on the shape and size of virion particles and symptoms of the disease, reverse transcription-PCR and quantitative-RT-PCR of stunted rice plants and prevalent white-backed planthopper (WBPH) were performed using specific primers targeting two genomic components (S9 and S10) of Southern rice black-streaked dwarf virus (SRBSDV), a double stranded RNA virus of genus *Fijivirus* and the results indicated its specific association with stunting disease of rice. Sequencing of the amplified S9 and S10 genomic components showed maximum identity of 97.90–100.00% and 98.04–99.48%, respectively with SRBSDV isolates from South Korea and Vietnam. To the best of our knowledge, this is the first conclusive evidence of association of SRBSDV with stunting disease of rice from India. The findings seek urgent attention and in-depth investigation on its establishment in the Indian subcontinent and necessary interventions.

Keywords: Rice stunting, white-backed plant hopper, Southern rice black-streaked dwarf virus

Introdution

India is the second largest producer and the first largest exporter of rice globally. North India particularly, Indo Gangetic plains is known for growing rice and is India's major basmati producing pocket. Several biotic stresses affect rice cultivation in India, which were addressed through systematic crop improvement and management efforts. This year (2022) for the first-time farmers from different parts of Haryana and Punjab reported the occurrence of a new disease manifested as severe stunting of rice plants during the last week of July 2022. The disease-affected areas of different rice varieties were surveyed during August 2022. Both basmati and non-basmati growing areas of the Haryana state were surveyed. A total of 24 rice fields (total of 63.5 acres of rice fields across the 9 locations) of 11 rice varieties [Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 1692, Pusa Basmati 1847, CSR-30, PR114, PR126, PR130, PR131, 28P67 (Pioneer), Arize Swift Gold (Bayer Crop Science)] were surveyed in Sonipat, Panipat, Karnal, Kurukshetra, Ambala, and Yamunanagar districts of Haryana (Table 1). The incidence of disease was recorded in the affected fields by randomly selecting a quadrat of 10´x10´ (comprising about 100 hills). The disease incidence varied from 1–10% in the affected fields (Table 1). In a severely affected field at Kutani, Panipat, up to 20% incidence was recorded.

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Fig. 1. (a-b) Appearance of severely stunted rice plants under field conditions (variety: Pusa Basamti-1692, location: Dipalpur, Murthal, Sonipat); (c) Extent of stunting observed on the rice (Variety: Pusa Basamti-1509, location: Kutani, Panipat); (e) Effect on root growth of stunted rice plants (Variety: Pusa Basamti-1692, location: Kutani, Panipat); (f) Formation of white streaked galls on stem of infected rice plants (variety: Swift Gold)

Symptoms of severe dwarfing/stunting along with darker green and stiff leaves were observed in the diseased rice plants (Fig. 1a-c). Stunting was observed either in the form of individual plants scattered at different parts of a field or as group of 4–10 dwarf plants occurring in the patches. The early (in nursery stage to within one month of transplanting) infected tillers exhibited severe stunting and infected tillers showed gradual withering from the periphery of the hill. Roots of infected plants were poorly developed and turned brownish in colour (Fig. 1d). Roots became fibrous and shortened in length. The diseased tillers were easy to pull out. The stems of infected plants exhibited small streaked white waxy galls, which increased in size when maintained under controlled environmental conditions at 27°C, 70% RH and 14 hours light - 10 hours dark photoperiod (Fig. 1e). A few stunted tillers exhibited black streaks on the lower portion of the sheath. The late sown crop (sown after the second week of July) had less incidence than the early sown (sown in second fortnight of June). Plants infected after the initiation of internode elongation stage did not exhibit detectable stunting symptoms. The late (after internode elongation stage) infected plants produced smaller panicles with chaffy grains. Low level (~5-10 per hill) of brown planthopper (BPH) and white-backed planthopper (WBPH) incidence was also recorded in some locations. BPH and WBPH from the infected tillers were collected in insect collection tubes and brought to laboratory. Both the basmati and non-basmati varieties were affected by stunting disease. Based on the symptoms, the infection of Southern rice black-streaked dwarf virus (SRBSDV) was suspected. Southern rice black-streaked dwarf virus is non-enveloped icosahedral virus under the genus *Fijivirus* and family *Spinareoviridae* having 10 linear double-stranded RNA (dsRNA) segments of 4.5 – 1.8 kb (S1 to S10) as genome (Zhou et al. 2008) with entire genome encoding for 13 open reading frames (ORFs).

