THE ELIMINATION OF VIRUSESAND PHYTOPLASMAS IN IN GRAPEVINE PLANTS BY HEAT TREATMENT AND TISSUE CULTURE

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Heat treatment can be performed in several ways but, regardless of the procedure used, testing of the treated material for assessment of its health status should follow. The culture of meristems, combined with thermotherapy offers encouraging results. Using these methods, we are get the grapevine plants free of harmful viruses and phytoplasma diseases.

Keywords*: heat therapy, tissue culture, grapevine free from virus and phytoplasma diseases*

Introduction. Among woody plants, grapevine, apple and peach are the most frequent targets of sanitation protocols because their health statusis strictly regulated. Chemotherapy is the most frequently used technique in grapevine. Tissue culture, usually adopted to regenerate plantlets in biotechnological breeding programs, represents the less used tool for eliminate viruses from plants. Good health of the produced plants, in terms of viruses, could only be assured by carefully selecting mother plants that were virus-free plants according to accepted diagnostic procedures and then propagating material that was guaranteed. The most dangerous viruses are addressed by these regulations which then lead to the production of plants characterized by good health status, and their use can be considered safe. Mother plants are critical as they are used by nurseries to produce propagation material and distribute it, perhaps worldwide: strictregulatory measures are important to protect agricultural systems and environments from the spread of viruses. However, in some cases, identification of heal thy plants among selected local varieties with limited diffusioncanbe difficultor impossible, making sanitation procedures necessary to recover useful healthy plants.

The culture of meristems, combined with thermotherapy offers encouraging results. However, application of these approaches, as well as other techniques, has not led to a definitive clarification of the mechanisms of action involved, probably due to in complete knowledge about the target virus and the mechanism of resistance activated by plants.

Thermotherapy treatment consists of keeping plants, or more frequently a part of them, at temperatures between 32°C and 54°C, within the physiological tolerance limits

of each plant, for an appropriate period. In practice, the selected temperature represents the best compromise between virus degradation and plant survival, taking into account that the threshold of thermal sensitivity of some viruses is lower than that of plant cells and that damage caused to plant tissues by the thermal stress can more easily be reversed than viral damage [5]. All known graft-transmissible infectious agents of grapevine, except viroids, can be eliminated from parts of infected plants with varying levels of efficiency by heat therapy. Heat treatment can be performed in several ways but, regardless of the procedure used, testing of the treated material for assessment of its health status should follow. A sufficient interval between sanitation of the material and the conclusion of virus testing is necessary in order to avoid false negatives. The use of RT-PCR technique will help in selecting the successfully sanitized material, avoiding the false negative recovery due to the very low virus concentration after the first step of sanitation.

Results of investigations. Place pot-grown vegetative vines (e.g. rooted cuttings 2years-oldor older) of each variety or rootstock type to be taken in to the scheme into a heat cabinet and maintain at constant temperature of 38 ±1°Cand 16–18 hartificial illumination.

Collect tips 0.5–1 cm long from vegetatives hoots after 4 weeks or more (up to 300 days if the vines survive) from the beginning of the treatment, and root in a heated (25°C)sand bench undermost, after surface sterilization, in agarized nutrient medium under sterile conditions. Pot rooted explants and let them growina glasshouse until ready for testing.

Hot water treatment of dormant canes is successfully used to eliminate phytoplasmas, partially *Agrobacterium vitis* and several other pests. The dormant material is immersed in agitated water at 50°C and treated for 45 minutes according to the method of Caudwell *et al*. (1991). The hot water treatment should be done immediately before grafting, at the end of storage in a specially designed equipment maintaining exactly the required temperature throughout the plant material by an efficient mixing system [2,3].

Testing of 123 cultivars on the presence of virus infections are revealed that 73.3% of the samples are infected by grapevine fleck virus (GFkV) and 4.4% - by grapevine fan leaf virus (GFLV). These viruses are dangerous, and that is why we started to use the virus's elimination program [4].

