VIR-005

Coat protein gene expression of *Grapevine virus B* in *Escherichia coli* and polyclonal antibody production. Radaelli P[1,3], Fajardo TVM[1], Nickel O[1], Eiras M[2], Pio-Ribeiro G[3]. [1]Embrapa Uva e Vinho, Bento Gonçalves, RS; [2]Instituto Biológico, São Paulo; [3]UFRPE, Recife, PE. E-mail: thor@cnpuv.embrapa.br. Expressão do gene da proteína capsidial do GVB em *E. coli* e produção de anticorpos policlonais.

Corky bark, a component of the grapevine rugose wood complex, caused by *Grapevine virus B*, GVB (*Flexiviridae, Vitivirus*), induces decrease of production, incomplete ripening of grapes and progressive decline. The coat protein (CP) gene (594 bp) of GVB was RT-PCR-amplified from total RNA of infected grapevine, cloned into the pGEM-T-Easy vector and sequenced (GenBank AF438410). The fragment was subcloned into the *Eco*RI site of the pRSET-C expression vector and the recombinant plasmid was used to induce the expression of the CP in *E. coli* 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 GVB and His. The *in vitro*-expressed recombinant CP had a MW of ca. 26 kDa (~3 kDa corresponding 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 GVB CP in Western blots and with infected grapevine extracts in dot-ELISA. While GVB purification is very difficult and labor-intensive, the procedure mentioned here is an interesting alternative that overcomes this and allows reliable GVB diagnosis in grapevines.