Detection of ‘Candidatus Phytoplasma oryzae’ in non-symptomatic sugarcane cultivars in eastern Uttar Pradesh, India

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Abstract Twenty-six non-symptomatic leaf samples from six sugarcane varieties (CoS07250, CoS96268, CoS91269, CoSe01424, CoS08272, CoS08279) grown at the Sugarcane Research Institute farm at Shahjahanpur were analyzed for presence of phytoplasmas by using P1/P6 and R16F2n/R16R2 primers pairs. Amplification products of 1.2 kb were obtained in 17 of the 26 non-symptomatic leaf samples in nested PCR assays. The amplified products were directly sequenced, aligned and used in a BLASTn search query of the GenBank database. The 16Sr DNA sequences of all the 17 sugarcane phytoplasma non-symptomatic isolates were identical (99-100%) among themselves and showed 99% sequence identity to member strains of 16SrXI group of phytoplasma that includes sugarcane grassy shoot phytoplasma (SCGS). Since the phytoplasma association was detected in non-symptomatic sugarcane cultivars, the results suggest that it may become serious at later stages of the crop growth or in ratoon crops and, consequently, this issue requires attention.

Key words Sugarcane, SCGS phytoplasma, non-symptomatic cultivars, ‘Ca. Phytoplasma oryzae’

INTRODUCTION

‘Candidatus Phytoplasma oryzae’, causing sugarcane grassy shoot disease, continues to be a problem for sugarcane and causes severe losses to sugarcane crops in many regions of India, especially in central and western Uttar Pradesh. The pathogen is reported to have become widespread in Uttar Pradesh in the last decade and to cause losses of up to 40% in many commercial sugarcane varieties (Tiwari et al. 2012). Different symptoms are produced by the pathogen on sugarcane such as white leaf, grassy shoot, green grassy shoot and stunted growth (Rao et al. 2014). Earlier workers recorded phytoplasma associations in non-symptomatic sugarcane varieties in fields infected with sugarcane grassy shoot disease (Tiwari et al. 2016; Soufi and Komor 2014). These results prompted us to study whether non-symptomatic sugarcane varieties may be potential reservoir sources of phytoplasmas in field conditions that, under favourable conditions and after a build-up of the phytoplasma titre, can cause serious losses in ratoon crops.

MATERIALS AND METHODS

During survey of sugarcane fields at Sugarcane Research Institute Shahjahanpur, UP, India in 2013-2015, 26 apparently healthy looking leaf samples from six sugarcane varieties (CoS07250, CoS96268, CoS91269, CoSe01424, CoS08272, CoS08279) were collected for phytoplasma indexing. Leaf samples were used for DNA extraction by method described by Ahrens and Seemuler (1992). PCR was performed with phytoplasma universal primers P1/P6 (Deng and Hiruki 1991) followed by nested R16F2n/R16R2 primers (Gundersen and Lee 1996). The PCR reactions were performed in BIOCHEM thermo cycler, using denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 50 s, 60°C for 45 s and 72°C for 90 s, and a final extension of 7 min at 72°C. In a nested PCR assay, the PCR conditions followed were: denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 50 s, 55°C for 45 s and 72°C for 90 s and a final extension for 7 min at 72°C. The PCR and nested PCR products were electrophoresed in 1% agarose gel with Lambda DNA ladder (Bangalore Genei Pvt. Ltd, India).

DNA isolated from periwinkle plants infected with sugarcane grassy shoot phytoplasma (group 16SrXI, rice yellow dwarf group phytoplasma; Rao et al. 2014) and maintained in a greenhouse was used as positive control. The leaf samples of all the six sugarcane varieties grown through tissue culture were used as negative controls. The amplified products in
nested PCR were eluted from gel by using Qiaquick Kit (Qiagen). PCR products were sequenced and sequences were assembled using DNA baser V4.2 program and aligned using CLUSTAL W method of Bio-Edit software (Bio-edit Sequence Align Editor). The sequences we generated and sequences of reference phytoplasma strains retrieved from GenBank were used to construct phylogeny through MEGA 5.0 version Software (Tamura et al. 2011).

RESULTS AND DISCUSSION

We detected phytoplasmas in 17 of 26 non-symptomatic leaf samples (from the fields nearby where symptomatic clumps were also seen) in nested PCR assays that yielded ~1.2 kbp amplicons. No amplifications were seen in PCR with P1/P6 primers and the leaves collected of non-symptomatic varieties from distant sugarcane fields grown out of tissue-culture raised plants. All the amplified product were directly sequenced and of 17 amplified products, 6 (one representative of each variety) were submitted to GenBank with the accession numbers KR092366, KR092367, KX160816, KX160817, KX160818 and KX160819. Submitted 16S rDNA sequences showed 99% identity with identified phytoplasma strains of ‘Ca. P. orzaye’ and 99-100% identity among themselves through BLASTn analysis. Phylogenetic analysis using MEGA 5.0 software also supported BLASTn analysis results where all the six phytoplasma isolates of non-symptomatic sugarcane clustered together in the phylogeny tree with members of ‘Ca. P. oryzae’ group (data not shown).

