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Agro-Bio Tech

Le Corps professoral de

Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Madame ZHANG Peipei,

Titulaire d'un diplôme de *master of science majoring in biochemistry and molecular biology*,

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 25 juillet 2018, à 10 heures précises (personne ne sera admis après cette heure),

en l'auditorium PhV (Physiologie Végétale, bât. 48),

Avenue Maréchal Juin, 2, à 5030 GEMBLOUX.

Cette dissertation originale a pour titre :

« Detection and identification of viruses infected in wheat and its pests ».

Le jury est composé comme suit :

Président : Prof. F. FRANCIS : Professeur Ordinaire,
Membres : Prof. S. MASSART (Promoteur), Prof. X. WANG (Promoteur - CAAS, Chine), Prof. H. VANDERSCHUREN, Prof. F. VERHEGGEN, Prof. H. JIJAKLI, Prof. Y. LIU (CAAS, Chine).

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Abstract — Wheat is the second important crop in the world, nevertheless its production is hampered by viral diseases causing severe economic losses. More than 50 wheat viruses have been reported so far. Identification of the species and biological characteristics of the wheat viruses is the key to control the occurrence of wheat viral diseases. Planthopper s are also the limiting factors in wheat production due to sapping the plants and transmitting wheat viruses. But there is little information about the commensal viruses in planthoppers. The objective of this thesis is to detect and identify the viruses infected in wheat and its pests to enlarge the understanding of wheat pathogen and insect viruses, providing a basis for prevention and control of wheat viral disease. The main contents and results are as follows:

(1) A multiplex RT-PCR system for simultaneous detection of four wheat viruses

Wheat dwarf virus (WDV), barley yellow striate mosaic virus (BYSMV), rice black-streaked dwarf virus (RBSDV) and northern cereal mosaic virus (NCMV) are four viruses infecting wheat and causing similar symptoms. A multiplex reverse transcription polymerase chain reaction (m-RT-PCR) protocol has been developed for the simultaneous detection and discrimination of these viruses. Annealing temperature, concentrations of dNTP, Taq polymerase and Mg^{2+} were optimized for the m-RT-PCR. The detection limit of the assay was up to 10^{-2} dilution. The amplification specificity of these primers was tested against a range of field samples from different parts of China, where RBSDV, BYSMV, WDV and NCMV have been detected. This study fulfills the need for a rapid and specific wheat virus detection that also has the potential for investigating the epidemiology of these viral diseases.

(2) Identification of a new wheat virus

To identify the pathogens responsible for leaf yellowing symptoms on wheat samples collected from Jinan, China, we tested for the presence of three known barley/wheat yellow dwarf viruses (BYDV-GAV, -PAV, WYDV-GPV) (most likely pathogens) using RT-PCR. A sample that tested negative for the three viruses was selected for small RNA sequencing. A novel polerovirus was discovered and temporarily named wheat leaf yellowing-associated virus (WLYaV). The full genome of WLYaV corresponds to 5,772 nucleotides (nt), with six AUG-initiated open reading frames, one non-AUG-initiated open reading frame and three untranslated regions, showing typical features of the family *Luteoviridae*. Sequence comparison and phylogenetic analyses suggested that WLYaV had the closest relationship with sugarcane yellow leaf virus (ScYLV), but the identities of full genomic nucleotides and deduced amino acid sequence of coat protein (CP) were 64.9% and 86.2%, respectively, below the species demarcation threshold (90%) in the family *Luteoviridae*. Furthermore, agroinoculation of *Nicotiana benthamiana* Domin leaves with a cDNA clone of WLYaV caused yellowing symptoms on the plant. Our study discovered a new polerovirus that is associated with wheat leaf yellowing disease, which would help to identify and control pathogens of wheat.

(3) Identification of two novel totiviruses in white-backed planthopper

Two novel double-stranded RNA virus species belonging to the family *Totiviridae* were identified using high throughput sequencing and tentatively named Sogatella furcifera totivirus 1 and 2 (SfTV1 and SfTV2). Their complete genomes consist of 6,310 and 6,303 nt, respectively, showing typical genomic features with viruses in the family *Totiviridae*. Identity, phylogenetic and sequence analyses showed that SfTV1, SfTV2 and three other insect viruses may form a proposed novel genus of family *Totiviridae*. Vertical transmission of the two viruses was highly efficient, and they were detected in all insect tissues and developmental stages, with the highest titers in the adult and in the hemolymph and reproductive organs. To our knowledge, this is the first report of viruses in the family *Totiviridae* found in a hemipteran insect.

(4) Identification of a new partitivirus in small brown planthopper

In the present study, a novel partitivirus, tentatively named laodelphax striatellus associated partitivirus (LsPV), was identified in *Laodelphax striatellus* Fallén by RNA-sequencing. Its genome contains two segments of double stranded RNA (dsRNA), dsRNA1 (1,775 nucleotides) encoded a putative RNA-dependent RNA polymerase (RdRp) of 538 amino acids. dsRNA2 (1,575 nucleotides) encoded a putative coat protein (CP) of 440 amino acids. LsPV has similar genomic size, conserved motifs, and close phylogenetic relationships with members of *Gammapartitivirus* genus within the family *Partitiviridae*, the identities were only 28.6-70.9% for RdRp and 20.2-59.5% for CP, indicating that it is a new species of this genus. This is the first report of a complete genomic sequences of partitivirus from an insect.

(5) Identification of a new iflavirus in small brown planthopper

A novel iflavirus, tentatively named laodelphax striatellus iflavirus 3 (LsIV3), was identified in *Laodelphax striatellus* Fallén by total RNA-sequencing, and its genome sequence was confirmed by Sanger sequencing. The complete genome consisted of 10,831 nucleotides with a poly-A tail and included one open reading frame, encoding a 361.7-kD polyprotein. Conserved motifs for structural proteins, helicase, protease, and RNA-dependent RNA polymerase were identified by aligning the deduced amino acid sequence of LsIV3 with several other iflaviruses. Results of the identities and phylogenetic analysis based on the deduced amino acid sequences of the complete polyprotein of LsIV3 and other iflaviruses, indicated that LsIV3 is a new species belonging to the family *Iflaviridae*.