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Research Article

Development of an Indirect ELISA and Dot-Blot Assay for Serological Detection of Rice Tungro Disease

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Rice tungro disease (RTD) is one of the most destructive diseases of rice in South and Southeast Asia. RTD is routinely detected based on visual observation of the plant. However, it is not always easy to identify the disease in the field as it is often confused with other diseases or physiological disorders. Here we report the development of two serological based assays for ease of detection of RTD. In this study we had developed and optimized an indirect ELISA and dot-blot assay for detection of RTD. The efficiency of both assays was evaluated by comparing the specificity and sensitivity of the assays to PCR assay using established primer sets. The indirect ELISA showed 97.5% and 96.6%, while the dot-blot assay showed 97.5% and 86.4% sensitivity and specificity, respectively, when compared to established PCR method. The high sensitivity and specificity of the two assays merit the use of both assays as alternative methods to diagnose RTD. Furthermore, the dot-blot assay is a simple, robust, and rapid diagnostic assay that is suitable for field test for it does not require any specialized equipment. This is a great advantage for diagnosing RTD in paddy fields, especially in the rural areas.

1. Introduction

Rice tungro disease (RTD), which causes reduction in rice production, is a widespread viral disease in South and Southeast Asia. In one of the worst reported outbreaks, it was estimated to cause annual losses in excess of about US$1.5 × 109 [1]. The disease is caused by infection of two different viruses [2]. The rice tungro bacilliform virus (RTBV) is a double-stranded deoxyribonucleic acid (DNA) virus from the family Caulimoviridae, of the genus *Tungrovirus* [3], and the rice tungro spherical virus (RTSV), a single-stranded ribonucleic acid (RNA) virus from the family Sequiviridae, of the genus *Waikavirus* [4]. RTSV has a single-strand polyadenylated RNA genome of about 12 kb that encodes a single large open reading frame (ORF). The structure of RTSV particles is spherical or icosahedral with a diameter of 30–33 nm. Its capsid comprises three coat proteins, namely, CP1, CP2, and CP3 [5]. On the other hand, RTBV has a circular double-stranded DNA genome of 8 kb that encodes four ORFs. RTBV has a bacilliform structure with width and length of 38 nm × 200 nm, respectively [6]. The symptoms and severity of this disease depend on these two viral agents. If rice is coinfected by both of the viruses, it will show the typical severe symptoms of yellow-orange leaf discoloration, plant stunting, and reduced yield [7]. On the other hand, if rice is infected only with RTBV, it shows milder symptoms. In contrast, rice plants will show no symptoms if they are infected only with RTSV [8].

Generally, except in advanced laboratories, RTD is commonly identified by visual observation of the symptoms. However, visual identification based on the symptoms alone is not reliable and often confused with other diseases and nonpathogenic disorders that can cause similar symptoms [9]. Conventionally, insect transmission assays had been used