Vitis 33, 179-180 (1994)

Research Note

Elimination of Grapevine Leafroll Associated Virus Type I in *Vitis vinifera* cv. Lemberger

G. Staudt and H. H. Kassemeyer

K e y w o r d s : virus elimination, GLRaV.

Introduction: In 1949 Limasset and Cornuet have demonstrated that in plants systemically infected by viruses the titer of the virus is decreasing to the shoot tips meristem. After confirming these results by Morel and Martin (1952) the method of meristem or shoot tip culture has been increasingly applied to eliminating viruses in various plant species.

The method was extended by the additional use of thermotherapy (Gifford and Hewitt 1961; Galzy 1964; Nyland and Goheen 1969). By combining these methods several investigators have succeeded in the elimination of various viruses of different grapevine cultivars (Stellmach 1980; Barlass *et al.* 1982; Altmaier 1990; Savino *et al.* 1990; Goussard *et al.* 1991).

The investigations in our laboratory differ with regard to the size of the explants used (Staudt and Kassemeyer 1990). Shoot tips and axillary buds were used for the *in vitro* culture. Shoot tips had a maximum length of up to 3 mm.A modified LS medium was used at 25 ± 2 °C and 16 h fluorescent light (0.15-0.35 mW m -2 nm -1) for a 6-week culture. Under these conditions virus elimination was successful as far as the nepoviruses GFLV and RRV are concerned. The temperature was not high enough to explain the results as a thermotherapy effect and the size of the explants did not meet the conditions of meristem culture. Elimination, therefore, was probably the result of the optimal growth conditions of the *in vitro* culture. Under these conditions the virus titer was most probably diminished in the apical meristems and subsequently elimination of the viruses was the result.

The investigations to eliminate closteroviruses with the same method showed no success. A clone of *V. vinifera* cv. Räuschling, systemically infected by GLRaV-I has been grown under the conditions of *in vitro* culture as mentioned above for 8 years and after 34 passages without any sign of virus elimination. Closteroviruses seem to behave quite different to the nepoviruses as far as virus elimination is concerned. This has already been supposed by Savino *et al.* (1990) and Monette (1983).

Great efforts have been made to eliminate closteroviruses by using additional methods. Chemotherapeutical and thermotherapeutical methods were applied. According to the former methods various antiviral substances were tested. Substances were included which had been shown to interfere with the RNA metabolism and which were successfully applied by Schuster (1982). But no effect was observed in our experiments with antiviral substances. Similar negative results were reported by Stevenson and Monette (1983) and Monette (1984) after using Ribavirin in an attempt to eliminate GLRaV.

Goheen *et al.* (1965) and Jákó (1986) have shown that GLRaV will be inactivated by high temperatures and Bass and Vuittenez (1977) and Bass and Legin (1981, 1984) have reported successful elimination by using high temperatures. Therefore, there was no other choice but to use a combined method of thermotherapy and *in vitro* culture for our further investigations.

Materials and methods: The clone of *V. vinifera* cv. Räuschling infected by GLRaV - I was kindly supplied by Dr. P. Gugerli, Federal Agricultural Station of Changins, Nyon, Switzerland.The clone of *V. vinifera* cv. Lemberger was collected at Beilstein, Württemberg, Germany and investigated to be systemically infected by GLRaV-I.

ELISA was carried out with GLRaV-I-specific antibodies raised from rabbits immunized with antigenes isolated according the method described by Kassemeyer (1991). The antibodies were purified by means of affinity chromatography with protein A sepharose and labelled with biotin. For the double-antibody-sandwich ELISA biotinylated antibodies and alkaline phosphatase labelled streptavidin was used. The leaf samples were homogenized in extraction-buffer according to Gugerli *et al.* (1984). LS medium was used modified according to Staudt (1984).

Results: On February 23, 1990 3-year-old plants of cv. Lemberger, systemically infected by GLRaV-I, well growing in 10 l buckets, were trained and subsequently transferred from the greenhouse with temperatures of 20-25 °C to growth chambers with 26 ± 2 °C and 12 h light (Osram HQIL 5.0 mW m-2 nm -1)/d. Each plant had been formerly tested positively several times for GLRaV-I. Within the first week of the treatment the temperatures were increased in the growth chambers up to 32 °C. This temperature was applied for 31 d, then raised to 34, 36 and 38 °C each for 1week. The plants resisted the treatment without any serious damage. A control of powdery mildew was necessary once and some yellowing of some leaves were observed. Because of the high evaporation rate sufficient watering was absolutely necessary.

