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Transfer and expression analysis of RTBV-O-Ds2 viral gene in Indian rice varieties against *Rice Tungro Virus* (RTV) using RNAi technology and chromosomal location of transgene

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Rice, *Oryza sativa* L., is one of the most important cereal crops in the world and rice tungro disease (RTD) is the most limiting factor for rice cultivation especially in South and South-East Asian countries. Rice tungro is a composite disease caused by simultaneous infection of plant with two unrelated viruses viz. rice tungro bacilliform virus (RTBV), a plant pararetro double-stranded (ds) DNA virus in the family Caulimoviridae, under the genus tungrovirus and rice tungro spherical virus (RTSV), a positive sense single-stranded (ss) RNA virus in the Sequiviridae family. Tungro viruses are efficiently transmitted in a semi-persistent manner by green leafhoppers (*Nephotetiix virescens*). In the present study RNAi based transgene construct, carrying RTBV ORF IV in both sense and anti-sense orientation, from a transgenic Pusa Basmati 1 line (PB1/RTBV-O-Ds2) was backcrossed with two popular rice varieties viz. Sadabdi (IET 4786) and Kheits (IET 4094). Functionally, the transgene construct targets homology-dependent selective degradation of RTBV transcripts employing RNAi mechanism. The backcross (BC) generations (BC3F4) were developed from O-Ds2 positive lines. The PCR-mediated detection of transgene indicated successful transfer of the RNAi into the rice cultivars through repeated backcrossing and a high frequency of amplification of fragments specific to ORF-IV (Ods-2 gene) and for hpt marker gene was detected in the successive backcrossed progenies of the rice varieties. The reverse-transcription PCR (RT-PCR) analysis of the backcrossed progenies for RTBV-O-Ds2 was also performed to check the activation of RNAi strategy in case of backcrossed lines. The expression of the transgene in both the varieties was analyzed by real-time PCR and attempt has been taken to identify the flanking sequence of the T-DNA and its characterization.

Keywords: Rice Tungro Disease, Ods-2 gene, expression, rice progenies

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