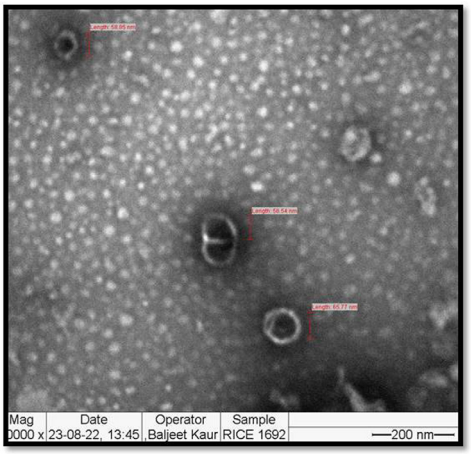

Fig. 2. Icosahedral virus particles observed under transmission electron microscope.

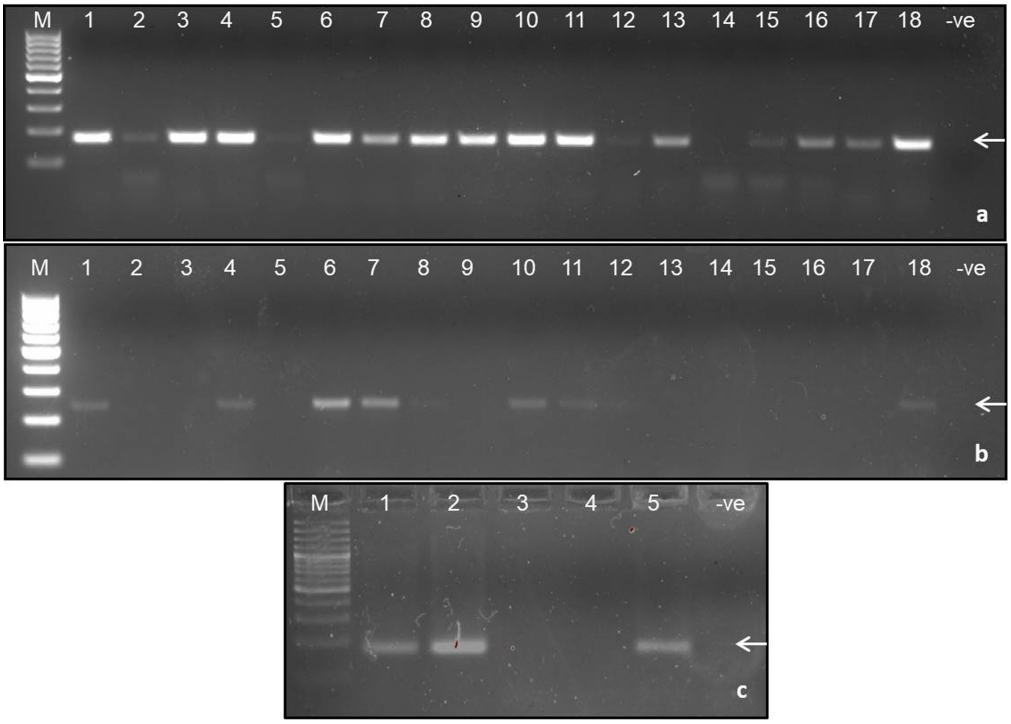

Fig. 3. RT-PCR amplification of (a) nonstructural protein (176 bp fragment of S9 genomic component) and (b) capsid protein (242 bp fragment of S10 genomic component) of Southern rice black-streaked dwarf virus (SRBSDV) in symptomatic and asymptomatic rice samples. Lane M: 100 bp Ladder, Lanes 1-13: symptomatic rice samples of varieties Pusa Basmati 1692, Pusa Basmati 1692, Pusa Basmati 1121, Pusa Basmati 1509, PR 114, PR 126, Pusa Basmati 1847, PR 131, PR 130, PR 126, 28P67 (Pioneer), Swift Gold, CSR-30; Lanes 14-18 asymptomatic samples of varieties Pusa Basmati 1692, Pusa Basmati 1121, Pusa Basmati 1509, 28P67 (Pioneer), Swift Gold, -ve: Negative control. (c) Detection of SRBSDV in plants, WBPH vector and developing seeds. Lane M: 100 bp plus DNA ladder, Lane 1: Pusa basmati 1692 stunted plant, Lane 2: Pusa Basmati 1509 stunted plant, Lane 3: Pusa basmati 1692 asymptomatic plant, Lane 4: developing seeds from stunted plant, Lane 5: WBPH, -ve: Negative control.

Table 1. Details of locations surveyed for rice stunting disease

S. no.	Variety	Percent incidence (%)	Locations	Area surveyed (acre)
1	PB 1692	10-11		4.0
2	PB 1718	3-4	Dipalpur, Murthal, Sonipat	3.5
3	PB 1121	1-2		4.0
4	PB 1121	1-2	Dehra, Hathwala road, Block Samalkha, Distt. Panipat,	3.0