Shoot tips 0.2 - 0.5 mm long were taken from the greenhouse grown grape plants infected by Grapevine Fan leaf (GFLV) and Grapevine Fleck Virus (GFkV). The tips were washed in detergent, then sterilized in 70 ethanol for 45 sec. followed by 0.10% (v/v) bleach for 10 min. The shoot tips were subsequently washed in three changes of

sterile distilled water. After sterilization the shoot tips were placed on the surface of Chee and Pool media supplemented by vitamins and minerals.

When the shoots achieved the length of 1 - 2 cm, the plants were transferred to the same media with 0.2 ml/1 NAA for root formation. After rooting the plants were transferred to boxes with a soil mixture and kept there until the plants reached 8 - 10 cm length. The heat treatment took place in a special thermostatically controlled temperature of +30+32 °C and lighting for about 16 hours. The plants were kept in the unit for 6, 12 and 15 weeks. Shoot tips 0.3-0.5 mm long from those plants were then taken and placed in the tubes on the previous media with correspondingly BAP and NAA hormones. The growing plants, which rooted, were transplanted to pots with soil mixture for adaptation under high humidity. The adapted plants were tested by the ELISA test for the presence of those viruses by which they were infected previously. Antibodies and conjugates were obtained from Agritest, Tecnopolis, Italy. Buffers, dilutions and tissue extracts were prepared following the instructions provided by the manufacturers. Absorbance was recorded at 405 nm using a micro plate reader (Labsystems Multiskan RC, Fisher Scientific).'The results suggest that with the combination of shoot apices and heat treatment, it is possible to eliminate: GFLV*)* an GFkV from the grapevine plants. There were no differences in virus elimination between the plants heat-treated 6,12 or 15 weeks. The percentage of recoveries was 80% for GFkV and 100% for GFLV.

For elimination of Bois noir phytoplasma grapevine wood, scions and rootstocks prior to grafting should be held in cold storage (1–5°C and high relative humidity) to maintain dormancy and enhance quality. However, grapevine plant material should be taken out of cold storage 12–24 h prior to treatment and stored at room temperature in a humid and aerated chamber. The hot water treatment should be done immediately before grafting, at the end of the storage period. Treatment before or during storage in a refrigerating chamber is strongly inadvisable.

The temperature (50°C) after immersion and the treatment duration (45 min) should be respected. It was found that in such treatment the dormant buds were usually damaged, but 3 weeks after the planting of seedlings from the treated plants in pots in a greenhouse, the substituent eyes began to germinate and the plants are completely caught up in growth the untreated seedlings. After verification by the polymerase chain reaction, the treated plants were not contained phytoplasma disease.

Conclusion. It was established that the culture of meristem from grapevines infected by harmful viruses, combined with thermotherapy gave us the possibility to obtain the plants free from fan leaf and leaf roll viruses. By the water heat treatment we obtained the plants free from Bois noir phytoplasma.

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Анотація

Мілкус Б.Н. ***Оздоровлення винограду від вірусів і фітоплазми за допомогою термотерапії та культури тканини.*** *Встановлено, що за допомогою культури меристеми в поєднанні з термотерапією можна оздоровити виноград від таких шкідливих вірусів , як коротковузля і скручування листків . За допомогою водної терапії нам вдалося оздоровити виноград від фітоплазмового захворювання - почорніння деревини*

Ключові слова: віруси винограду, фітоплазма, сухоповітряна термотерапія, ПРЦ- діагностика, водна терапія.

Аннотация

Милкус Б.Н. ***Оздоровление винограда от вирусов и фитоплазмы с помощью термотерапии и культуры ткани.*** *Установлено, что с помощью культуры меристемы в сочетании с термотерапией можно оздоровить винограда от таких вредоносных вирусов, как короткоузлие и скручивание листьев. С помощью водной терапии нам удалось оздоровить виноград от фитоплазменного заболевания – почернение древесины.*

Ключевые слова: вирусы винограда, фитоплазма, суховоздушная термотерапия, ПРЦ- диагностика, водная терапия.