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ÁREA VEGETAL E INVERTEBRADOS

MOLECULAR DETECTION OF HONEY BEE VIRUSES IN APIARIES OF SOUTHERN BRAZIL

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Resumo

Bees are very important insects for agriculture, fulfilling an important role in pollination and renewal of the ecosystem. However, in several countries significant losses of colonies and population decline of honeybees and native bees have been reported, which are influenced by biotic and abiotic factors, including the effects of multiple pathogen infection and/or pesticide exposure. The majority of the viruses that have already been isolated and characterized in bees are classified as positive-sense single-stranded RNA viruses within the order *Picornavirales*, comprising the families *Dicistroviridae* and *Flaviviridae*. Prominent viruses include acute bee paralysis virus (ABPV), deformed wing virus (DWV), black queen cell virus (BQCV), sacbrood bee virus (SBV) and israeli acute bee paralysis (IAPV). Thus, the objective of this study was to detect the main bee viruses in apiaries in southern Brazil. Samples of honeycomb (larva and pupa) and adult bees were collected in the apiaries and kept under refrigeration until transportation to the Laboratory. Immediately the larvae and pupae were homogenized to TRIzol Reagent and frozen at -70 °C. Adult bees collected and stored in closed bottles were directly frozen. Six pupae, larvae and adult bee abdomen were submitted to RNA extraction and cDNA synthesis, followed by two multiplex polymerase chain reaction (PCR) (1: ABPV, CBPV and SBV; 2: BQCV, DWV and IAPV). All cDNA samples were tested with the endogenous control (GAPDH) to verify the efficiency of the whole process. RNA extracted from the bees' *pool* was used as negative control and gBlock® Gene Fragments were used as positive control of honey bee viruses. To date, 75 samples were obtained, mainly from southern Rio Grande do Sul, two of which were positive for IAPV (3,5%), three for ABPV (5,4%) and twenty-five for BQCV (33,3%), totaling 40% (30/75) of positive samples. All detected viruses were obtained from adult bees, and the identity of these viruses was confirmed by nucleotide sequencing. No viruses were detected in samples of larvae and pupae. These results demonstrate that ABPV, BQCV and IAPV viruses are present in apiaries in the South region of Brazil, with a high percentage of positivity for BQCV, and may, together with other factors, contribute for the bee population decline. This study is underway in order to increase the number of samples collected and phylogenetic characterization of the viruses detected.

PEPPER MILD MOTTLE VIRUS (PMMOV) DETECTED IN IMPORTED QUARANTINE CHILI PEPPER GERMPLASM

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Resumo

Recently (2019) the presence of *Pepper mild mottle virus* (PMMoV) was detected in imported chili pepper (*Capsicum* sp.) seeds through visual analysis of symptoms in chili pepper plants (germinated seeds), mechanical inoculation of indicator plants with symptomatic leaf extract of chili pepper and Enzyme-Linked Immunosorbent Assay (ELISA). A symptomatic chili pepper plant was identified, whose extract mechanically inoculated in indicator plants generated typical symptoms of virus in sweet pepper (*Capsicum annuum*). ELISA confirmed the presence of PMMoV as well as absence of *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tomato bushy stunt virus* (TBSV) and *Potyvirus*. Polymerase Chain Reaction (PCR) molecular tests confirmed absence of *Potyvirus*, *Tobamovirus* and *phytoplasma*. As PMMoV is a non-quarantine pest and is already present in Brazil, the corresponding chili pepper seed samples were released for importation, with the information of PMMoV presence. Consultation of scientific publications and discussion with virologist colleagues corroborated decision making. Therefore, the detection of PMMoV in this quarantine importation process of chili pepper seeds exemplifies the relevance of the plant quarantine service of import / export of materials for research. In addition, this service is effective preventive control of plant viruses and contributes to impacting scientific research on plant genetic improvement and phytosanity as well as for agriculture and national food security within Brazil. This work is aligned with the Brazilian policy, which promote prevention and surveillance of quarantine absent pests, according to the "Portaria nº 131, 27/jun/2019 - Programa Nacional de Prevenção e Vigilância de Pragas Quarentenárias Ausentes (PNPV-PQA)".

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