46 d after the beginning of the heat treatment leaf samples were taken from the treated plants for a first ELISA-test. Each sample consisted of 3 middle sized leaves. Already after that time no viruses could be detected.

After 60 d of heat treatment shoot tips were taken from all plants and transferred to *in vitro* culture. From each shoot 2 lines were initiated, one from the tip itself consisting of a length of up to 3 mm and the other from the first axillary bud. After 45 d the *in vitro* plants had about 12 leaves and were transplanted in pots in the greenhouse for further investigations.

When the *in vitro* plants in the pots had reached a high of about 45 cm 10 of them were tested by ELISA. No viruses could be detected in the samples. All plants were grown in the greenhouse until to next spring and tested several times with ELISA. 4 plants turned out to be infected by GLRaV. In July 1992 all plants were tested again.

Staatliches Weinbauinstitut, Merzhauser Str. 119, D-79100 Freiburg, Germany

Only in 3 plants of the formerly infected plants the ELISA-test was still positive. The positive testing plants were derived from shoot tips as well as from axillary buds. In July 1993 the remaining 62 plants were tested again but without any positive reaction. After grown for 3 years and thoroughly tested, the plants can be denoted as most probably GLRaV-free. They will be grafted on virus-tested rootstocks and planted in the field for further investigations.

As soon as the plants in the field will come to flower the yield will be carefully investigated. In case of higher yield, which should be expected as consequence of the missing viruses, yield should be limited in order to preserve the high reputation of the Lemberger wines.

The 4 plants treated with thermotherapy were subsequently grown in a greenhouse. After 3-month cultivation from all shoots grown out, leaves were sampled for the ELISA test. All tests of the 4 plants each showed positive reactions. From these results it can be concluded that during the 3-month growth reinfection of the shoots had taken place originating from the old wood or the rootstock respectively.

Discussion: The cv. Lemberger is one of the famous varieties for red wine production grown in Württemberg. Excellent quality wines are produced by this variety. Wine growers have complained for a long time about extensive berry dropping in many years by which the yield is considerable decreased. Our investigations of numerous samples of cv. Lemberger from various regions of Württemberg have proved all to be infected by GLRaV-I (KASSEMEYER, 1991). Therefore, it was of great interest to make all efforts to get the variety free of viruses, especially free of GLRaV-I.

The described method of combined thermotherapy and *in vitro* culture has proved to be successful and reliable as well. Presently 62 plants can be denoted as virus-free, including the closterovirus GLRaV - I. Although the artificial elimination of pathogenic viruses will probably not be significant in the running production of certified plant material, it will be without question a valuable tool to regenerate varieties which show decreased growth and yield as a result of virus infection. An instructive example can be reported from the investigations of LEGIN and WALTER (1986). They succeeded making virusfree 'Klevner von Heiligenstein' which is a famous subvariety of Gewürztraminer grown in Alsace.

To explain the different behaviour of the nepo- and closteroviruses to the different methods of virus elimination, their molecular structure may serve as a solution. Nepoviruses are relatively small single-stranded RNA viruses, closteroviruses on the other hand are relatively long double-stranded RNA viruses. Dispersal within plants may be interpreted in both types by cell-to-cell spreading via plasmodesms, for the long-distance dispersal the phloem is concerned. A difference could exist in the different sensitivity of the viruses to high temperatures in the meristem of the shoot tips. While the nepoviruses investigated were eliminated already by temperatures of 25 °C this happened with the closteroviruses only when the temperatures were > 36 °C.

Acknowledgements: We thank Mrs. C. STEINMETZ, M. BAUER and P. BOHNERT for their skilful assistance during the course of the investigations.

ALTMAIER, B.; 1990: The use of *in vitro* apical culture of grapevines to eliminate pathogens (different viruses, *Agrobacterium tumefaciens*). Proc. 5th Intern. Symp. Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG. Vitis, Special Issue, 463.

BARLASS, M.; SKENE, K. G. M.; WOODHAM, R. C.; KRAKE, L. R.; 1982: Regeneration of virus-free grapevines using *in vitro* apical culture. Ann. Appl. Biol. **101**, 291-295.

BASS, P.; LEGIN, R.; 1981: Thermothérapie et multiplication *in vitro* d' apex de vigne. Application à la séparation ou à l' élimination de diverses maladies de type viral et à l'élevation des dégats. C. R. Acad. Agric. France **67**, 922-933.