5	PB 1509	18-20	Kutani, Panipat	2.5
6	PB 1509	10-12	Kutani, Panipat	4.0
7	PR 114	7-8		3.5
8	PB 1121	1-2	Newal, Kunjpura, Distt. Karnal	3.0
9	PB 1509	7-8		3.5
10	PR 126	3-4		4.5
11	PB1847	4-5		3.0
12	PR114	8-10	Bachki, Block Pehowa, Kurukshetra	3.5
13	PR131	7-8		4.0
14	PR130	2-3		3.0
15	PR126	4-5	Tepla, Ambala	5.0
16	28P67 (Pioneer)	10		2.0
17	Arize Swift Gold (Bayer Crop Science)	8-10	Sherpur, Baroda, Ambala	3.0
18	CSR-30	6-8	Talakaur, Saraswatinagar, Yamunanagar	4.5

Table 2. Primers used for detection of different viruses in present study

Primer	Sequence (5′ - 3′)	Target virus	Genomic region	Fragment size	Reference
P9-2-F	AATCCTTGCTGTATATCATTCTT	Southern rice black-streaked dwarf virus (SRBSDV)	S9 (non structural protein)	176 bp	He et al. 2013
P9-2-R	TACCTCCATTGAACACTTGT				
S10F	CTCCGCTGACGGTTTAGAAG	SRBSDV	S10 (capsid protein)	242 bp	He et al. 2013
S10R	TAACCGCCATAGTGT				
RBSDV (376F)	GATAGACAGGCAAATATAAGCGT	Rice black-streaked dwarf virus (RBSDV)	S9 (non structural protein)	1200 bp	Zhou et al. 2010
RBSDV (1462R)	GGATTACAACACACACAACGAAA				
RDVCP 1F	TAGCGATACCAAGCCTACCG	Rice dwarf virus (RDV)	Capsid protein	572 bp	Designed in present study
RDVCP 1R	TGATGTTCCCACCACCAAGT				

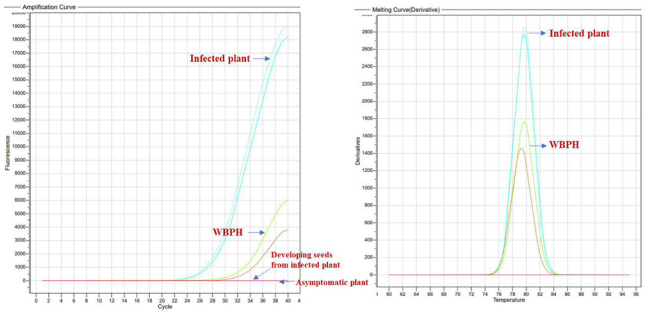

Fig. 4. Real time qRT-PCR fluorescence curves and melting curves of SRBSDV in infected plants, WBPH, developing seeds of infected plants and asymptomatic plant

