Crop Protection 114 (2018) 143–147

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Short communication

First report of *Rice stripe necrosis virus* in Argentina

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ARTICLE INFO

Keywords:
Oryza sativa
Benyvirus
RSNV
Plant virus

ABSTRACT

Since 2008/2009, we have seen symptoms of viral infection in rice plants from crop regions in Argentina. The main objective of this work was to identify the causal agent of the rice plants' crinkling. We therefore analyzed samples of symptomatic rice plants from Berón de Astrada (Corrientes province) and San Javier (Santa Fe province) by serological, electron microscopy and RT-PCR methods, and determined the presence of *Rice stripe necrosis virus* (RSNV). Helically constructed rod-shaped virus particles, with an axial canal with an estimated modal length of 228 nm, 370 nm and a last interval of 87–181 nm and a diameter of 20 nm, were observed in ultrathin sections and ISEM-D from rice leaf samples. Transmission of RSNV by viruliferous soil was achieved. Phylogenetic analysis, based on a partial sequence (828 nt) of the helicase gene of 10 RSNV isolates, grouped the Argentinian sequences in the same clade as the African, rather than with the sequence from Colombia. This is the first report not only of the presence of RSNV in Argentina, but of a viral infection in rice plants in the country.

1. Introduction

Rice (Oryza sativa L.) is fifth in the ranking of Argentina's cereal production (https://datos.agroindustria.gob.ar/dataset/estimaciones-agricolas). It is grown mainly in the provinces of Corrientes (45%), Entre Ríos (37%) and Santa Fe (14%) (Buryaile et al., 2016). Diseases are one of the main limitations to rice productivity. Several species of fungi infecting rice have been reported In Argentina, but there were no reports of viruses infecting rice in the country (http://www.sinavimo.gov.ar/plagas?populate=arroz). Since 2008/2009, we have seen symptoms of viral infection in rice plants like those described for “Rice Crinkle Disease” (Louvel and Bidaux, 1977; Fauquet and Thouvenel, 1983) from several locations of the provinces of Corrientes, Santa Fe and Tucumán. In Berón de Astrada, around 28% of rice crops (3270 of 11700 has) presented symptoms during 2017/2018, with up to 65 symptomatic plants per m2 (Carbajal, L., ADECOAGRO, personal communication). There are approximately 30 diseases caused by viruses in rice plants (Abo and Fadhila, 1998; Yang et al., 2017). Rice stripe necrosis virus (RSNV) is the causal agent of “Rice Crinkle Disease”, one of the most severe viral infections in rice (Fauquet et al., 1988; Morales et al., 1999). Rice plants infected with RSNV present conspicuous chlorotic stripes and systemic leaf necrosis, growth retardation, panicle malformation and low seed production (Fauquet and Thouvenel, 1983).

RSNV is a virus of *Benyvirus* genus, transmitted by *Polymyxa graminis* (Fauquet et al., 1988; Kanyuka et al., 2003), an obligate parasite of plant roots, protist of the order *Plasmodiophorales* (Dick, 2001). This parasite has recently been reported in Argentina in rice plants from Berón de Astrada (Cundom et al., 2018). Up to five genomic RNAs have been reported for *Benyvirus*, though only two have been found for RSNV: RNA1 with 6614 nucleotides (nt) in length and RNA2 with 4631 nt in length (Gilmer and Ratti, 2011; Lozano and Morales, 2009).

Several papers and technical reports relate the presence of symptoms of RSNV infections and yield losses in rice fields. The effect of RSNV on production depends on the developmental stage in which the plant was infected, the number of infected tillers on each plant, the severity of the symptoms and the rice variety. Among 73 rice varieties tested in Côte d'Ivoire between 1981 and 1983, the percentage of infected plants ranged from 0 to 100%, depending on the variety (Fauquet et al., 1988). In Colombia and Ecuador, infected rice fields showed between 20% and 40% of yield losses (Pardo and Muñoz, 1994; Paz et al., 2009). The presence of RSNV was reported in Colombia

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https://doi.org/10.1016/j.cropro.2018.08.012
Received 27 February 2018; Received in revised form 25 July 2018; Accepted 8 August 2018
0261-2194/ Published by Elsevier Ltd.

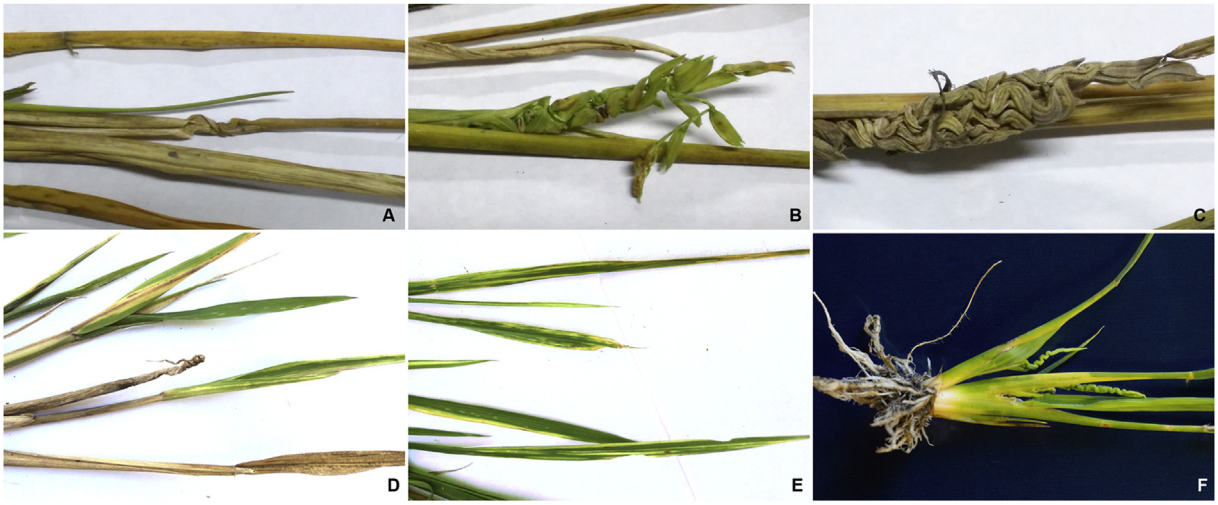

Fig. 1. Rice plants (*Oryza sativa* L.) showing symptoms of a viral infection. A) Leaf crinkling and necrosis; B and C) Panicle deformation; D) Leaf crinkling, chlorotic striping and necrosis; E) Leaf striping; F) Striping and crinkling in seedlings.

