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AKADÉMIAI KIADÓ

Variability of phytoplasma associated with weeds grown in and around sugarcane crop in Uttar Pradesh, India

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RESEARCH ARTICLE

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ABSTRACT

During survey from 2013 to 2018, *Cynodon dactylon* at Shahjahnapur, Azamagarh, *Oplismenus burmannii* at Shahjahnapur, *Arundo donax* at Deoria and *Ocimum cannum* at Gorakhpur were observed with phytoplasma suspected symptoms. The maximum disease incidence (34%) was recorded at Deoria District in case of *A. donax* plant with witches' broom symptom. The nested PCR using universal primer pairs (R16F2n/R16R2) confirmed the phytoplasma association with all the suspected samples. Further BLASTn and phylogenetic analysis of the sequences revealed the association of 16SrXIV-A subgroups phytoplasma with *C. dactylon*, *O. burmannii* and phytoplasma belonging to 16SrI-B subgroups with *A. donax* and *O. cannum* plants in the present study.

KEYWORDS

weeds, secondary host, diversity, 16SrXIV, 16SrI

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INTRODUCTION

Weeds growing in and around fields of sugarcane may cause considerable economic losses because of ample space between sugarcane lines and because of the weeds compete with the sugarcane forcibly than other agricultural crops (Tiwari et al., 2022). According to Singh et al. (1978), over 60 weeds can be found in sugarcane fields during monsoon periods causing yield losses upto 35% (Sadruddin and Srivastava, 1983; Singh et al., 2021). Phytoplasmas cause diseases in several weeds, which serves as natural host of phytoplasma, facilitating the spread of phytoplasmas to other economically important plants, resulting in increasing economic losses. *Cynodon dactylon* was the first weed reported as host of phytoplasma in India in 1978 (Singh et al., 1978; Rao et al., 2017a; Tiwari et al., 2022). So far, over 60 weed species were reported as a host of phytoplasma belonging to six different phytoplasma ribosomal groups, from Asian countries and most of them (40) from India (Mall et al., 2010; Rao, 2021). Sequence studies at nucleotide have revealed that weed-infecting phytoplasmas mostly belong to 16SrI, 16SrII, 16SrIV, 16SrV, 16SrVI, 16SrVIII, 116SrIX, 6SrXI and 16SrXIV groups and weeds are the second largest host of phytoplasma in India after ornamental plants (Mall et al., 2010; Rao et al., 2017b) Among the reported phytoplasma groups, 16SrI followed by 16SrXIV phytoplasmas have a wider occurrence in nature all over the world especially in Asian countries (Mall et al., 2010; Tiwari et al., 2022).

Sugarcane, a highly commercial crop in India, especially in Uttar Pradesh, faces several biotic and abiotic stress, the most important biotic diseases are caused by fungus and phytoplasma (Tiwari et al., 2010). Moreover, weeds have been confirmed as hosts of several phytoplasma groups (16Sr-I, XIV groups) and sugarcane viruses etc. (Chaube et al., 2015; Rao et al., 2017b; Maurya et al., 2017, 2020; Tiwari et al., 2022). The Sugarcane grassy shoot (SCGS), leaf yellow (SCYLP), and white leaf (SCWL) caused by phytoplasma are the most important phytoplasmal diseases of sugarcane because of significant yield losses. The SCWL, SCGS are caused by 16SrXI-B (Rao et al., 2014) however SCYLP in India is reported with 16SrI-B & 16Sr-XII group of phytoplasma (Gaur et al., 2008; Kumar et al., 2015a). Early detection of these phytoplasmas associated with weeds is cardinal to hinder the spread of phytoplasma diseases to other commercial crops. In the present study survey of four important sugarcane growing districts was performed to collect weeds suspected to be infected with phytoplasma near and in the sugarcane fields. Their phytoplasma etiology was confirmed through PCR, nested PCR, sequencing, and phylogeny.

MATERIAL AND METHODS

From 2013 to 2018, a survey was conducted in sugarcane cultivating districts: Shahjahanpur, Deoria, Azamgarh and Gorakhpur of Uttar Pradesh state, India. The diseases incidence was calculated on the basis of the ratio of healthy and symptomatic plants in the field. For the study and etiology, no. of three leaf samples from symptomatic plants species (showing white leaf, excessive branching or little leaf with witches' broom symptoms) and one leaf form non-symptomatic sample were collected from weeds grown in and around fields of sugarcane (Fig. 1).

