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Research Note

Influence of meristem culture and virus elimination on phenotypical modifications of grapevine (*Vitis vinifera* L., cv. Refošk)

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Treatments of virus-infected plants with *in vitro* chemotherapy and thermotherapy are still in the preliminary research phases without conclusive results (BASS and LEGIN, 1981; BRENDEL 1988; BARBA *et al.* 1990; KRIEL 1990;). Therefore meristem culture remains one of the most suitable methods for the elimination of virus and viruslike diseases of grapevine (KRIEL 1990). It enabled a complete removal of leafroll associated closteroviruses (ALTMAYER 1989; BARBA *et al.* 1990) whereas in the elimination of nepoviruses this technique was less (50—60 %) efficient. Beside this, the *in vitro* meristem culture also makes possible the elimination of other grapevine pathogens: bacteria, MLO and viroids (ARREGUI *et al.* 1988; DURAN-VILLA *et al.* 1988; ALTMAYER 1989).

The problem of introduction of plant tissues in the *in vitro* conditions is that the phenotype of regenerated plants can be modified: stronger anthocyanin pigmentation of shoots, leaf petioles and leaf veins, changes of leaf sinus shape, and increased pubescence of lower leaf surface. Anomalies were even more frequent if the thermotherapy *in vitro* was used (GRENAN 1984). In cv. Corvina veronese CANCELLIER and COSIO (1988) have also recorded changes in leaf pubescence and shape, coloration of shoot and lower productivity, whereas CHÉE and POOL (1982) and LINH LE (1987) did not record any modifications in Rougeon and Frappato morilla.

The purpose of our research was to study the specific requirements of cv. Refošk with regard to micropropagation by *in vitro* culture and regeneration of virus-free plants and to check the identity of vines thus obtained relative to their mother plants and standard ampelographic description of the cultivar discussed.

High incidence of grapevine fanleaf (GFV) and grapevine leafroll associated viruses (GLRaV type I and III) was found in the local vine cultivar Refošk (*Vitis vinifera* L.) grown in the Slovene Karst region. Healthy stock material was required for the successful start of the clonal selection program. Treatment by *in vitro* meristem culture was 100 % effective in eliminating closteroviruses, while only 60 % of explants were free of GFV. Enzyme-linked immunoassay (ELISA) was used for virus detection.

The best proliferation was achieved on the modified Murashige and Skoog medium, supplemented with 2.0 mg/l BA and 0.3 mg/l IAA. Without exogenously added cytokinin there was no new bud formation. Regeneration of meristems smaller than 0.3 mm was not successful. The early rooting of tips developed from proliferated buds was necessary to ensure their further growth. The continued subculturing of explants on cytokinin added medium inhibited their elongation and accelerated the aging of cultures. The best rooting of the tips was performed on the same basal

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medium supplemented with 2.0 mg/l IAA. More than 80 % of shoots were easily rooted in whole plantlets that were successfully transplanted to soil.

Various phenotypical modifications were noted after transfer into *in vivo* conditions: double nodes, modified leaf shape, irregular distribution of tendrils on the shoot as well as the absence of prostrate hairs on the lower leaf blade. The incidence of these changes was determined visually and with measurements of leaf parameters before and after the first pruning.

Five months after the first pruning the majority of the above mentioned modifications had disappeared. The morphology of the vines became adequate to the standards of cv. Refošk and no double nodes or irregular phyllotaxy were noticed. Formerly, completely hairless leaves began to obtain cover of prostrate hair which was visibly thicker on leaves of the upper 1/3 of young shoot. In spite of the fact that the pubescence was still essentially less intensive in comparison to that in mother vines it can be concluded that these changes were not entirely irreversible, their carriers being primarily the lower buds of the shoot.

Phenotypical modifications of cv. Refošk can be partly attributed to the influence of the *in vitro* technique (changes of leaf pubescence, coloration of shoot tip etc.) and partly to the elimination of virus GFV (changed phylometrical characteristics).

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