In Uttar Pradesh, typical symptoms of sugarcane phytoplasma (white leaf/yellow leaf/green grassy shoot) are a regular occurrence (Rao et al. 2008; Tiwari et al. 2012), but the presence of phytoplasma in symptomless plants that we recorded suggested that either the phytoplasma perpetuates through infected mother cane setts that were vegetatively propagated, or in these fields some efficient vector is present that was responsible for spread of the phytoplasma from nearby infected fields. Earlier studies already confirmed the association of phytoplasma in different leafhopper vectors and their role in natural spread of SCGS phytoplasma in nature at the same location (Rao et al. 2014; Tiwari et al. 2016).

Our findings alert us to check primarily for the phytoplasma indexing for the sugarcane setts to be used for planting. The thermotherapy used for elimination of SCGS phytoplasma as suggested by earlier workers (Rao et al. 2012; Soufi and Komor 2014) may also be applied for sugarcane setts to be used as seed material. However, there was no report available on impact of low titre of phytoplasma in non-symptomatic sugarcane varieties, but it is quite possible that under favorable weather conditions, the pathogens would have a significant impact on yield and quality of non-symptomatic (phytoplasma positive) sugarcane varieties. In conclusion, rigorous hot-water treatments and selection of healthy seed material of sugarcane are recommended before planting to manage phytoplasma infections.

ACKNOWLEDGMENT

We thank DST-SERB, New Delhi for the financial assistance.

REFERENCES


Détection de ‘*Candidatus Phytoplasma oryzae*’ dans des cultivars de canne à sucre asymptomatices dans l’est de l’Uttar Pradesh (Inde)

Résumé. Vingt-six échantillons de feuilles asymptomatices de six variétés de canne à sucre (CoS07250, CoS96268, CoS91269, CoSe01424, CoS08272, CoS08279) cultivées à la ferme du Sugarcane Research Institute à Shahjahanpur ont été analysés pour la présence de phytoplasmes en utilisant les paires d’amorces P1/P6 et R16F2n/R16R2. Des produits d’amplification de 1,2 kb ont été obtenus dans 17 des 26 échantillons de feuilles asymptomatices lors de tests de PCR nichée. Les produits amplifiés ont été séquencés directement, alignés et utilisés pour une recherche d’homologie avec le logiciel BLASTn de la base de données Genbank. Les séquences d’ADN 16Sr des 17 isolats asymptomatices de phytoplasmes de canne à sucre étaient identiques (99-100%) entre eux et présentaient 99% d’identité de séquence avec des souches du groupe de phytoplasmes 16SrXI qui comprend le phytoplasme du grassy shoot (SCGS). Etant donné que l’association avec des phytoplasmes a été détectée dans des cultivars de canne à sucre sans symptômes, ces résultats suggèrent que cela puisse devenir plus sérieux à des stades ultérieurs de croissance de la culture ou bien en repousse et, par conséquent, ce problème mérite l’attention.

Mots-clés: Canne à sucre, phytoplasme SCGS, cultivars asymptomatiques, ‘*Ca. Phytoplasma oryzae*’

Detección de ‘*Candidatus Phytoplasmas oryzae*’, en variedades de caña de azúcar asintomáticas, al este de Uttar Pradesh, India

Resumen. Veintiséis muestras de hojas asintomáticas de seis variedades de caña de azúcar (CoS07250, CoS96268, CoS91269, CoSe01424, CoS08272, CoS08279) obtenidas en la granja del Instituto de Investigación de la caña de azúcar en Shahjahanpur se analizaron para detectar la presencia de fitoplasmas mediante el uso de las parejas de iniciadores P1/P6 y R16F2n/R16R2. Mediante pruebas de PCR anidado se obtuvieron productos de amplificación de 1,2 kb, en 17 de las muestras de hojas de las 26 asintomáticas. Los productos amplificados fueron directamente secuenciados, alineados y usados en la búsqueda con BLASTn de la base de datos del GenBank. Las secuencias de ADN 16Sr de todos los 17 aislamientos de fitoplasmas de caña de azúcar, que no se asociaron con síntomas, fueron idénticos entre sí (99-100%) y mostraron un 99% de identidad de secuencia con los variantes de fitoplasmas miembros del grupo 16SrXI que incluye el fitoplasma de los brotes herbáceos de la caña de azúcar (SCGS). Debido a que se detectó la presencia de fitoplasmas en variedades de caña de azúcar que no presentan síntomas, se sugiere que esta condición puede llegar a ser grave en las etapas posteriores del crecimiento o socas de cultivo, por lo que este problema requiere atención.

Palabras clave: Caña de azúcar, SCGS fitoplasmas, variedades asintomáticas, ‘Ca. Phytoplasmas oryzae’