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A Multiplex PCR Assay for Rapid Identification of three Major Thrips Vectors Present in India

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Abstract—*Thrips are the principal insect pests of vegetable and ornamental plants worldwide and act as vectors of economically damaging tospoviruses. Thrips transmit tospoviruses in a persistent-propagative manner. To date, four thrips species have been reported to transmit five tospoviruses in India and their identification at 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 nucleotide sequence-based identification tools which are accurate and rapid independent of developmental stages. The present study reports a multiplex PCR assay to identify three major thrips vector species viz. Thrips palmi, T. tabaci and Scirtothrips dorsalis.*

Isofemale lines of T. palmi, T. tabaci and S. dorsalis were generated on brinjal, onion and chilli plants, respectively within insect rearing cages under controlled conditions. The species were initially identified based on morphometric keys and nucleotide sequences of cytochrome oxidase subunit I (COI). In case of thrips species, ITS and COIII sequences exhibit additional advantages for identification at species level because of a larger interspecific distance than COI. In the present study, COIII and ITS1 region were utilised to design species-specific primers. Among the 13 pairs of primers tested, primer pairs AG47F-48R, AG 35F-36R and AG 87F-88R amplified 560 bp, 689 bp, and 361 bp products of T. palmi, S. dorsalis, and T. tabaci respectively at same PCR conditions without any cross-reactivity. The amplified products were further sequenced to substantiate the species identity. 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. PCR assay with a cocktail of all three primer pairs detected three thrips species efficiently and could discriminate all the three species even in mixed populations. The assay will be useful in rapid and simultaneous identification of major thrips vectors and ascertaining their distribution profile.