Representative whole plant samples (both asymptomatic and symptomatic) were collected and brought to laboratory of ICAR-IARI, New Delhi. The symptomatic and asymptomatic plants were maintained in the National Phytotron facility, ICAR-IARI, New Delhi under controlled conditions. The leaf samples were subjected to transmission electron microscopy (TEM) (JEOL JFC-1100, Tokyo, Japan) after staining with 2% aqueous uranyl acetate (UA) at the Plant Virology Unit, Division of Plant Pathology, ICAR-IARI, New Delhi. Examination of leaf dip prepared grids of infected plant sheaths showed the association of typical icosahedral virions of ~65–75 nm in diameter (Fig. 2). The aggregates of virus particles were also seen in lattice structure typical of Fijivirus infection in rice. The results indicated the association of Fijivirus with the diseased samples.

In order to further characterize the actual virus species associated with stunting symptoms, the samples were subjected to reverse transcription-PCR (RT-PCR) based amplification using primers specific to two genomic components viz. S9 and S10 of SRBSDV. The WBPH population collected from the stunted tillers were also tested for presence of virus using SRBSDV specific primers in RT-PCR. Total RNA was extracted from leaf sheath of symptomatic and asymptomatic rice plants using an RNA isolation kit (Sigma-Aldrich, USA) following the manufacturer's instructions. The purified RNA was electrophoresed on agarose gel using TBE Buffer. Total RNA from WBPH was collected using NucleoSpin RNA XS, Micro kit (Macherey-Nagel, Düren, Germany). Primer pairs P9-2-F and P9-2-R targeting nonstructural protein (176 bp) of S9 genomic component and pair S10F and S10R targeting partial capsid protein (242 bp) of S10 genomic segment of SRBSDV respectively were used (He et al. 2013) (Table 2).

First-strand cDNAs were synthesized by using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, USA) as per He et al. (2013). The reverse transcription-PCR (RT-PCR) reaction was performed using 100 ng of cDNA template and 10 µM gene-specific primers in a Mastercycler nexus GX2 thermal cycler (Eppendorf, Hamburg, Germany). The nonstructural protein (176 bp) and partial capsid protein (242 bp) of SRBSDV were amplified following the thermal cycling conditions reported by He et al. (2013). The amplicons were electrophoresed on 1.6% agarose gels and visualized under gel documentation system (Bio-Rad, USA). The specific amplicons were gel eluted and sequenced bi-directionally at a commercial facility (Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka, India). The viral identity of obtained sequences was confirmed through nucleotide BLAST analysis. The sequences were assembled and consensus sequences were deposited in NCBI database.

RT-PCR targeting S9 genomic component (nonstructural protein) showed amplification in all the 17 samples of 11 varieties including symptomatic (Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 1692, Pusa Basmati 1847, CSR-30, PR114, PR126, PR131, PR130, 28P67, Arize Swift Gold) and asymptomatic samples (Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 1692, 28P67, Swift Gold) (Fig. 3a). However, RT-PCR targeting S10 genomic component (capsid protein) showed amplification only in seven symptomatic (Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 1847, Pusa Basmati 1692, PR 126, 28P67, Arize Swift Gold) samples and an asymptomatic sample (Arize Swift Gold) (Fig. 3b). This variation in amplification of different genomic components of SRBSDV has been previously reported (He et al. 2013; Matsukura et al. 2013). The nonstructural protein coded by S9 genomic fragment involved in viroplasm formation is usually expressed in higher concentration and this could be the reason of its detection in all the tested samples. The sequences of 17 partial nonstructural protein of all the positive samples (Deposited to GenBank: accession numbers OP627883-OP627899) and capsid protein of 4