Total DNA was extracted from the midribs of leaves from symptomatic and asymptomatic plants using the method reported by Ahrens and Seemuller (1992) and DNA was used as a template for PCR assays. The extracted genomic DNA was amplified by direct and nested polymerase chain reactions (PCR) for 16SrRNA gene amplification of phytoplasma by using

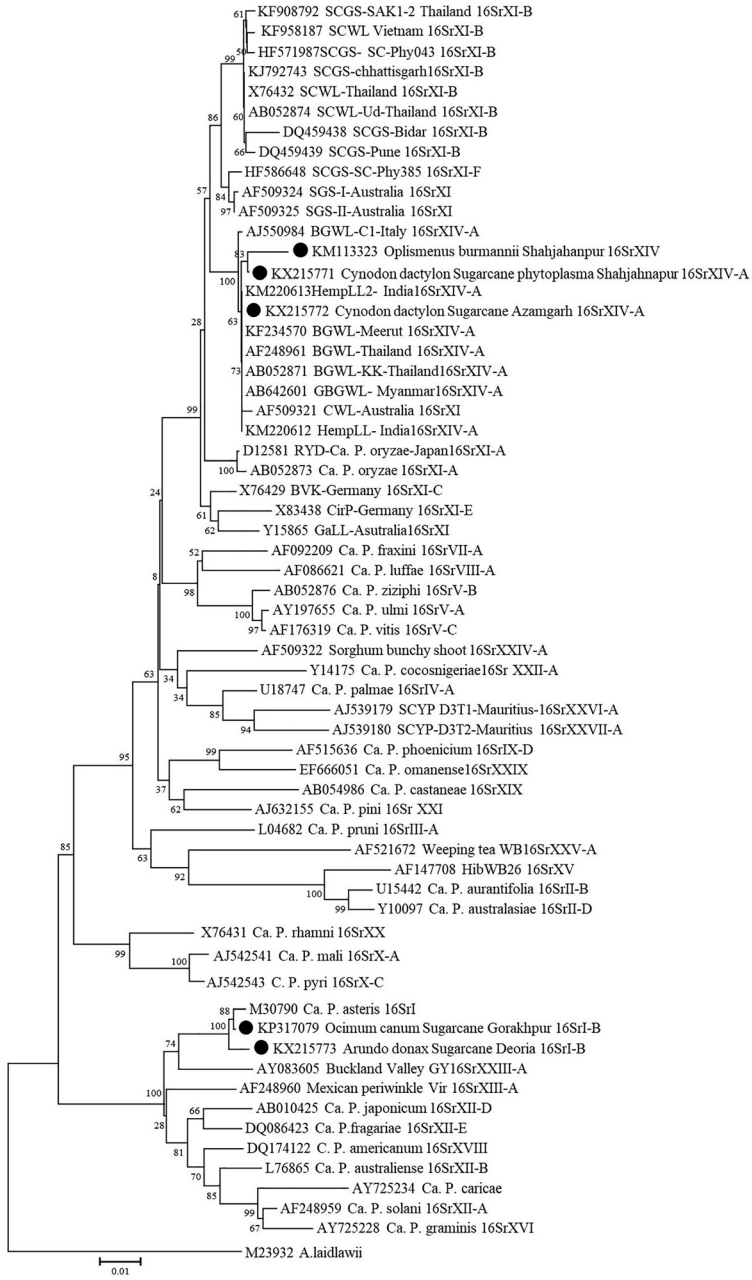

Fig. 1. Phylogenetic tree based on 16S rDNA constructed by neighbor joining method showing the relationships among *Cynodon dactylon* (KX215771, KX215772; *Arundo donax* (KX215773)); *Ocimum cannum* (KP317079); *Oplismenus burmannii* (KM113323) and selected phytoplasma strains. GenBank accession numbers are specified in the tree. *Acholeplasma. laidlawii* was used as outgroup

universal primer P1/P7 (Deng and Hiruki, 1991) followed by nested PCR primers R16F2n/R16R2 (Gundersen and Lee, 1996). The product of direct PCR was a template for nested PCR after 1:20 dilution using primer pair R16F2n/R16R2. The partial phytoplasma 16S rRNA gene amplified by R16F2n/R16R2 primers was purified from agarose gel using NucleospinR Gel (Macherey-Nagel) and or directly the PCR product was cleaned by PCR Cleanup System (Macherey–Nagel).

At least two direct PCR amplified products were sequenced directly in both directions using the same set of primers as for the PCR amplification at BiokartPvt., Ltd., Bengaluru. CLC Genomics Workbench 7.04 was used for assembling the forward and reverse sequence data of each test samples and BLASTn analysis was employed for the pair-wise sequence comparison analysis and phytoplasma related sequences were deposited in GenBank (NCBI, Bethesda, MD, USA) and accession numbers were received (Table 1). Different isolates of phytoplasma groups/subgroups were aligned with phytoplasma sequences available in GenBank using Clustal W. After multiple sequence alignment using MEGA version X (Kumar et al., 2016) phylogenetic trees were constructed with the neighbor-joining method and bootstrap testing using 1,000 replications for the evaluation of branching validity.

