

VIR-004

Expression of *Grapevine leafroll-associated virus 2* coat protein gene in *Escherichia coli* and polyclonal antibody production.

Fajardo TVM¹, Radaelli P^{1,3}, Nickel O¹, Eiras M², Pio-Ribeiro G³.
¹Embrapa Uva e Vinho, Bento Gonçalves, RS; ²Instituto Biológico, São Paulo; ³UFRPE, Recife, PE. E-mail: thor@cnpuv.embrapa.br.
Expressão do gene da proteína capsidial do GLRaV-2 em *E. coli* e produção de anticorpos policlonais.

Grapevine leafroll-associated virus 2, GLRaV-2 (*Closteroviridae*, *Closterovirus*) causes leafroll symptoms in grapevines and seems also to be involved in the aetiology of a widespread graft-incompatibility condition. The coat protein (CP) gene (597 bp) of GLRaV-2 was RT-PCR-amplified from total RNA of infected grapevine, cloned into the pCR2.1 vector and sequenced (GenBank EU053126). The fragment was subcloned into the *EcoRI* site of the pRSET-A expression vector and the recombinant plasmid was used to induce the expression of the CP in *E. coli* cells strain BL21:DE3. The CP, fused to a 6-His-tag, was purified from *E. coli* total protein extract by affinity chromatography using a Ni-NTA resin. Identity of the purified protein was confirmed by SDS-PAGE and Western blot, using commercial antibodies against GLRaV-2 and His. The *in vitro*-expressed recombinant CP had a MW of ca. 25 kDa (~3 kDa correspondig to the tag sequence). The purified protein was quantified and 2 mg used for the immunization of a rabbit. The obtained antiserum reacted with expressed GLRaV-2 CP in Western blots and with infected grapevine extracts in dot-ELISA. The production of recombinant coat protein offers an alternative for the development of antibodies for the reliable serological diagnosis.