

ZIKA VIRUS NON-STRUCTURAL RECOMBINANT PROTEIN 1 (NS1 ZIKV) EXPRESSION, PURIFICATION AND EVALUATION OF VACCINAL POTENTIAL.

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Resumo

Zika virus (ZIKV) is an arbovirus that belongs to the *Flavivirus* genus and is transmitted to humans by *Aedes* mosquito bites. ZIKV has been responsible for outbreaks in several countries, including Brazil. The disease caused by ZIKV is usually acute and self-limited, however, some cases may lead to more severe disease forms, such as microcephaly and Guillain-Barré syndrome. Several studies are underway to develop alternatives to prevent the infection by ZIKV, since there are no current commercial vaccines available. In this context, the search of antigens with potential to induce protective immunity is fundamental. Thus, the present study aimed to express and purify the ZIKV non-structural protein 1 (NS1 ZIKV) in a prokaryotic system, with preserved antigenicity and immunogenicity aiming its use as an antigen in murine model. The expression of NS1 ZIKV protein was performed on recombinant *E. coli* BL21 (DE3)-RIL strain followed by its purification by nickel affinity chromatography. NS1 ZIKV recombinant protein was expressed only in the insoluble pellet fraction of the bacterial lysate and was submitted to an optimized renaturation stage (*refolding*). The refolded protein was tested on different buffers (HEPES and sodium phosphate) and protein stability was evaluated after the dialysis. The final protein yield was approximately 1 mg per liter of culture. The antigenicity of the recombinant protein was measured in ELISA after testing the protein reactivity with antibodies generated after ZIKV infection. NS1 ZIKV protein immunogenicity was evaluated after immunization of C57BL/6 and AG129 mice (three subcutaneous doses), associated or not with two different adjuvants: Poly (I: C) and LT-B adjuvants. In both mouse lines, high IgG antigen-specific antibody responses were achieved particularly with the C57BL/6 mice. Thus, the recombinant protein was obtained with good stability and preserved its antigenicity and immunogenicity. After the optimization process, we concluded that the recombinant protein had the required quality of a model antigen for vaccine studies of potential ZIKV vaccines based on non-structural recombinant proteins.

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Palavras-chaves: FLAVIVIRUS, NS1 PROTEIN, ZIKA, VACCINE, RECOMBINANT PROTEIN

SEED TREATMENTS ON WHEAT STRIPE MOSAIC VIRUS MANAGEMENT AND MOLECULAR CHARACTERIZATION OF PLASMIDIOPHORID VECTOR

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Resumo

The Wheat stripe mosaic virus (WhSMV) is a new characterized viral species associated with soil-borne wheat mosaic disease (SBWMD) in Brazil. The SBWMD management is difficult because the plasmodiophorid vector of the virus forms resistance spores and remains viable in the soil for several years. The aim of this study was to test seed treatments for vector control and plant development in the field with a history of this disease and characterize molecularly the plasmodiophorid associated with wheat samples infected by WhSMV. The trial was conducted in the field during the winter in 2017 season, in Passo Fundo, Rio Grande do Sul State, southern Brazil. The experimental design was a randomized block, with five replications and two cultivars (TBIO Toruk and BRS Guamirim). Thirteen seed treatments were used, including seeds treated with water + polymer (control); *Bacillus subtilis*; *Bacillus amyloliquefaciens*; *Trichoderma asperellum*; acibenzolar-s-methyl; azoxystrobin; pyraclostrobin + thiophanate methyl + Fipronil; thiophanate methyl + fluazinam; phenamidone; fludioxonil + metalaxyl + thiabendazole + azoxystrobin; fluxapiroxade; metalaxyl; and dimetomorph. The effectiveness of seed treatment was evaluated by incidence of SBWMD at wheat flowering stage and grain yield. Azoxystrobin showed the lowest incidence, when compared with control, other fungicides, biological control agents and resistance inducer. Seed treatment using thiophanate methyl + fluazinam showed higher grain yield. Wheat roots of the cultivar TBIO Toruk and BRS Guamirim were collected and the molecular characterization of partial nuclear 5.8S and internal transcribed spacer 1 (ITS 1) of the *P. graminis* ribosomal DNA sequences was performed. Additionally, the presence of WhSMV was confirmed by RT-PCR using specific primers and sequencing. Nucleotide identities and phylogenetic analysis supported the classification of plasmodiophorid found in the wheat roots as *P. graminis*. This is the first molecular characterization of *P. graminis* in Brazil. The results presented here indicated the association of *P. graminis* with roots of wheat plants infected by WhSMV in Brazil and suggests that the new virus WhSMV could be transmitted by plasmodiophorid. Financial Support: EMBRAPA, UDESC, CAPES.

Palavras-chaves: *Triticum aestivum*, *Polymyxa graminis*, Virus, Incidence, Detection

EVOLUTIONARY DYNAMICS OF BIPARTITE BEGOMOVIRUSES: ONE GENOME, TWO HISTORIES

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Resumo

An intriguing aspect of virus evolution is the emergence of viruses with segmented genomes. A special case of genome segmentation are viruses which have their genomes segments packed into separate