- -; - -; 1984: Thermotherapy and *in vitro* multiplication of grapevine apexes. Its use for the elimination of virus diseases and for the estimation of damages. Progr. Agric. Vitic. **101**, 270-274.

- -; VUITTENEZ, A.; 1977: Improvement of thermotherapy of the grapevine by cultivation of shoot apices on nutritive media or by grafting on aseptic grape seedlings *in vitro*. Ann. Phytopathol. **9**, 539-540.

GALZY, R.; 1964: Technique de thermothérapie des viroses de la vigne. Ann. Epiphyt. **15**, 245-256.

GIFFORD, E. M.; HEWITT, W. B.; 1961: The use of heat therapy and *in vitro* shoot tip culture to eliminate fanleaf virus from the grapevine. Amer. J. Enol. Viticult. **12**, 129-130.

GOHEEN, A. C.; LUHN, C. F.; HEWITT, W. B.; 1965: Inactivation of grapevine viruses *in vitro*. Proc. Intern. Conf. Virus Vector Perennial Hosts, with Special Reference to *Vitis*, 255-265. Davis, California.

GOUSSARD, P. G.; WIID, J.; KASDORF, G. G. F.; 1991: The effectiveness of *in vitro* somatic embryogenesis in eliminating fanleaf virus and leafroll associated viruses from grapevines. S. Afr. J. Enol. Viticult. **12**, 77-81.

GUGERLI, P.; BRUGGER, J. J.; BOVEY, R.; 1984: L'énroulement de la vigne: mise en évidence de particules virales et développement d'une méthode immuno-enzymatique pour le diagnostic rapide. Rev. Suisse Viticult. Arboricult. Hort. **16**, 299-304.

JÁKÓ, N.; 1986: Erzeugung symptomfreier Pflanzen aus Triebspitzenmeristem blattrollkranker Stöcke bei *Vitis vinifera* L. cv. Blauer Burgunder. Mitt. Klosterneuburg **33**, 15-17.

KASSEMEYER, H. H.; 1991: Investigations about the occurrence of closterovirus-like particles in grapevines in Germany. Proc. 10th meeting ICVG, Volos, Greece, Sept. 3-7, 1990, 81-88.

LIMASSET, P.; CORNUET, P.; 1949: Recherche du virus de la mosique du tabac dans les méristèmes des plantes infectées. C. R. Acad. Sci. Paris **288**, 1971-1972.

MONETTE, P. L.; 1984: Virus eradication: *in vitro* heat therapy; *in vitro* drug therapy. Research Highlight 1984, Saanichton Research and Plant Quarantine Station, Sidney, B. C., Canada.

MOREL, M.; MARTIN, C.; 1952: Guérison de Dahlias atteints d' une maladie à virus. C. R. Acad. Sci. Paris **235**, 1324-1335.

NYLAND, G.; GOHEEN, A. C.; 1969: Heat therapy of virus diseases of perennial plants. Ann. Rev. Phytopathol. **7**, 331-354.

SAVINO, V.; BOSCIA, D.; D' ONGHIA, A. M.; MARTELLI, G. P.; 1990: Effect of heat therapy and meristem tip culture on the elimination of grapevine leafroll associated closterovirus type III. Proc. 10th Meeting ICVG, Volos, Greece, Sept. 3-7, 1990, 433-436.

SCHUSTER, G.; 1982: Antiphytovirale Verbindungen mit Guanidinstruktur. Phytopathol. Z. **103**, 77-86.

- -; 1982: Improvement in the antiphytoviral chemotherapy by combining ribavirin (Virazole) and 2,4-dioxo-hexahydro-1,3,5-trazine (DHT). Phytopathol. Z. **103**, 323-328.

STAUDT, G.; 1984: The effect of myo-Inositol on the growth of callus tissue in *Vitis*. J. Plant Physiol. **116**, 161-166.

- -; Kassemeyer, H.-H.; 1990: Elimination of virus diseases by *in vitro* culture. Proc. 5th Intern. Symp Grape Breeding, 12-16 Sept. 1989, St. Martin/Pfalz, FRG. Vitis, Special Issue, 465.

STELLMACH, G.; 1980: Propagation of healthy plants from green tips produced on vines infected with NEPO-viruses. Z. Pflanzenkrankh. Pflanzensch. **87**, 92-96.

STEVENSON, J. H.; MONETTE, P. L.; 1983: Delay of onset of leafroll symptom expression in *Vitis vinifera* Liemberger from ribavirin-treated *in vitro* cultures. Can. J. Plant. Sci. **63**, 557-560.