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A multiplex PCR assay for rapid identification of major tospovirus vectors reported in India

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Abstract

Background: To date, four thrips vectors have been reported to transmit five different tospoviruses in India. Their identification at an early stage is crucial in formulating appropriate pest management strategies. Since morphometric key-based thrips identification based on the adult stage is time-consuming, there is a need to develop diagnostic tools which are rapid, accurate, and independent of developmental stages. Here, we report a multiplex PCR assay to identify four major thrips vectors viz. *Thrips palmi*, *T. tabaci*, *Scirtothrips dorsalis*, and *Frankliniella schultzei* present in India.

Results: Cytochrome oxidase subunit III and internal transcribed spacer region 2 were utilized to design species-specific primers. Of 38 pairs of primers tested, primer pairs AG35F-AG36R, AG47F-AG48R, AG87F-AG88R, and AG79F-AG80R amplified 568 bp, 713 bp, 388 bp, and 200 bp products from the DNA templates of *T. palmi*, *S. dorsalis*, *T. tabaci*, and *F. schultzei*, respectively at same PCR conditions. The specificity of the primer pairs was validated with a large number of known specimens and no cross-reactivity was observed with other thrips species. The multiplex PCR assay with a cocktail of all the four primer pairs detected four thrips vectors efficiently and could discriminate all of them concurrently in a single reaction.

Conclusion: The multiplex PCR reported in this study could identify the major thrips vectors reported in India. The assay will be useful in ascertaining distribution profile of major thrips vectors, disease epidemiology, screening large samples, and quarantine.

Keywords: *Thrips palmi*, *Thrips tabaci*, *Scirtothrips dorsalis*, *Frankliniella schultzei*, Tospovirus transmission, Diagnosis

Background

The tiny, fringed-winged insects belonging to order Thysanoptera are the sole transmitters of economically damaging tospoviruses (family *Tospoviridae*, order *Bunyavirales*) in ornamental, legume, and vegetable crops. Infection of tospoviruses results in necrotic lesion, stunting, wilting, reduced yield, diminishing quality of produce, and eventually death of plant. Tospoviruses like tomato spotted wilt virus (TSWV) infects more than 900 plant species and impatiens necrotic spot virus (INSV) can be found in over 300 plant species [1]. To date, 16 thrips vectors transmitting more than 29 tospoviruses have been reported [1–4]. Among them, four thrips vectors viz. *Thrips palmi* Karny

[5, 6], *Scirtothrips dorsalis* Hood [7], *T. tabaci* Lindeman [8], and *Frankliniella schultzei* Trybom [7] are reported to transmit five tospoviruses in India [4]. Although *F. occidentalis* (Pergande) has been recently reported in India [9, 10], its distribution and role in spreading tospoviruses under Indian conditions are yet to be studied.

A single thrips species may transmit more than one tospovirus and mixed infestation of more than one thrips vector in a host plant is common. Therefore, identification of thrips vectors at very early stages of infestation is necessary to understand the disease epidemiology and development of appropriate pest management strategies. Since morphometric key-based identification is time consuming, laborious, skill-based, stage-specific, and cannot resolve species ambiguities, there is a need to develop molecular diagnostic techniques that are rapid, specific and independent of developmental stage. PCR-based techniques

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