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Insect vectors

Insect vectors of phytoplasmas in soybean fields: discovery of *Recilia dorsalis* and *Exitianus indicus* through feeding medium assay

Kiran Kirdat[1,2], Bhavesh Tiwarekar[1], Shivaji Sathe[2] and Amit Yadav[1]

[1]National Centre for Cell Science, SPPU Campus, Ganeshkhind, Pune 411007, India;
[2]Department of Microbiology, Tuljaram Chaturchand College, Baramati, Maharashtra 413102, India

Abstract

The inoculative potential ability of *Recilia dorsalis* and *Exitianus indicus* in soybean fields was detected through a feeding medium assay and TaqMan-quantitative PCR. The study inferred a seasonal pattern in detecting the phytoplasmas in the feeding medium. Phytoplasmas were consistently detected in the insect bodies throughout the season, suggesting also a possible role of alternate weed hosts as reservoirs. The PCR test of artificial diets provides an easy and reliable way to assess insect potential inoculative ability and enables large-scale testing.

Keywords: qPCR, insect transmission, seasonal phytoplasma pattern, artificial diet

Introduction

Phytoplasmas are associated with numerous plant diseases, affecting a wide range of plants worldwide (Lee and Davis, 2000) and are transmitted by phloem-feeding insect vectors belonging primarily to *Cicadellidae*, *Derbidae*, and *Cixiidae*, commonly known as leafhoppers, planthoppers, and psyllids (Weintraub and Beanland, 2006). The impact of phytoplasma diseases and their distribution is essentially dependent on the feeding behaviour of the insect vectors (Bosco *et al.*, 1997).

In India '*Candidatus* Phytoplasma aurantifolia' (16SrII-B) and '*Ca.* P. australasia' (16SrII-D) are commonly associated with phyllody and witches' broom diseases in various pulse crops (Thorat *et al.*, 2017). The no pod disease incidence in these crops resulting from phytoplasmas infection has led to significant crop yield losses (Thorat *et al.*, 2016). In addition to crops, 16SrII phytoplasmas had also been reported from weeds (Duduk *et al.*, 2018; Kirdat *et al.*, 2020). The large distribution of phytoplasmas in crops and weeds underscores the contribution of insect vectors that allow pathogen transmission (Duduk *et al.*, 2018; Rao *et al.*, 2017). While several studies have detected phytoplasmas in insect DNA using PCR and suggested potential insect vectors for 16SrII phytoplasmas (Yadav *et al.*, 2015; Salehi *et al.*, 2007), only a few studies have confirmed the vectoring ability of insects through transmission assays (Phookan *et al.*, 2019).

This study aimed to investigate the diversity of *Cicadellidae* insect vectors in soybean fields and determine their vectoring ability using feeding medium assays. The assay used in this study was a PCR-based method for detecting phytoplasma in insect feeding medium.

Materials and Methods

Insects belonging primarily to the *Cicadellidae* family were collected using a white light trap from soybean fields in western Maharashtra, India. The samples were collected in September and October 2017 (n=212) and 2018 (n=260). They were divided into 55 groups based on morphological characters, and representatives of each group were DNA barcoded using the cytochrome c oxidase (COI) gene (Folmer *et al.*, 1994). All 472 samples were screened for phytoplasma presence using a TaqMan-qPCR assay (Christensen *et al.*, 2004). *Recilia dorsalis* (Motschulsky) and *Exitianus indicus* (Distant) were selected for transmission studies owing their abundance and percentage of positivity to phytoplasma presence.

A feeding medium assay was used to assess the vectoring ability of *R. dorsalis* (n=318) and *E. indicus* (n=341), collected periodically from July to October 2019 and 2021, following the protocol of Tanne *et al.* (2001). In brief, each insect was placed in a sterile 1.5 ml Eppendorf tube filled with 200 µl of sterile TE sucrose (5%) in their caps and sealed with parafilm. The field-collected insects were placed directly in these tubes and allowed to feed on the medium for 4 to 5 days. The TaqMan-qPCR assays was used to detect the presence of phytoplasmas in both insect body and feeding medium.

Results and Discussion

The insect samples (n = 472) were identified morphologically and confirmed with DNA barcoding (GenBank accession numbers MW221037 to MW228467). *R. dorsalis* (n = 162)

Corresponding author e-mail: Amit Yadav (amityadav@nccs.res.in)

and *E. indicus* (n = 86) were found predominantly infected with phytoplasmas in the soybean fields. The qPCR analysis revealed that 50.41% of the insects (n = 472) carried phytoplasmas. Moreover, this study identified known phytoplasma vectors, such as *Hishimonus phycitis*, *Nephotettix virescens*, and *Cofana unimaculata*, in soybean fields carrying phytoplasmas. Additionally, this study reports *Balclutha incisa* and *Yamatotettix sexnotatus* as potential phytoplasma vectors.

This study confirmed the inoculative ability of *E. indicus* and *R. dorsalis* through feeding medium assay. The qPCR assays confirmed the presence of phytoplasmas in the feeding medium and insect bodies with an average positivity rate of 15.03% and 27.35%, respectively. The detection of phytoplasmas in the feeding medium showed a seasonal pattern (Figure 1).

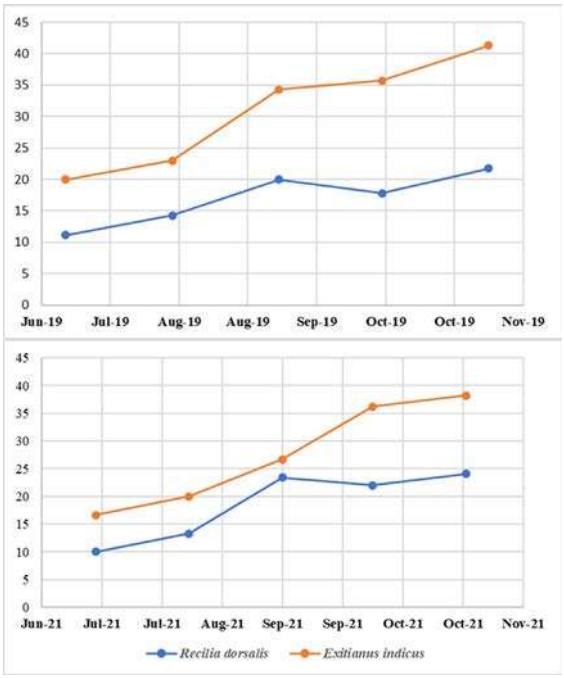

Figure 1. Inoculative ability of *E. indicus* and *R. dorsalis* through feeding medium assay assessed using qPCR assays from July to October 2019 (top) and 2021.

In contrast, phytoplasma detection in the insects' bodies did not indicate a season-related pattern. Phytoplasmas were found in the insect bodies throughout the season, suggesting a role of alternate weed hosts as phytoplasma reservoirs. Identification of insect vectors and their transmission parameters is crucial for controlling vector-transmitted diseases. However, large-scale biological assays for vector identification can be impractical, making the PCR test of artificial diets a reliable and easy tool for assessing insect inoculative ability.

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