(Morales et al., 1999), Ecuador (Paz et al., 2009), Côte d'Ivoire (Louvel and Bidaux, 1977), Liberia, Nigeria and Sierra Leone (Fauquet et al., 1988), Panama (Lozano and Morales, 2009), Brazil (Maciel et al., 2006), Burkina Faso (Sereme et al., 2014), Benin (Oludare et al., 2015) and Mali (Decroës et al., 2017).

The main objective of this work was to confirm the identity of the causal agent of rice plants crinkling in crop regions in Argentina.

2. Material and methods

2.1. Rice sample collection

In February 2017, five symptomatic rice plants were collected from San Javier (Santa Fe Province) showing leaf veins thickening, leaf blades crinkling and young leaves unfolding from the spindle, dwarfism, lax and compact panicles, and leaf blades with a darker color or with chlorotic stripes that eventually turn necrotic (Fig. 1). These plants were used for the serological, molecular and microscopic identification of the causative agent of the disease. Subsequently, 58 symptomatic rice plants were collected from affected rice crops from Berón de Astrada (Corrientes Province).

Additionally, six plants were collected of different weed species that grow in rice crops: three plants of *Ludwigia* sp., one *Panicum* sp., one *Eleusine* sp. and one *Echinochloa* sp., from the surroundings of symptomatic rice plants in San Javier and Berón de Astrada. These also presented symptoms of leaf mosaic and twisting. The plant material collected was maintained under fresh conditions until arrival at the laboratory, where it was stored at 6 °C until analysis.

Rice plants obtained from seeds sown in sterile soil, maintained under controlled conditions in the greenhouse and without symptoms, were used as a negative control.

2.2. Serological detection

Seven rice plants, the five symptomatic plants from San Javier and two negative controls, were analyzed by Plate-trapped antibody enzyme-linked immunosorbent assay (PTA-ELISA) according to Lommel et al. (1982), using RSNV antiserum at a final dilution of 1:250 (v/v) (Fauquet and Thouvenel, 1983). The antiserum to an isolate of RSNV from Côte d'Ivoire (Fauquet and Thouvenel, 1983) was kindly provided by Dr. Eugénie Hébrard, Institut de Recherche pour le Développement (IRD), Montpellier, France.

2.3. Electron microscopy

Leaf samples of the five symptomatic rice plants from San Javier were fixed in a modified (2% paraformaldehyde, 2.5% glutaraldehyde in 0.05 M pH7 phosphate buffer) Karnovsky solution (Karnovsky, 1965) for at least 24 h. After being rinsed in a 0.05 M pH 7 phosphate buffer, the samples were post-fixed for 2 h in 1% osmium solution, contrasted in 0.5% uranyl acetate for 24 h, dehydrated in serial dilutions of acetone and included in Spurr resin (Spurr low viscosity embedding media kit, Polysciences, INC.). Ultrathin sections were contrasted in 2% uranyl acetate and examined under a Jeol JEM EXII (Jeol, Tokio, Japan) transmission electron microscope (Kitajima and Nome, 1999).

Additionally, the plants were analyzed by leaf dip and immunosorbent electron microscopy plus decoration (ISEM-D) methods, according to Milne and Luisoni (1977). Briefly, specific RSNV antiserum (Fauquet and Thouvenel, 1983) was used to coat grids (diluted 1:500, v/v) and to decorate the virus particles (diluted 1:20, v/v). The viral decorated particles were observed with a JEOL 1200 transmission electron microscope. From a sample with decorated particles (SF10), measurements and virion modal lengths were estimated using InfoStat software (version 2013, Universidad Nacional de Córdoba, Córdoba, Argentina) by comparing 300 viral particles with scales of known size.

2.4. Detection of RSNV by RT-PCR

Total nucleic acids were extracted from leaf tissue of all the plants, the five symptomatic plants from San Javier (Santa Fe), 58 rice plants collected from Berón de Astrada (Corrientes) and six weed plants, using the CTAB method (Hoisington et al., 1994). Reverse transcription-polymerase chain reaction (RT-PCR) was performed using specific primers, RSNV1-3827R/and RSNV1-3827R, targeting the helicase gene of RSNV with expected amplicons of 927 base pairs (bp) (Oludare et al., 2015).

The amplicons obtained from three symptomatic rice plants (isolate "Cor4" from Corrientes and isolates "SF5" and SF10″ from Santa Fe Province) were cloned into pGEm-T Easy Vector System (Promega, Madison, WI, USA), following the manufacturer's instructions. High efficiency cells were transformed using the recombinant plasmids, and the colonies containing the insert were then selected and multiplied. Plasmid DNA was purified from the clones of interest using Wizard Plus SV Minipreps DNA Purification System (Promega). One clone of each isolate was sequenced in both senses using universal primers M13 F/R and Sanger technology (Instituto de Biotecnología, CICVYA-INTA, Buenos Aires).