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Over expression of Recombinant CP3 Protein of Rice Tungro Spherical Virus in Prokaryotic Expression System

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ABSTRACT

Currently, serological based diagnosis of rice tungro disease (RTD) is being limited by the availability of antisera. This study aims to produce recombinant protein from the coat protein (CP)3 of rice tungro spherical virus (RTSV) as a potential antigen in producing high titre antibodies for use in detection of RTD. Amplified RTSV CP3 gene of approximately 900 bp was cloned into pETSUMO vector. The recombinant plasmid was transformed into cloning host Mach1™-T1® *E.coli* cells and screened for recombinant gene in the correct orientation. Plasmid DNA from positive transformants was isolated and transformed into expression host BL21 (DE3) *E. coli* cells. One positive clone was selected for large scale expression where the expressed protein was purified and analysed by SDS-PAGE and Western blot. This recombinant RTSV CP3 protein, 46 kDa in molecular mass with 6x His-tag and SUMO protein fused to its N-terminal, was found to be antigenic when it reacted with our in-house generated polyclonal antibodies against tungro viruses.

INTRODUCTION

Rice tungro spherical virus (RTSV) is a plant picornavirus from the family *Sequiviridae* and the genus of *Waikavirus*. It assists in the transmission of rice tungro bacilliform virus (RTBV), the major causative agent of expression of rice tungro disease (RTD) symptoms, by green leafhopper, *Nephotettix* sp. [1-3]. Rice plants infected by RTSV alone are either asymptomatic or showed mild growth reduction [4].

RTSV has a positive-sense single-stranded, polyadenylated RNA genome of approximately 12 kb contained within a capsid composed of three coat proteins (CP) [5]. The genome encodes a single large polyprotein of 3473 amino acids which included the three CPs namely CP1, CP2 and CP3. The molecular mass of CP1, CP2 and CP3 are estimated to be 22.5 kDa, 22.0 kDa and 33 kDa respectively. Among the three CPs, CP3 is probably the major determinant on the virus surface [6]. Currently the most common method used for the diagnosis of RTD is by visual observation, however the results can be unreliable and not specific.

Another common diagnosis is by using molecular methods however these techniques can be costly especially for screening large amount of samples [7-10]. Used of serological methods had also been reported such as enzyme-linked immunosorbent assay (ELISA) and latex flocculation test [11-13]. Although the serological methods has been shown to be more reliable and cost effective, the availability of high titre antisera against tungro viruses for use in serological identification of RTD is limited by low concentration of tungro viruses in plants for antibody production [7, 14].