Poster Presentation 4

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# Identification of internal ribosomal entry sites in the genome of a fungal virus conferring hypovirulence to the white root rot fungus

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**Purpose:** Fungal viruses have a potential to control fungal diseases. The prototypic megabirnavirus, Rosellinia necatrix megabirnavirus 1 (RnMBV1), confers hypovirulence to its natural host, the white root rot fungus *Rosellinia necatrix* that causes destructive diseases on fruit trees. The RnMBV1 genome consists of two dsRNA segments of 9 and 7 kbp in length (dsRNA1 and dsRNA2), and each encodes two ORFs. Unique features of RnMBV1 include extremely long 5' untranslated region (5' UTR) spanning 1.6 kbp that share significant sequence identity between the segments. Therefore, the 5' UTRs are suspected to carry essential roles in replication and translation. This study explored internal ribosomal entry site (IRES)-mediated translation of the RnMBV1 genes.

**Methods:** IRES activities were evaluated by transgenic expression of a bicistronic luciferase cassette where a codon-optimized Renilla luciferase (ORluc) gene was cap-dependently translated while a codon-optimized firefly luciferase (OFluc) coding domain by IRES-dependently. Viral sequences were inserted in between the ORluc and OFluc genes and subjected to dual luciferase assay.

**Results and conclusions:** The 5' UTR of two dsRNA segments of RnMBV1 showed almost equal IRES activities. A series of deletion mutation analyses on dsRNA1 5' UTR revealed that fully functional IRES required a region positioned at 434-1401 but not other non-coding or coding sequences. Interestingly, there are no potential AUG start codons from nucleotide 1402 to 1678 (the first AUG), although 23 AUGs are found in the region 1-1401. The 1 kb RnMBV1-IRES is one of the largest viral IRES elements reported so far.