RESULTS AND DISCUSSION

During survey from 2013 to 2018 at four districts (Shahjahanpur, Deoria, Azamagarh, Gorakhpur) of Uttar Pradesh, *C. dactylon* at Shahjahnapur, and Azamagarh, *Oplismenus burmannii* at Shahjahnapur, *Arundo donax* at Deoria and *Ocimum cannum* at Gorakhpur were found with phytoplasma suspected symptoms (Table 1). The highest disease incidence (34%) was recorded in case of *A. donax* at Deoria. The *O. cannum* and *O. burmannii* at Gorakhpur and Shahjahanpur respectively found with minimum incidence, i.e. 1%. All the three symptomatic leaves of each plant's species showed ~1.2 kbp amplicons in nested PCR analysis with R16F2n/R16R2 primers however, it was absent in non symptomatic plants. The amplified products were eluted, purified and sequenced and submitted into GenBank. *C. dactylon* isolate from Shahjahanpur and Azamagarh showed highest 99–100% sequence identity with several isolates of 16SrXIV group of phytoplasma in BLASTn searches. The *O. burmannii* from Shahjahnapur also found maximum identical to 16SrXIV groups in BLASTn searches. The *A. donax* isolate from Deoria, and

Table 1. List of phytoplasma sequences obtained in the present study and 16Sr group/subgroup classification based on sequence similarity

S. No	Samples name	Location	Disease Incidence (%)	Symptoms	16Sr-Group	Accession
1	*Cynodon dactylon*	Shahjahnapur	18%	White leaf	16SrXIV-A	KX215771
2	*Cynodon dactylon*	Azamgarh	6%	White leaf	16SrXIV-A	KX215772
3	*Arundo donax*	Deoria	34%	Excessing branching	16SrI-B	KX215773
4	*Ocimum cannum*	Gorakhpur	1%	Little leaf	16SrI-B	KP317079
5	*Oplismenus burmannii*	Shahjahanpur	1%	White leaf	16SrXIV	KM113323

Ocimum cannuum isolate from Gorakhpur shared 99–100% identity with 16SrI group isolates. Further phylogenetic analysis of studied isolates confirmed the BLASTn results and *Cynodon* and *Oplismenus* isolates grouped with 16SrXIV-A subgroup of phytoplasma, however, *Arundo* and *Ocimum* isolate made a close relationship with 16SrI-B subgroup phytoplasma.

Over sixty weeds have been reported and identified as a host of various groups of phytoplasma and most phytoplasma groups involved with weeds are 16SrXIV followed by 16SrI group of phytoplasma (Tiwari et al., 2015, 2017; Rao et al., 2017b; Rao, 2021). In the present study, two phytoplasma groups, i.e. 16SrXIV and 16SrI, are identified in five isolates of four weed species from four districts. The *Cynodon* was already confirmed as host of 16SrXIV subgroup of phytoplasma from India (Rao et al., 2007), Iran (Hemmati et al., 2017), Iraq (Alkuwaiti et al., 2017), Saudi Arabia (Omar et al., 2016) and Turkey (Çağlar et al., 2013), which supports the present study results of isolates from Azamagarh district of Uttar Pradesh, India with the symptoms of white leaf. The infected plants were reported to show bushy growth, whitening of leaves, little leaves, and plant death (Hemmati et al., 2017).

Results from *Oplismenus* isolate collected from Shahjahanpur with leaf discolouration were supported the earlier findings of Rao et al. (2010) from Gorakhpur, Uttar Pradesh confirming a phytoplasma '*Ca.* Phytoplasma cynodontis'. This taxon has been in the past reported in India in Bermuda grass (Rao et al., 2007; Khanna et al., 2015).

A. donax and *Ocimum canum* isolates with phytoplasma symptoms like witches' broom, shoot proliferation and little leaf disease confirmed 16SrI-B subgroup phytoplasma which supported the findings of Tiwari et al. (2015) and Rao et al. (2017a) from India with similar symptoms of the disease.

While the weeds recognised as phytoplasma hosts often propagate profusely nearby field crops, the probabilities of transmission of phytoplasmas associated with important agricultural, economical and horticultural crops from weed to crops and vice versa cannot be overlooked (Tiwari et al., 2012, 2015, 2022). It suggests that the variability of phytoplasma in weeds plays an active role in the transmission of phytoplasma from diseased plant to healthy via vectors and because of this reason phytoplasmas can survive in many potential economical crops (Harrison et al., 2008).

Previous research has also suggested that a single plant species can host a variety of distinct strains; one strain can infect multiple plant species. It is imperative to characterise the vectors of numerous phytoplasmas in order to to enlarge effective management programs. Vectors transmitting the 16SrI (*Hishimonus phycitis*) and SrXIV (*Exiniatus indicus*) phytoplasma groups are already reported from India (Kumar et al., 2015b; Tiwari et al., 2021). Additional efforts should be initiated to categorise the function of alternate host species in phytoplasma diseases epidemiology; the host-vector interaction and the role of secondary host species. Hence forward, it is essential to concentrate future investigations on improving integrated management practices of phytoplasma diseases.

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