Production of polyclonal antiserum using recombinant coat protein of Rupestris stem pitting-associated virus. Basso, M. F.[1]; Fajardo, T. V. M.[1]; Eiras, M.[2]; Ayub, R. A.[3]; Nickel, O.[1] - [1]Embrapa Uva e Vinho - Lab. de Virologia; [2]Instituto Biológico - Lab. de Fitovirologia; [3]Universidade Estadual de Ponta Grossa - UEPG. *E-mail: marcosbiotec@gmail.com*. Production of polyclonal antiserum using recombinant coat protein of Rupestris stem pitting-associated virus.

Rugose wood is a disorder of grapevine and one of the most economically important graft-transmissible viral diseases. The symptoms are characterized by the development of pitting in the woody cylinder; affected vines usually decline and exhibit yield losses. RSPaV (genus Foveavirus) is one of the causal agents of this disease. The coat protein (CP) gene (780 bp) of RSPaV, isolate CF207, was previously RT-PCR-amplified from total RNA of infected grapevine, cloned into the pGEM-T Easy vector and sequenced (EF636804). The fragment was subcloned into the EcoRI site of the pRSET-B 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 antibodies against histidine. The in vitro-expressed recombinant CP had a MW of ca. 31 kDa (ca. 3 kDa from the tag sequence). The purified protein was quantified and 2.55 mg used for the immunization of a rabbit. The obtained polyclonal antiserum reacted with expressed RSPaV CP in Western blot and with infected grapevine extracts in indirect ELISA. The production of recombinant coat protein offers an alternative for the development of specific antibodies for the reliable serological diagnosis.