selected samples (Pusa Basmati 1692, Pusa Basmati 1121, PR 126 and Pusa Basmati 1847) (Deposited to GenBank: accession numbers OP627900-OP627903) out of the total 18 samples tested in present study shared an identity of 97.9-100% and 98.04–99.48%, respectively with earlier reported isolates of SRBSDV (GenBank accession no: MF356700 and KM454855) from South Korea and Vietnam. This confirmed the association of SRBSDV with severe stunting disease of rice in North West India. The viral RNA was also amplified from the WBPH using primer pairs P9-2-F, and P9-2-R targeting S9 genomic component of SRBSDV (Fig. 3c). RNA was also extracted from developing seeds of stunted plants as described above. However, no amplification of viral RNA was observed in RT-PCR (Fig. 3c). Simultaneously, two other viruses associated to be reported with similar symptoms viz. rice black-streaked dwarf virus (RBSDV) and rice dwarf virus (RDV) and were also tested in RT-PCR (Table 2) (Zhou et al. 2010; Zhou et al. 2013), but no amplification specific to RBSDV and RDV was recorded. Thus, the association of RBSDV and RDV with the diseased samples was overruled.

Further, the presence of SRBSDV in stunted plants, developing seeds, and WBPH was tested in quantitative RT-PCR (qRT-PCR). Total RNA was extracted and cDNA was synthesized as described above. The qRT-PCR was carried out in a Insta Q48 Real-Time PCR System (Himedia, India) as per He et al. (2013). In qRT-PCR, an average CT value of 23.6 was recorded for infected plant samples (Fig. 4). Whereas, an average CT value of 29.3 was recorded for WBPH (Fig. 4). No CT was recorded in healthy plants and developing seeds of the infected plants up to 40 cycles. As the CXR dye non-specifically binds to any dsDNA, the melting curve was analyzed. The SRBSDV amplicon produced a single peak at 79°C without any secondary peak, which indicated specificity of the reaction.

By employing the three independent methods, present study provides the first conclusive evidence of the association of SRBSDV with stunting disease of rice in the region of North-West India. The SRBSDV genome was reported to be highly conserved as evident from the similarity in genome sequences obtained from different parts of East and South East Asia (Zhou et al. 2013), which indicated its spread after having evolved in Southern China. SRBSDV is closely related to the other *Fijivirus* i.e., RBSDV but its specific transmission by WBPH vectors in persistent propagative manner makes it distinct from latter. After first report of SRBSDV in 2001, it remained confined to Southern China for next few years. It was only by the end of 2009 that its infection spread to large areas of Southern China, North and Central Vietnam, and Japan (Zhou et al. 2013). Since then, its varying degree of incidence is known to occur on yearly basis in East and South East Asia. Epidemiological studies have indicated its spread through long distance migration of WBPH, which is a very efficient vector of SRBSDV. There is yearly overseas migration of viruliferous WBPH vectors from Southern China and Vietnam to Japan (Matsukura et al. 2013) which is responsible for regular disease occurrence in Japan. The overwintering of SRBSDV happens either through viruliferous WBPH vectors or through the alternate hosts like rice, maize and other grassy weeds (Zhou et al. 2013). SRBSDV is not seed transmitted and in our study, we did not find its infection in immature seeds of affected plants. It is, however, a matter of investigation how does the viruliferous WBPH migrate to the affected rice belt across the North-West India and establish the primary inoculum. The alternate and overwintering virus and vector hosts need to be studied in detail to combat the emerging disease in rice.

Author's contribution

Conceptualization of research (VKB, AKS); Designing of the experiments (VKB, SKS, AG); Collection of experimental materials (VKB, SKS, AG); Execution of lab experiments and data collection (DD, PT, SJ, NG, SKS, AG), Analysis of data and interpretation (SKS, AG, NG, VKB); Preparation of the manuscript (SKS, AG, NG, VKB).

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