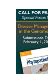
View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Horizon / Pleins textes

# First Report of Rice stripe necrosis virus Infecting Rice in Benin

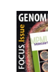
APS apsjournals.apsnet.org/doi/10.1094/PDIS-11-14-1126-PDN

Plant Disease, May 2015, Volume 99, Number 5
Page 735



http://dx.doi.org/10.1094/PDIS-11-14-1126-PDN

DISEASE NOTES



A. Oludare, M. Sow, and O. Afolabi, Africa Rice Center, 01 BP 2031, Cotonou, Bénin ; A. Pinel-Galzi and E. Hébrard, UMR RPB, Institut de Recherche pour le Développement (IRD), BP 64501, 34394 Montpellier cedex 5, France ; and D. Silué, Africa Rice Center, Cotonou, Bénin.

Open Access.

## ABSTRACT

In December 2012 and March 2013, rice plants with unusual leaf symptoms were observed on AfricaRice research fields at Cotonou, Benin. Some plant accessions died because of severe attacks. Surveys conducted in March 2014 (300 to 400 plants/accession assessed) indicated that 51 out of 57 lowland and 55 out of 62 upland accessions were diseased with an incidence of 0 to 37% in upland (respectively on e.g., accessions NERICA L-20 and IR68702-07-2-1-4-B) and 0 to 26.6% in lowland (respectively on e.g., NERICA L-34 and NIL 130-1-3). The symptoms consisted of crinkling yellow foliar stripes and severe plant malformation. These symptoms suggested the presence of *Rice stripe necrosis virus* (RSVN), a nonenveloped rod-shaped multipartite virus from the genus *Benyvirus* in the family *Benyviridae* that is transmitted by *Polymyxa graminis*, a plasmodiophorid protist. First described in 1977 in Côte d'Ivoire, the disease was reported in Nigeria, Liberia, and Sierra Leone (Fauquet et al. 1988), in several countries of America ( Lozano and Morales 2009), and more recently in Burkina Faso (Sérémé et al. 2014). RSNV was identified after total RNA extraction and specific RT-PCR amplification from symptomatic accessions. Briefly, RNA extraction was performed on 0.05 g of leaves with RNeasy Plant Mini Kit (Qiagen); the reverse transcription step was performed with 5 µM of the reverse primer RSNV1-3827R (5′-TGTGGCGTTTCCAGACCTAAA-

3′) and 10 U/µl of M-MLV-reverse transcription (Promega). A specific fragment of 927 bp from the ORF1 of RNA1 was obtained from five samples after PCR with 1 µM primers RSNV1-2901F (5′-TGAATTTGGTGCTCTCTTG-3′) and RSNV1-3827R and 10 U/µl of Dynazyme (Finnzyme). The enzymes and kits were used according to manufacturer's instructions. Direct sequencing (GenBank accession number KP099623, Line NIL 130) confirmed the identification with 95.8% nucleotide identity with the unique complete RSNV genome available in the database (EU099844). The presence of *P. graminis* was checked in the roots of the attacked plants. For this purpose, roots were cut, surface sterilized with 70% alcohol, rinsed in 10% hypochlorite, and further rinsed three times with distilled water. Total DNA was then extracted using the ZR fungal/bacteria DNA miniprep Zymo research kit (Epigenetics). The *Polymyxa*-specific primers PxRealF and PxRealR (Sérémé et al. 2014) were used in a PCR with 30 cycles of 30 s at 94°C, 1 min at 60°C, and 2 min at 72°C. The roots of the affected plants showed the *P. graminis*-specific amplicon of 127 bp. Virus transmission assays were performed with disinfected rice seeds of eight varieties, the susceptible control Oryzica 3, and the resistant control MG 12 (Gutierrez et al. 2010). Four replicates of four conditions were conducted: plants grown in infected soil, in sterilized soil only or mixed with roots of PCR-confirmed (virus and protist) infected plants (2 entire fully developed roots per pot), or mechanically inoculated with sap of PCR-confirmed RSNV infected plants (grinding 1 g of leaves in 5 ml of phosphate buffer, pH 7.5). Characteristic foliar stripping and crinkling malformation were observed 20 to 30 days after treatment on all plants of all accessions grown either on infected soil or on soil mixed with roots of RSNV-affected plants. No symptom was observed on mechanically inoculated plants nor on those grown on sterilized soil and *Oryza glaberrima* MG 12 plants grown on infected soils. Further studies are needed to assess the spatial distribution and the incidence of the disease, the biological and molecular diversity of RSNV, and the resistance level of the African rice accessions.

***References***: Section:

(1) Fauquet, C., et al. 1988. Develop. Appl. Biol. 2:71.

(2) Gutierrez, A. G., et al. 2010. BMC Plant Biol. 10:6. 10.1186/1471-2229-10-6

(3) Lozano, I., and Morales, F. 2009. Eur. J. Plant Pathol. 124:673. 10.1007/s10658-009-9453-z

(4) Sérémé, D., et al. 2014. Plant Dis. 98:1451. 10.1094/PDIS-06-14-0626-PDN [Abstract]

(5) Ward, E., et al. 2005. New Phytol. 165:875. 10.1111/j.1469-8137.2004.01291.x